Master of Science in BIOMEDICAL SCIENCES
UNIVERSITÀ DEGLI STUDI DELL'INSUBRIA
Academic Year 2021/22

THESIS PROJECTS

UNIVERSITÀ DEGLI STUDI DELL'INSUBRIA - DBSV

Laboratorio di fisiologia Cellulare e Molecolare

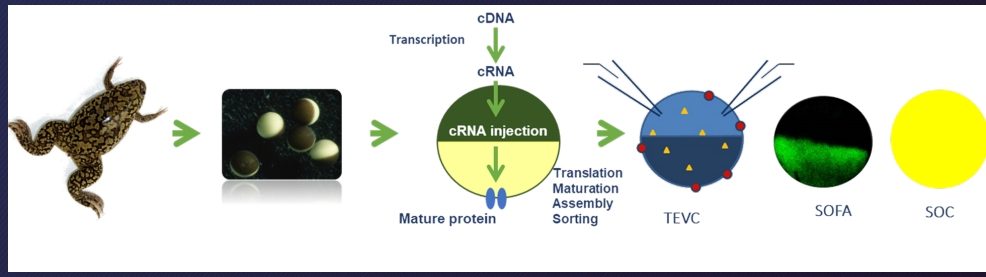
Elena Bossi, Cristina Roseti, Cinquetti Raffella, Tiziana Romanazzi, Manan Bhatt, Angela Di Iacovo

Master Thesis Project

1 «The role of neurotransmitter transporter in health and disease" (NeuroTrans Project). More information→ SCAN ME

2 «The regulatory pathway of nucleoside transporters» (In Collaboration with University of Barcelona) scan me

- Electrophysiological Techniques
- Molecular Biology Techniques
- Immunochemistry



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UNIVERSITÀ DEGLI STUDI DELL'INSUBRIA - DBSV
Laboratorio di Neurofisiologia Cellulare
Lia Forti

Master Thesis Project

"The effects of acute stress on glutamatergic neurotransmission in pyramidal neurons of the medial prefrontal cortex"

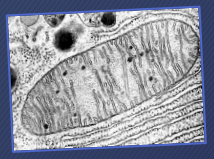
- Electrophysiological Techniques

  Patch-clamp: single cell studies

UNIVERSITÀ DEGLI STUDI DELL'INSUBRIA - DiSAT

Laboratory of Biochemistry & Functional Proteomics

Profs. Mauro Fasano & Tiziana Alberio



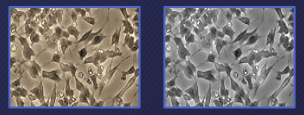
Vesicles trafficking dysregulation has been implicated in several neurodegenerative diseases, among which is **Parkinson's disease (PD)**. The early pathogenesis of PD also involves **dopamine (DA)** dyshomeostasis and **mitochondrial dysfunction**.

The aim of our project is to obtain an in-depth characterization of the vesicular trafficking alterations in a cellular model of disrupted DA homeostasis in **SH-SY5Y neuroblastoma cells**.

A combination of **proteomics** and **targeted molecular approaches** will be applied.

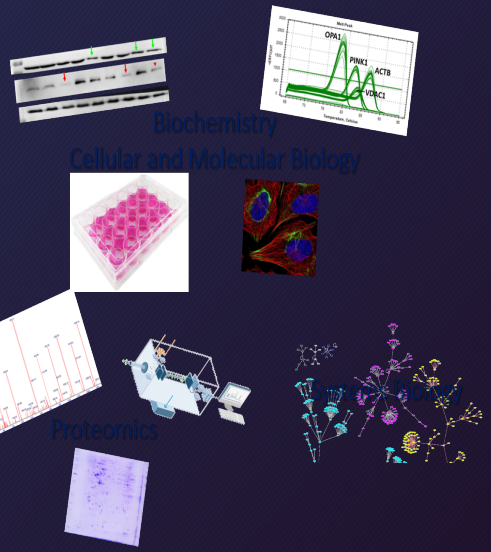
Several aspects involved in vesicle trafficking will be investigated: **anterograde and retrograde transports**, **VPS proteins** and **mitochondria-derived vesicles (MDVs)**.

SH-SY5Y



CTRL **DA**

Investigation of vesicular trafficking



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PROJECT TITLE

Prostate cancer: disentangling the relationships with tumor microenvironment to better model and target tumor progression

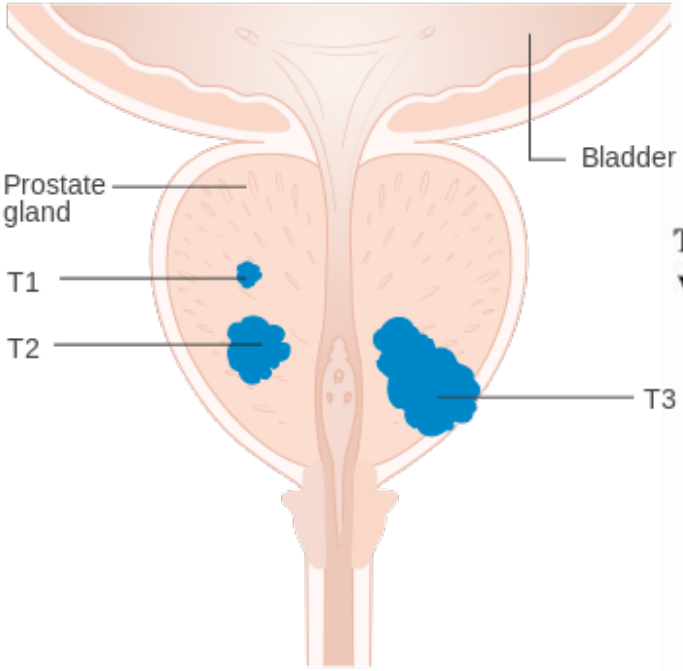
Laboratory of Tumour Epigenetics - University of Insubria
Villa Manara, Busto Arsizio

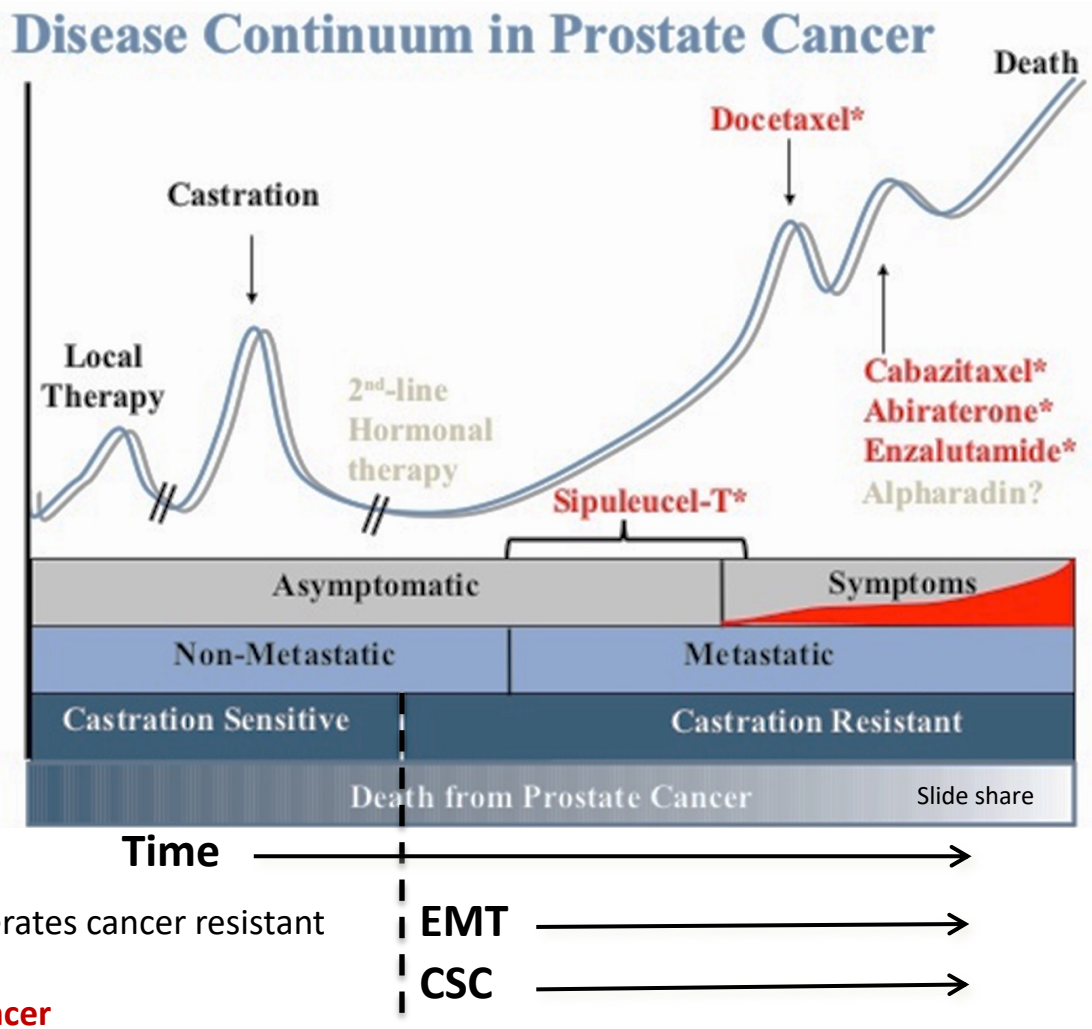
Prof. Ian Marc Bonapace
Dr. Serena Pagliara
Dr. ZaibunNisa
Dr. Michele Zocchi

Contacts: Prof. Ian Marc Bonapace
Email: ian.bonapace@uninsubria.it
Lab: 0331 339405

Progetto PRIN 2017 - External Collaborators:
Prof. Valeria Poli and Prof. Gontero: Univ. of Torino
Prof. Andrea Lunardi, Univ. of Trento
Prof. Licio Collavin: Univ. of Trieste
Prof. Marco Gaspari: Univ. of Catanzaro
Dr. Serena Chiriacò: Univ. of Salento (Lecce)

Castration resistant prostate cancer





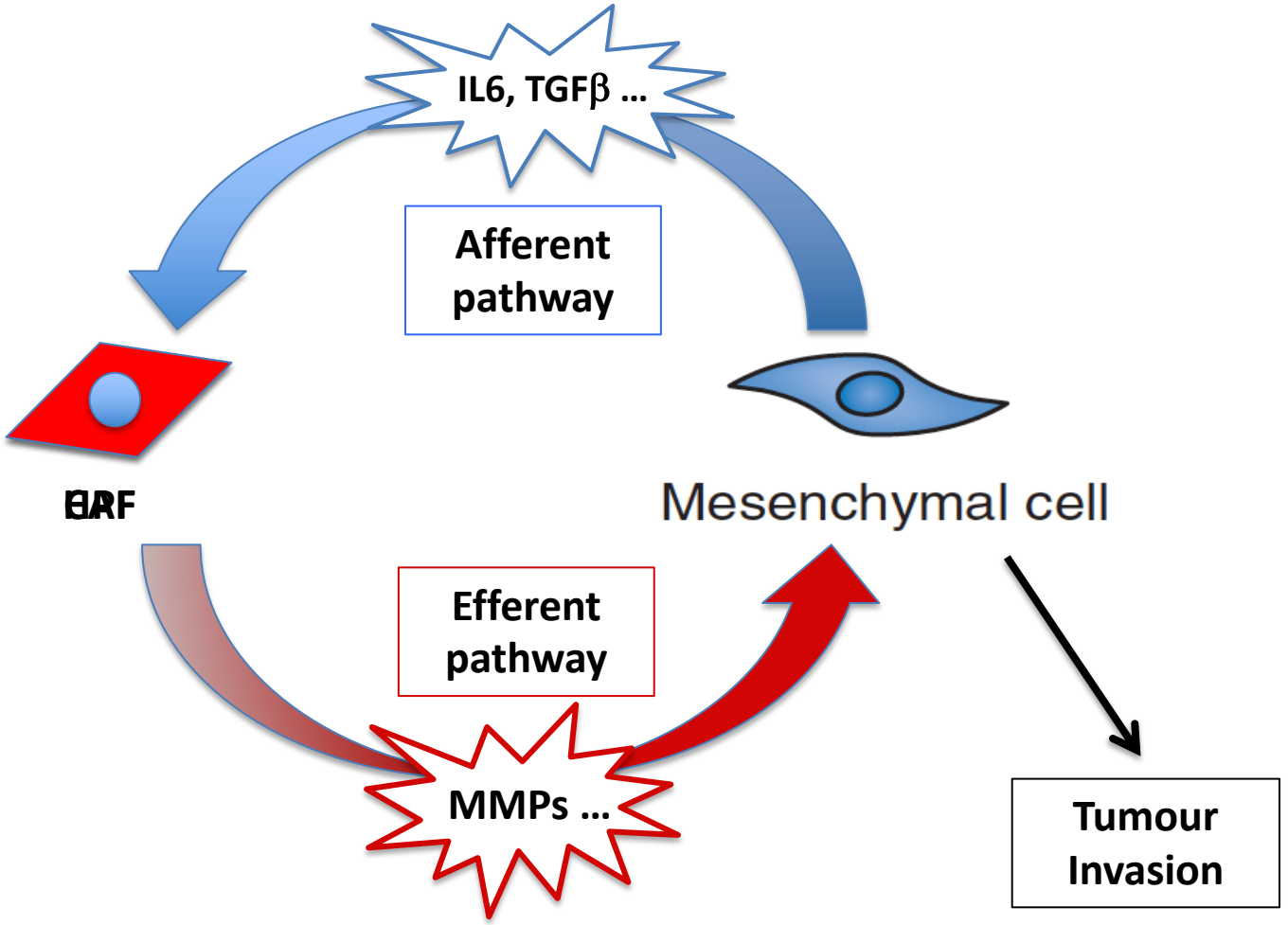
Prostate cancer treatment with anti-androgens generates cancer resistant tumours:

Castration resistant prostate cancer

which is resistant to anti-hormonal treatment and tends to produce cells undergoing **Epithelial to Mesenchymal Transition (EMT)** and cells with stemness features (**Cancer Stem Cells: CSC**)

Good evidence that CSC and EMT with their biomarkers contribute to drug resistance in prostate cancer

Cancer associated fibroblast induce EMT and stemness



There is a crosstalk between the tumour and the microenvironmental cells, mediated by diffusible factors, which helps the tumour cell to invade other tissues

In vitro 2D and 3D models for PCa progression

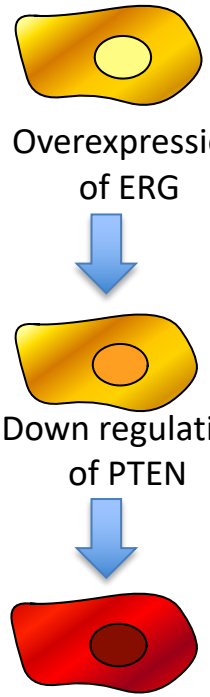
Collaboration with Prof. Andrea LUNARDI - UniTN

We will work on two in vitro models: 2D and 3D models of prostate cancer (PCa) to investigate the relationship between cancer cells and tumour microenvironment:

1) 2D model: normal prostate immortalized cells (RWPE-1) inducible for ERG and downregulated for PTEN
2) Organoids derived from RWPE-1 as in 1).

2D model

Prostate cells



Overexpression of ERG

RWPE1
Normal immortalized PCa cell

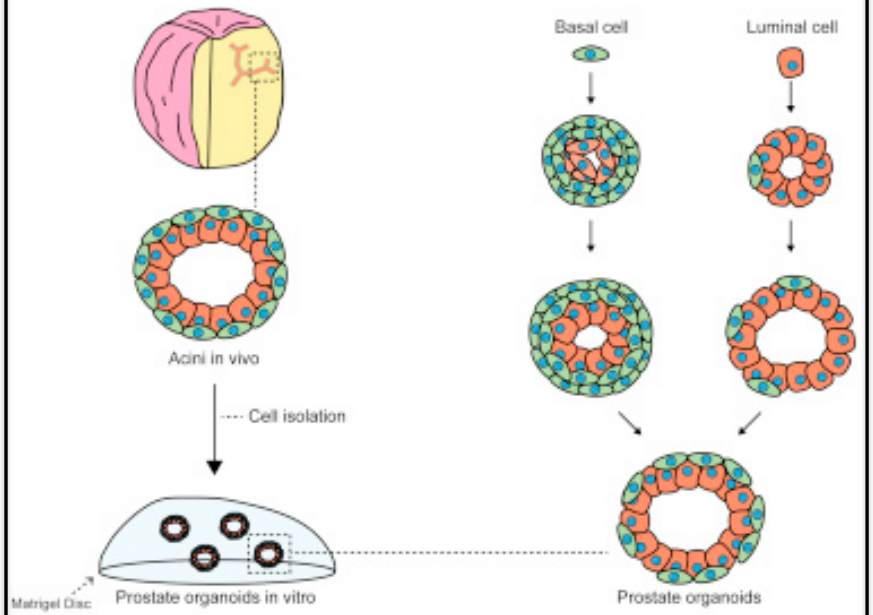
Down regulation of PTEN

RWPE1-ERG
Pre-cancerous PCa cells

RWPE1-ERG-PTEN
Cancerous PCa cells

3D model

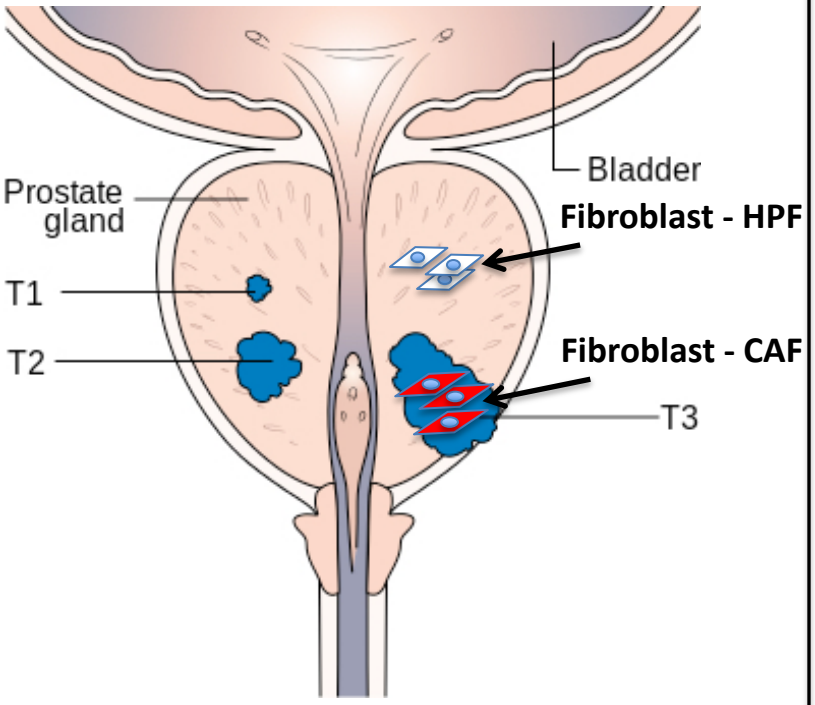
Prostate organoids



- Long term expansion
- Glandular morphology
- Basal & Luminal epithelium
- Androgen responsive
- Genetically stable

Isolation of Normal and Cancer associated Fibroblasts from Advanced Prostate Cancer (PCa)

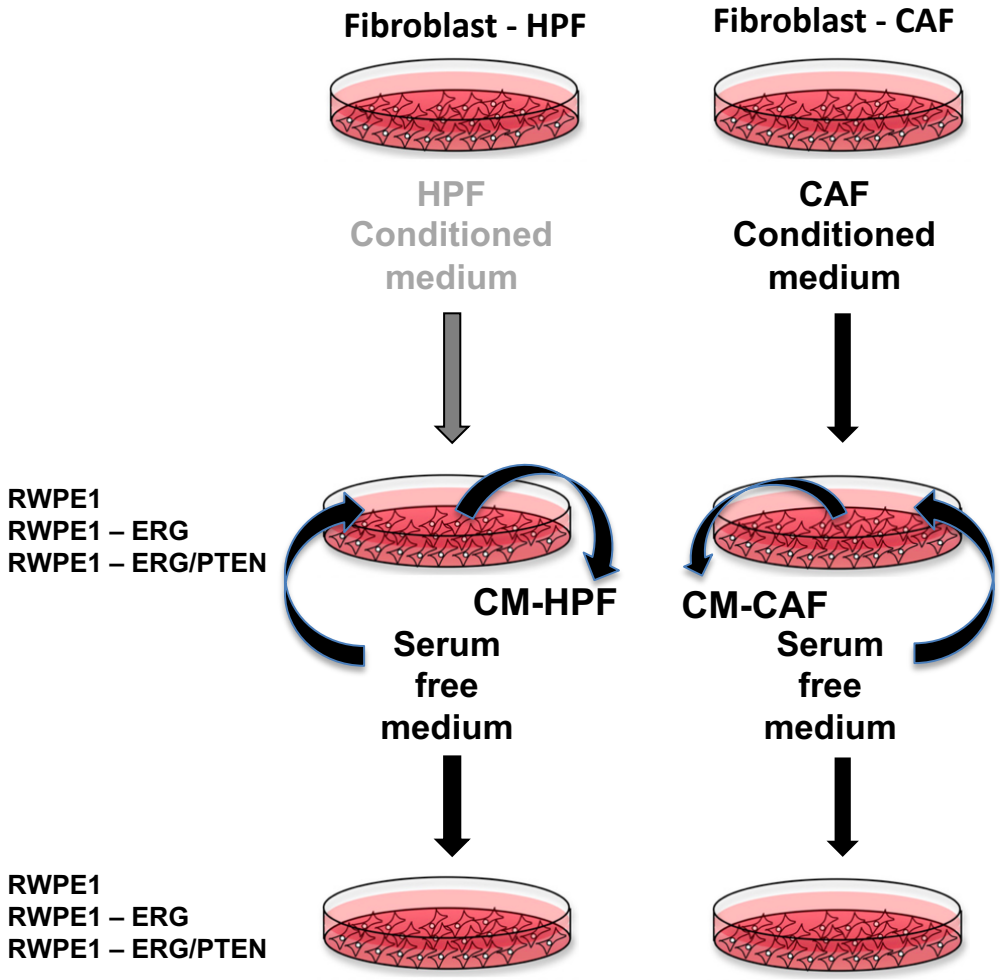
In collaboration with Prof. Gontero of the University of Turin we will isolate Normal (HPF) and Cancer Associated Fibroblasts (CAF) from radical prostatectomy of patients affected by high grade Prostate cancer (Gleason score ≥ 8)



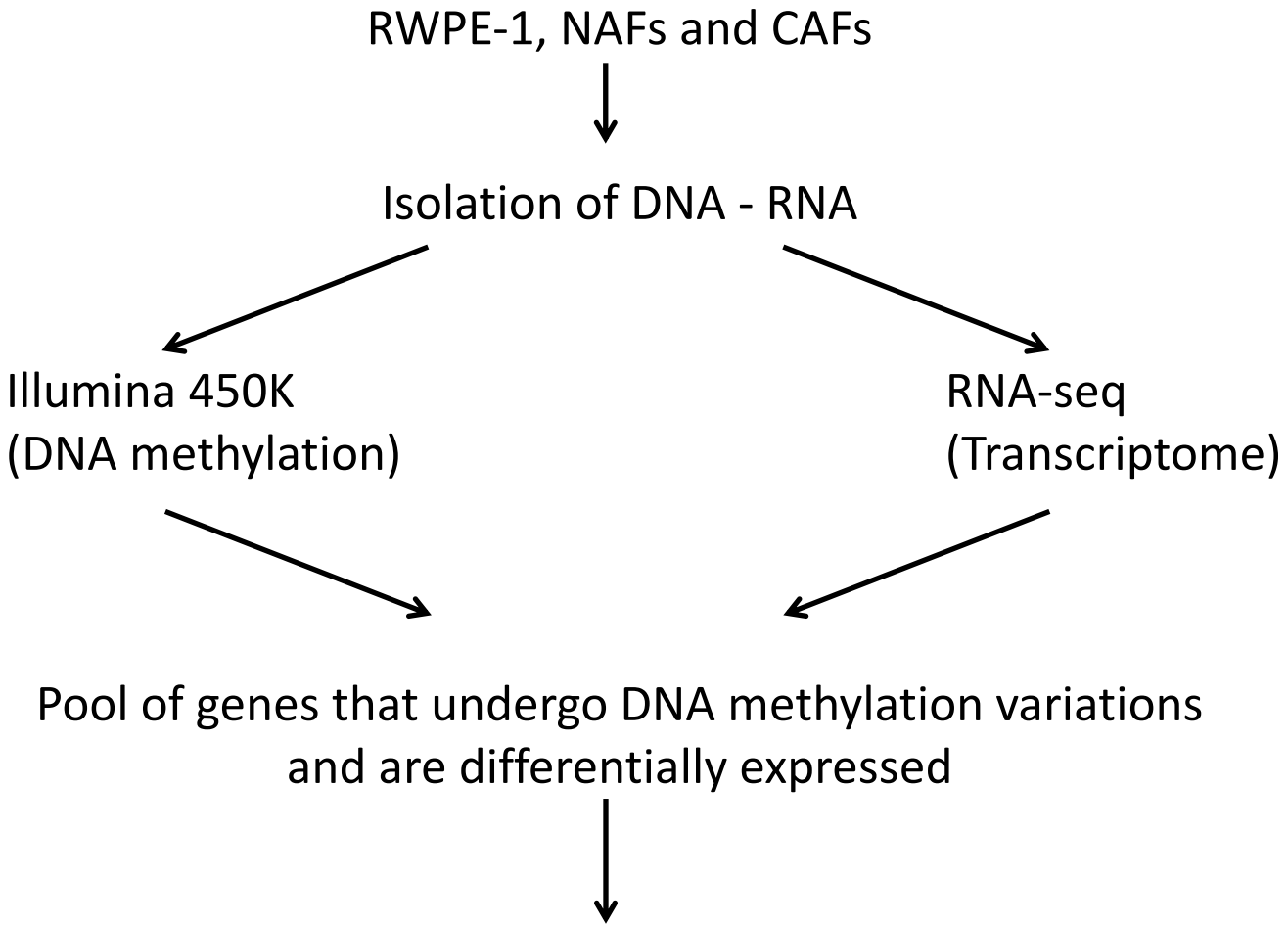
"Diagram showing T1-3 stages of prostate cancer CRUK 278" by Cancer Research UK - Original email from CRUK. Licensed under CC BY-SA 4.0 via Wikimedia Commons

Collaboration with Prof. Paolo GONTERO UniTO

HPFs and CAFs will be cultivated in vitro to obtain conditioned media that will be added to the 2D and 3D models induced with ERG and downregulated for PTEN to study the effects of the fibroblast secretome on tumour progression



Cross talk between 2D and 3D models of PCa and patients derived NAFs and CAFs



UNIVERSITÀ DEGLI STUDI DELL'INSUBRIA - DBSV

Laboratory of

Prof. Tiziana Rubino, Dr. Erica Zamberletti

Ongoing research lines in the lab are aimed at evaluating:

1) The therapeutic potential of some cannabinoids in models of autism
2) The ability of cannabidiol to modulate the long-term negative consequences of adolescent delta-9-tetrahydrocannabinol exposure on the brain
3) The role of the endocannabinoid system in adolescent brain maturation/remodeling

Main techniques:

- Behavioral tests in rodents
- Western blot analysis
- Immunofluorescence studies

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UNIVERSITÀ DEGLI STUDI DELL'INSUBRIA - DBSV

Laboratory of Molecular Neurobiology

Prof. Charlotte Kilstrup-Nielsen

Mutations in CDKL5 cause a severe neurodevelopmental disorder characterized by synaptic defects. In order to elucidate the underlying mechanisms and propose target-based therapies the focus of the laboratory is to study the role of CDKL5 in regulating microtubule dynamics and $GABA_A$-Receptor expression.

Project 1: Characterization of $GABA_A$-R expression and trafficking in *Cdkl5*-KO brains/neurons.
We have found $GABA_A$-R surface levels to be reduced in *Cdkl5*-KO neurons possibly explaining the epileptic phenotype. The student will use various biochemical approaches and immunofluorescence staining to analyze $GABA_A$-R trafficking in *Cdkl5*-KO neurons, the molecular mechanism underlying altered $GABA_A$-R expression, and GABAA-R expression in brains of KO mice.

Project 2: Characterization of microtubule-based transport in CDKL5-deficient cells.
We have identified the +TIP CLIP170 as an important downstream effector of CDKL5 on microtubule dynamics and as a druggable target allowing the restoration of CDKL5-related defects. The student will use various biochemical approaches and immunofluorescence staining (also live imaging) to analyse how loss of CDKL5 affects microtubule-based transport and CLIP170 functioning.

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Laboratory of Molecular Neurobiology
University of Insubria

UNIVERSITÀ DEGLI STUDI DELL'INSUBRIA - DBSV
Human GeneticsLab
Roberto Taramelli, Francesco Acquati

The Human Genetics Lab is involved n the molecular and functional characterization of of the human RNASET2 oncosuppressor gene, which plays a key role in the pathogenesis of several human cancer types.

The role of RNASET2 is being investigated by means of several multidisciplinary approaches, based on both 2D and 3D *in vitro* cellular models and xenograft-based in vivo assays with genetically engineered human cancer cell lines.

UNIVERSITÀ DEGLI STUDI DELL'INSUBRIA - DBSV
Laboratory of Molecular Genetics
Paola Campomenosi

1. **Regulation of the expression of the mitochondrial enzyme Proline Dehydrogenase (PRODH) and characterization of its role in non-small cell lung cancer (NSCLC)**
2. Quantification of specific circulating microRNAs as markers for the early diagnosis of lung cancer

Techniques: cellular and molecular biology techniques (DNA and RNA extraction, qPCR, Droplet digital PCR, luciferase assays, Western blot, ELISA); gene cloning; human cell cultures, stable and transient transfections, phenotypic assays on cells (proliferation, apoptosis, invasion, etc.).

UNIVERSITÀ DEGLI STUDI DELL'INSUBRIA - DBSV
Lab. BIOLOGY OF INVERTEBRATES
Annalisa Grimaldi http://dipbsf.uninsubria.it/invertebrati/

Identification and characterization of inflammatory factors playing a key role in promoting a rapid tissue regeneration by establishing a functional cross-talk between inflammatory response and connective tissue remodelling process

Techniques used in the laboratory: optical microscopy, transmission and scanning electronics, immunohistochemistry, enzymatic histochemistry, Western blot, use of biopolymers for setting up cell cultures

Collaborations with:
Laboratory of human genetics, prof. Francesco Acquati, DBSV; Laboratory of microbilogy, prof. Viviana Orlandi, DBSV;
Institut NeuroMyoGène, Universite Claude Bernard Lyon1, Dr Bénédicte Chazaud.

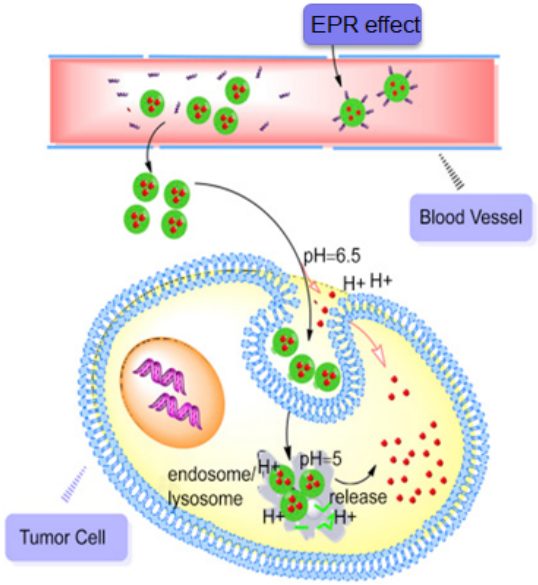
UNIVERSITÀ DEGLI STUDI DELL'INSUBRIA - DBSV
Polymer Therapeutics Laboratory
Lorella Izzo

Study of "smart" nanoparticles for anti-cancer drug-delivery

prof. Lorella Izzo – Polymer Therapeutics and Nanotechnology Laboratory
lorella.izzo@uninsubria.it

Nanomedicine represents a new generation of drug delivery and molecular imaging techniques with an enormous potential for improving human health. **Polymer Therapeutics** are nano-constructs that combine **bioactive agents and polymeric carriers** and are considered one of the most successful nanomedicines.

The development of **smart polymeric nanocarriers** deriving from mimicking the "nature's technologies" of self-assembling (polymer nano-assembling) has received an increasing attention for the creation of new systems, such as **micelles and polymersomes**, for precise and controlled drug delivery.



Owing to the wide range of pH gradients in physiological systems, **we are interested in the fabrication of pH-sensitive micelles and polymersomes able to convey different therapeutics payloads** such as chemotherapeutics (e.g. doxorubicin, paclitaxel) or biomolecules (siRNAs, peptides, protein) **to tumor tissue.** Once up-taken by cells, **the pH-sensitive carriers will release their payload in a controlled manner at the endosomal/lysosomal pH**.

Polymersomes: similar to lyposomes, nanoparticles containing a hydrophylic inner space separated from the outside environment by a double-layer membrane.

Micelles: nanoparticles with a hydrophobic core and a hydrophilic shell.

polymer ● hydrophylic drug ▲ hydrophobic drug

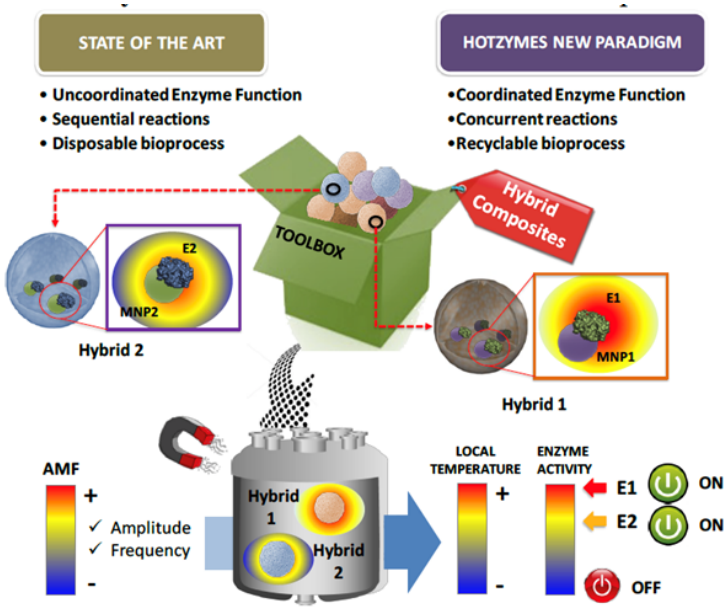
UNIVERSITÀ DEGLI STUDI DELL'INSUBRIA - DBSV
Cell Biology Laboratory

Giovanni Bernardini, Rosalba Gornati, Roberto Papait

Nanoparticle system activation by alternating magnetic fields, role of the enzyme orientation (Supervisor G. Bernardini)

We investigate the mechanisms of nanotoxicology, with particular attention to the effects of nanomaterials on cell proliferation and the interaction between nanoparticles and biological molecules.

Acquired skills:
Dedicated chemical synthesis.
Biochemical methodologies focalized on enzymes activity assays.
Cell culture maintenance, cultured cell tests.
Optical and electronic sample preparation and observation.



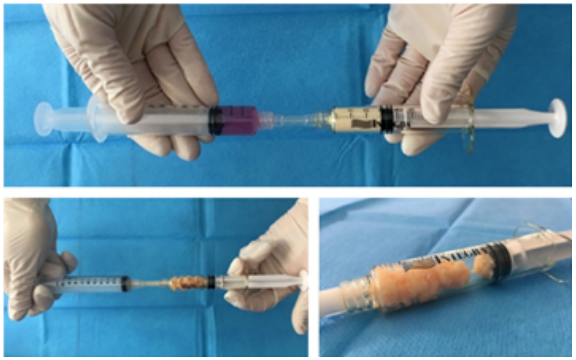
UNIVERSITÀ DEGLI STUDI DELL'INSUBRIA - DBSV
Cell Biology Laboratory

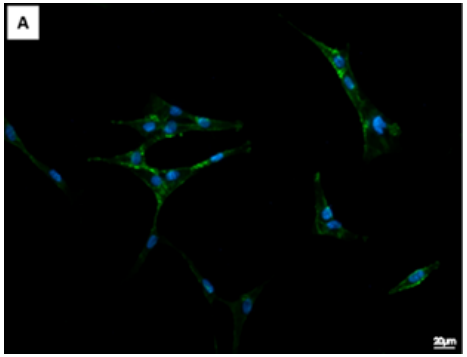
Giovanni Bernardini, Rosalba Gornati, Roberto Papait

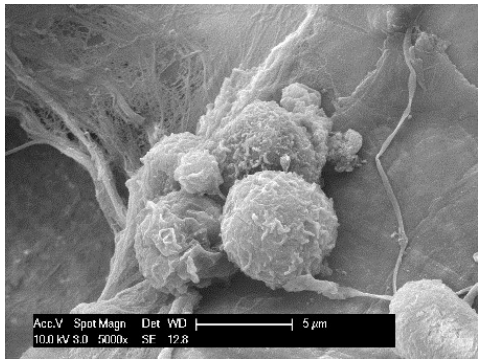
Human mesenchymal stem cells (hMSCs) (Supervisor R. Gornati)

We study the angiogenic potential of mesenchymal stem cells associated with biocompatible scaffold, and the effect of conditioned media for use in regenerative medicine.

Acquired skills: RNA extraction, cloning in plasmids, qualitative and quantitative PCR, gel electrophoresis. Cell extraction, maintenance, characterization by ELISA and viability tests. Optical and electronic sample preparation and observation.







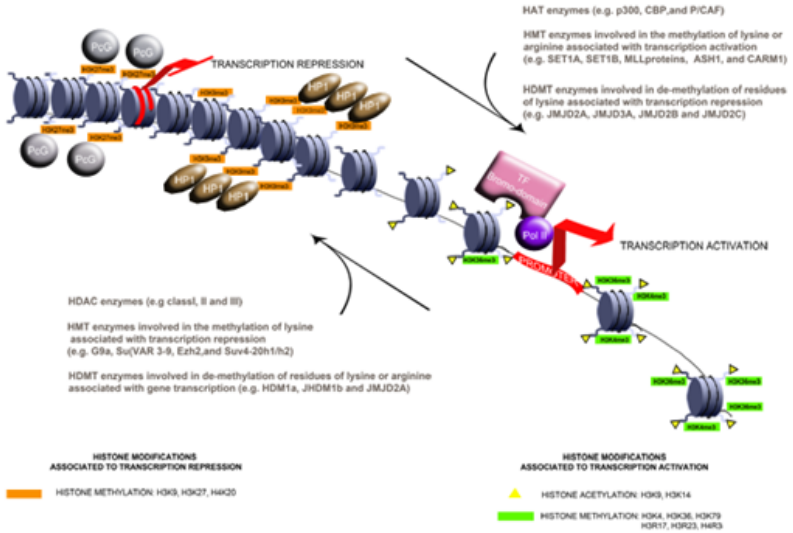
UNIVERSITÀ DEGLI STUDI DELL'INSUBRIA - DBSV
Cell Biology Laboratory

Giovanni Bernardini, Rosalba Gornati, Roberto Papait

The role of epigenetics in cardiac aging (Supervisor R. Papait)

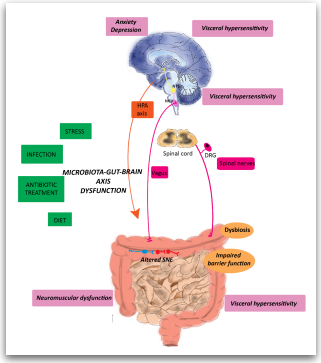
Investigate the relationships between some histone modifications, gene expression and ultrastructure of cardiomyocytes during cardiac aging, through the study of the epigenome and transcriptome by ChIp-seq, RNA-seq, and ultrastructural observations with TEM.

Acquired skills:
DNA and RNA extraction,
qualitative and quantitative PCR,
gel electrophoresis, western blot.
Optical and electronic sample preparation and observation.



UNIVERSITY OF INSUBRIA
DEPARTMENT OF MEDICINE AND THERAPEUTICS
DIGESTIVE SYSTEM PHARMACOLOGY GROUP

Cristina Giaroni , Annalisa Bosi, Davide Banfi



Baj et al., Int J Mol Sci 2019

- The group is mainly interested in investigating the adaptive machanisms underlying the interplay among the enteric nervous system (ENS), the gut microbiota and the enteric immune system along the microbiota-gut-brain axis and the possible participation of these changes in the pathogenesis of gut diseases and the related neuropsichiatric disorders, such as irritable bowel syndrome and inflammatory bowel disease.

- At the moment, we are focusing on the role of probiotics and of extracellular matrix molecules (i.e. hyaluronic acid) in the cross talk among the gut microbiota, the ENS and the enteric immune system in a dysbiotic mouse model obtained after chronic antibiotic treatment and in a murine model of Inflammatory Bowel Disease.

- The applied methods consist in biomolecular approaches (qRT-PCR, Western blotting), immunohistochemistry (HC on cross sections and IF on intestinal whole-mounts), pharmacological approaches (*in vitro* organ bath experiments on isolated intestinal segments and *in vivo* measurement of the efficiency of the intestinal transit)

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UNIVERSITÀ DEGLI STUDI DELL'INSUBRIA - DIPARTIMENTO DI MEDICINA E CHIRURGIA
Microbiology Laboratory

Andreina Baj - andreina.baj@uninsubria.it

The measure of total and species-specific Torquetenovirus (TTV) viraemia as a predictive marker of immune system function

Once thought to be only present in the host during disease, viruses have been recently demonstrated to be numerous in various districts in healthy subjects, and the term "virome" has been coined to describe the collection of viral species present in each human organ, as a kind of viral "flora" made up of bacteriophages, endogenous retroviruses, eukaryotic viruses not associated with disease and viruses able to cause acute, chronic or latent illness. More recently, monitoring the human virome has been suggested as a promising and novel area of research for identifying new biomarkers which would help physicians in the management of diseased patients. Thanks to the next-generation sequencing, the human virome has been studied in several districts, like the respiratory tract, gut, and skin, and in different clinical conditions. Thus, to date, we know that some components of the human virome are identified only in few districts of a limited number of individuals, while others are present in almost all body districts of a very high percentage of people.

Torquetenovirus (TTV) is the prototype of these latter components being the most representative and abundant virus of the human virome. TTV is presently classified in the Anelloviridae family, and it possesses several remarkable properties, including a particularly small single-stranded circular DNA genome, an extremely high degree of genetic heterogeneity (at least 29 TTV species have been identified so far), a remarkable ability to produce chronic infections with no associated clinical manifestations, and a high prevalence in the populations worldwide regardless of age, sex, and socio-economic status. Starting from these properties, evidence is increasing regarding the successful interplay of TTV with its host, and the control of TTV replication exerted by the immune system.

The goals of this project are to expand our knowledge in the TTV/immune system interplay investigating TTV and its genetic species as candidate surrogate markers to infer immune depression level, immune-reconstitution process, graft and/or infections risk, and, finally, the overall clinical outcome in adult patients receiving immunomodulant drugs. A precise understanding of how and how much immunity modulates TTV replication is of utmost importance given the intriguing idea of using PCR monitoring of TTV viremia as a robust way of assessing global immune function.

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Dept. Medical Biotechnology and Translational Medicine
University of Milan
Lab. Molecular and Cellular Biology Applied on Neurodevelopmental Disorders
Professor Nicoletta Landsberger, Dr. Angelisa Frasca

Our lab is focused on the study of Rett syndrome (RTT) and CDKL5 Deficiency Disorder (CDD), two neurodevelopmental diseases caused by mutations in the X-linked MECP2 and CDKL5 gene, respectively. Since no cure exists, the demand for innovative therapies is a real emergency.
This is the driving force of our studies aimed at revealing therapeutic targets and developing novel therapeutic strategies

Project 1: **Searching in cholesterol synthesis a novel and druggable pathogenic mechanism of Rett syndrome**

The project aims at characterizing in mouse models of RTT the recently identified defects in Nsdhl, an enzyme involved in one of the later steps of cholesterol biosynthesis, to reveal if and to what extent defects in cholesterol metabolism participates to the clinical symptoms of RTT, therefore providing novel avenues for treatment.

The student will use neuronal and astrocyte cultures and cerebral mouse tissues combined with approaches of molecular biology (real time PCR, western blotting, Immunohistochemistry and viral transduction to alter gene expression) to investigate the defects in Nsdhl expression, and its influence on neuronal maturation and functionality.

Project 2: **Nonsense suppressor tRNA therapy for the treatment of *CDKL5* premature stop codons**

The recent discovery that *CDKL5* is dosage sensitive poses concerns on conventional gene augmentative therapies. Thus, RNA based therapeutic approaches might be preferred. We have recently reported a partial efficacy of a read-through therapy on *CDKL5* premature termination codons (PTCs) that correspond roughly to 15% of all CDD patient mutations. To overcome the limits associated with a drug-induced read-through therapy, we propose a novel study aiming at testing *in vitro* the therapeutic potential of suppressor tRNAs (sup-tRNAs) that recode PTCs with the correct amino acid. The student will become familiar with molecular techniques (western blot, real time PCR, cloning) and cellular cultures (cell transfection and transduction, primary neuron culturing, imaging).

UNIVERSITÀ DEGLI STUDI DELL'INSUBRIA - DBSV

The Protein Factory 2.0

Lab. The Protein Factory 2.0

(http://www.dbsm.uninsubria.it/proteinfact2/)

Loredano Pollegioni, Gianluca Molla, Silvia Sacchi, Luciano Piubelli, Elena Rosini

- Study of pathophysiological states related to D-amino acids
- Study of cerebral metabolism of serine
- HPLC determination of serum and tissue D-amino acid contents (identification of early biomarkers for Alzheimer's disease and chronic diseases, and during aging)

- Optimization of the expression/production in *E. coli*of biomedically relevant recombinant enzymes, and biomedical characterization thereof.
- Molecular evolution of of biomedically relevant enzymes
- Structural bioinformatics of proteins

For further details write to luciano.piubelli@uninsubria.it

UNIVERSITÀ DEGLI STUDI DELL'INSUBRIA – DMS
Laboratorio di Anatomia Patologica
Daniela Furlan

Strikingly Different Immune Infiltration in *BRAF* Mutant Advanced Colorectal Cancer: prognostic and therapeutic implications

BRAF mutant Colorectal Cancer (BRAFCRC) has a poor prognosis and BRAF mutational status is a strong predictor for overall survival, particularly in advanced stage. BRAFCRC accounts for less than 10% of all CRC and are correlated with specific clinico-pathological and biological factors such as female gender, older age, right-sided tumor locations, frequent lymph node involvement, advanced primaries and high frequency of Mismatch Repair (MMR) defect associated with microsatellite instability (MSI). Despite BRAFCRC usually being considered a unique clinical entity, recent trials targeting selected BRAFCRC cohorts show strong heterogeneity, suggesting that further biological subdivisions may exist.
This study aims to investigate multigene immune signatures using Nanostring technology (IO360 panel) in 100 stage IV BRAFCRC samples, encompassing a comparable number of MSI and MSS (microsatellite stable) tumors. These variables will be correlated with MSI status, histological features and inflammatory profiles of the tumors. Additionally, all data will be correlated with patient survival (DFS, OS, PFS).

Daniela Furlan,
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