Call 1 Annex III: CIVIS Cancer and Immunology Blended Mobility Network (2021-2024)
Cancer and Immunology Institute (AMU)

Technical innovations in basic and translational research
Applications to immunology-oncology
DETAILLED PROGRAM (Digital capsules are 60 minutes each, about 1 day per course)

Course 1: Cytometry (mass, flow and spectral)

1 .1 **Multi-color flow cytometry for phenotypic characterization of tumor-infiltrating T lymphocytes.** We will describe an innovative platform for multicolor analysis that can detect up to 13 colors simultaneously and is used for characterizing tumor-infiltrating lymphocytes.
Silvia Piconese, Sapienza Universita di Roma silvia.piconese@uniorma1.it

1.2 **Real-time cell analysis in immuno-oncology field**. General objectives: this course will provide an overview on interaction of proliferating tumor cells with non-adherent immune cells based on specific applications like cell health and proliferation, cell function and cell movement. In this regard cell counting, viability, apoptosis, cytotoxicity and immune cell killing, all very important tests in cancer cell pathway analysis and testing of drugs for efficacy and safety, will be evaluated using a real-time cell analysis system that allows observation, fully automated, hands-free operation/measurement and analysis of cell cultures during uninterrupted incubation.
Mihaela Economescu, Stefan S. Nicolau Institute of Virology, Bucharest Romania
mihaela.economescu@virology.ro

1.3 **Multi-parametric flow cytometry analysis for phenotypic characterization of aged-associated changes in circulating T cell subsets.** We will describe an innovative multiparametric analysis by flow cytometry for characterizing circulating T cell immune-senescence.
Maria Mittelbrunn, Universidad Autonoma de Madrid mmittelbrunn@cbm.csic.es

1.4 **Evaluation of Neutrophils Functions in the Tumor Microenvironment.** General objectives: This course will provide an overview regarding functions of neutrophils in the tumor microenvironment. Will we provide training in evaluation of mouse neutrophils oxidative burst using flow cytometry analysis and luminol-enhanced chemiluminescence assay.
Luminita Marutescu, University of Bucharest luminita.marutescu@bio.unibuc.ro

1.5 **Cancer immunosurveillance- NK cells and cancer.** General objectives: This course will present guidelines for setting up and analyzing cancer animal models as well as insights into cancer immune-surveillance. We will provide training in isolation of NK cells from mouse spleen and from human whole blood and characterization using multicolor flow cytometry.
Gratiela Gradisteanu, University of Bucharest gratiela87@gmail.com

1.6 **Detection of immune checkpoints and their ligands in tumor environment by multi-color flow cytometry**. General objectives: this course will provide an overview on tumor environment characterization based on immune checkpoints and their ligands that play a crucial role in the inhibition or stimulation of tumor-infiltrating immune cells, thus influencing the antitumor immune response. Flow cytometry allows the analysis of immune system deficiency, as well as the ability of immune target inhibitors to facilitate the association of T cells to its target tumor cells.
Lilia Matei, Stefan S. Nicolau Institute of Virology, Bucharest Romania liliamatei@yahoo.com

1.7 **Evaluation of the immune check-point in tumors using xMAP technology.** General objectives: this course will provide an overview on detection of checkpoint proteins (CD27, CD28, CD137 (4-1BB), GITR, HVEM, BTLA, CD80, CD152 (CTLA4), IDO, LAG-3, PD-1, PD-L1, PD-L2, and TIM-3) in tumor tissue and their soluble forms, together with inflammatory proteins (cytokines, chemokines, growth factors), providing an insight on antitumor microenvironment and immune response.
Coralia Bleotu, Stefan S. Nicolau Institute of Virology, Bucharest Romania cbleotu@yahoo.com

1.8 **Multi-color imaging flow cytometry for characterization of cancer response to drug treatments.** We will demonstrate the effects of applying drug treatments (as such or embedded in delivery vehicles, such as nanoparticles) on cancer cells apoptotic response and cell signaling pathways activation status, using the Amnis FlowSight imaging flow cytometer. This platform allows quantitative analysis of cellular markers, as well as imaging of every cell, thereby providing detailed information on cell phenotype and function.
Livia Elena Sima, Institute of Biochemistry of the Romanian Academy lsima@biochim.ro, livia_e_sima@yahoo.com

1.9 **Computational methods for flow and mass cytometry data analysis.** General objectives: provide an overview of machine learning algorithms used for automatic identification and visualization of immune cell populations. High throughput cytometry generates high dimensional datasets. A panel of 30 markers defines up to 2^{30} (~1 billion) potential immune populations and prevents the use of standard tools classically used by cytometrists. In parallel to this technical development, workflow using machine-learning algorithms for data analysis emerged and rapidly became the standard for data analysis. This course will provide an overview of the main algorithms used for automatic clustering of immune populations, intuitions regarding the principle of these algorithms and keys to read and understand figures in articles.
AS Chretien, Aix-Marseille Université anne-sophie.chretien@inserm.fr

1.10 **Introduction to spectral flow cytometry using Cytek Aurora and Northern Lights.** Spectral flow cytometry is a technology based on conventional flow cytometry, in which the full emission spectrum of every fluorescent molecule is recorded, leading to a spectral signature specifically associated to each fluorochrome. This technology enables to detect up to 30 parameters on a single cell. This course will provide elements on the technical principle as well as examples of applications to exploration of immune alterations in solid tumors.
Olivier Jaen, European Technical Application Specialist ojaen@cytekbio.com

Keynote speaker: Roberto Spada, Fluidigm roberto.spada@fluidigm.com
imaging by mass cytometry: applications to immuno-oncology research

Course 2: Organoids and in vivo models

2.1 **Organoids: New biological models for research in developmental biology and cancer.** *In vitro* 3D culture of adult stem cells has revolutionized cell culture by bringing this innovative study model more faithful to the biology of the original tissue making the organoids a highly valuable tool to study organ homeostasis and tumorigenesis and to test drug response in different cancers paving the way to personalized medicine. In this session we will define 1. What are organoids and how to cultivate them; 2. What are their applications in cancerology; 3. How to use them in immunotherapy? 4. How to use them in drug screening.
Géraldine Guasch, CRCM, Aix-Marseille Université geraldine.guasch-grangeon@inserm.fr

2.2 **Ex vivo culture of organoids for personalized medicine & regenerative medicine.** Examples of the fields dealing with organoids/cancer personalized medicine and organoids/inflammatory bowel diseases will be provided.
Marie Isabelle Garcia, Université Libre de Bruxelles mgarcia@ulb.ac.be

2.3 **3D in vitro models of the human nervous system: challenges and opportunities**. Moving from conventional cultures to more complex 3D systems is regarded as an important step towards the production of more physiological in vitro models of human development and disease, which are particularly relevant for neurological diseases, including cancer and inflammatory neurodegenerative disorders. In addition to self-assembled brain organoids, 3D bioprinting represents an innovative approach to guide the assembling process of a 3D model, combining cells and matrices into a single construct with a defined pattern at the micrometric scale.
Alessandro Rosa, Sapienza Università di Roma alessandro.rosa@uniroma1.it

2.4 **Measuring mitochondrial activity by Seahorse technology.**
2.5 **XCELLigence System: a label-free technology for cell behavior analysis.**
We will combine the use of a Seahorse platform, and an xCELLigence System. The Seahorse technology has revolutionized the characterization of cellular metabolism in live cells. The equipment determines mitochondrial oxygen consumption and glycolytic rates in real-time based on changes in oxygen concentration and the acidification of the cellular medium using different respiratory substrates. It represents an excellent platform to characterize metabolic changes in cancer and in the reprogramming of immune cells, and for the finding potential therapeutic targets in drug discovery and repurposing. The Xcell system consist of an electrical bioempedance-based system that allows label-free, real-time, and continuous monitoring of cell adhesion, morphology, and rate of cell proliferation, and are particularly suited for properly characterizing the influence of drugs on cell growth and survival. Advantages of bioempedance measurements with the xCELLigence System over the other characterization methods for dynamic study of cell growth, morphological plasticity, adhesion and migration will be highlighted.
Petronila Penela ppenela@cbm.csic.es **Angela Albitre** angela.albitre@uam.es **Laura Formentini** lformentini@cbm.csic.es

2.6 **Animal Models for Tumor Immunology/Mouse Models for Cancer Immunotherapy Research.** We have established various syngeneic mouse tumor models with cell lines stably expressing the luciferase gene for *in vivo* cell imaging using the IVIS Lumina system available in the CBMSO. This IVIS Lumina system provides the possibility of monitoring from the same day of inoculation the subcutaneous, intravenous, intraperitoneal or orthotopic inoculation of tumor cells (syngeneic in immunocompetent or xenografts in immunodeficient mice), with the ability of *in vivo* tumor growth/survival monitoring and migration towards lymph nodes. In some of the models, it would be possible to detect and isolate circulating tumor cells. Technical expertise of performing histological and immunohistochemical detection of immune infiltrates will be also provided.
Catalina Ribas, Universidad Autónoma de Madrid cati.ribas@uam.es **Kostas Stamatakis** k.s@csic.es **María Sanz** maria.sanz@cbm.csic.es

Keynote speaker: Maxime Mahé, University of Nantes, Maxime.mahe@inserm.fr
Human intestinal organoid models to study gastrointestinal diseases
Keynote speaker: Federico Mayor, Universidad Autónoma de Madrid, fmayor@cbm.csic.es
Alterations of Signaling Pathways in cancer cells and the tumor microenvironment: Therapeutic molecular targets and approaches

Course 3: Genomics (CRISPR, RNAseq, scRNAseq and TCR Seq)

3.1 **Functional genomics using CRISPR Screening :** Among the major technological breakthroughs which emerged in the last 10 years in biomedical research, the clustered regularly interspaced palindromic repeats-(CRISPR-) associated (Cas) protein 9 (known as CRISPR/Cas9) constitute a revolution in genome engineering allowing researchers to manipulate the genome of virtually any organism (any cell type or tumor) and investigate the consequences of perturbing gene or regulatory elements at unprecedented resolution. We will describe the power and implementation of high-throughput CRISPR screening approaches at the genome-wide level using pooled guide RNA lentiviral approaches (>75,000 gRNA tested at once) to functionally study coding gene or regulatory element functions as well as to identify novel cancer vulnerabilities than can be exploited therapeutically in various cancer subtypes. We will also discuss recent CRISPR approaches allowing to combine CRISPR screens with single-cell RNA sequencing to provide rich phenotypic data at the scale of thousands separately perturbed cells and address the molecular mechanisms by which gene perturbations mediate their functional effects.
Sandrine Roulland, Aix-Marseille Université roulland@ciml.univ-mrs.fr

3.2 **DNA microarrays and RNAseq in transcriptomics in cancer.** We will describe the use of DNA microarrays in transcriptomics explaining how they are designed, produced and hybridized with probes. We will then discuss the way data are acquired, quantified and validated. We then describe RNAseq platforms for transcriptomics and their pipelines. Finally, we will compare the two approaches and discuss their use in cancer biology.
Rodolfo Negri, Sapienza Università di Roma rodolfo.negri@uniroma1.it
Valerio Licursi, Sapienza Università di Roma valerio.licursi@uniroma1.it

3.3 **Introduction to scRNAseq: how does it work? What can we learn?** scRNAseq is a recent technology which provides the transcriptomic profile of a single cell. This technology can be applied to the exploration of immune and cancer cell subsets and enables to decipher mechanisms involved in cancer cell evasion to immune surveillance. scRNAseq recently asserted itself as a major tool for the exploration of the immunome, with growing examples of application in the field of fundamental and translational oncology.
Pierre Milpied, CIML, Aix-Marseille Université milpied@ciml.univ-mrs.fr

3.4 **TCR/CDR3 sequencing: insight into the development of T cells and their anti-cancer response.** T cells (αβ T cells and γδ T cells) use V(D)J gene rearrangement with the potential to generate a set of highly diverse T cell receptors (TCRs) to recognize antigens. This diversity is generated mainly in the CDR3 of the TCR via combinatorial and junctional diversity. High-throughput sequencing of the CDR3 regions of TCRs allows the assessment of thousands of CDR3 sequences. This method can be applied to study T cell development and to investigate and track T cell responses in the periphery, such as in cancer patients. Furthermore, TCR/CDR3 sequencing can be combined with single-cell RNA sequencing (scRNAseq), allowing to identify paired chains (α and β, γ and δ) and to investigate the association at the single-cell level between particular TCRs and functions of T cells.
David Vermijlen, Université Libre de Bruxelles David.Vermijlen@ulb.be

3.5 **Hands-on Introduction to RNA-Seq.** In this course, you will perform analysis of bulk RNA-Seq dataset without any prior knowledge of coding. For this, you will utilize freely available online tools to perform the analysis and generate publication quality figures. The course is hands-on, thus you are strongly encouraged to perform the analysis in parallel with the presentation. For analysis, we will perform differential gene expression analysis of PBMCs (peripheral blood mononuclear cells) collected from heathy individuals and from patients with COVID19. The latter samples are further characterized by the severity in the disease state. The analysis will investigate the changes in gene expression based on severity of COVID19 disease status. The data analyzed here was published in Arunachalam et al.,

Science 2020. DOI: 10.1126/science.abc6261 and shared publicly. At the end of this course, you should be able to analyze other publicly available or your own bulk RNA-Seq datasets.
Sumeet Pal Singh, IRIBHM, Université Libre de Bruxelles sumeet.pal.singh@ulb.be

Course 4: Proteomics

4.1 **Protein analysis by mass spectrometry.** Mass spectrometry (MS) can provide information to identify, characterize and quantify proteins even in very complex protein mixtures. We will show how such investigations can be carried out by applying different MS-based strategies and describing the example of proteins involved in phosphorylation pathways altered in colon cancer cells.
Marco Crescenzi, Sapienza Università di Roma, Istituto Superiore di Sanità marco.crescenzi@iss.it
Serena Camerini, Istituto Superiore di Sanità serena.camerini@iss.it

4.2 **Glycosylation characterization of IgG and its impact in cancer and immunology.** Glycosylation of antibodies can have a large impact on their effector functions. Here the methods will be explained to determine the glycosylation pattern of IgG antibodies (using mass spectrometry and other techniques) and examples will be provided in the context of infection and cancer.
Cédric Delporte, Université Libre de Bruxelles Cedric.Delporte@ulb.be
Yosra Helali, Université Libre de Bruxelles yosra.helali@ulb.be

4.3 **Reverse Phase Protein Arrays: a platform for biomarker indentification and the follow-up of therapeutic intervention.** Reverse phase protein array technology is a high-throughput quantitative immunological technique to assess the expression level of proteins in large cohorts of clinical samples. The technology allows the establishment of reliable correlations with the clinicopathological information, survival and therapeutic responses of the patients. It has been successfully used for the identification of biomarkers of disease and potential therapeutic targets in cancer and other disorders, in phenotyping mouse models of disease, as well as in the follow-up of the effect of therapy.
José M. Cuezva, Universidad Autónoma de Madrid jmcuezva@cbm.csic.es

4.4 **Targeted proteomics: Development of parallel reaction monitoring (PRM)-based quantitative proteomics applied to her2-positive breast cancer**. The methods of targeted proteomics will be explained and then an example applied to HER2-positive breast cancer will be highlighted.
Luc Camoin, Université of Aix-Marseille luc.camoin@inserm.fr

4.5 **Maldi imaging on biological samples and Maldi imaging on glioma.** The methods of Maldi imaging and in source decay will be described and then will be applied to glioma and therapeutic antibodies.
Daniel Lafitte, **Université of Aix-Marseille** daniel.lafitte@univ-amu.fr

Keynote speaker: Isabelle Fournier, University of Lille
isabelle.fournier@univ-lille.fr
Expert in mass spectrometry, mass spectrometry imaging, proteomics, oncology et intraoperative mass spectrometry

Course 5: Microscopy-imaging

5.1 **Cryo-electron microscopy (Cryo-EM) for studying fine cellular structures and protein complexes in immune cells and cancer cells.** Cryo-EM is a method for imaging frozen-hydrated specimens at cryogenic temperatures by electron microscopy. Specimens remain in their native state without the need for dyes or fixatives, allowing the study of fine cellular structures, viruses, and protein complexes at molecular resolution. Cryo-electron tomography, single-particle cryo-electron microscopy, and electron crystallography are all sub-disciplines of cryo-EM that have been used successfully to analyze biological structures in cancer and immune cells.
Luciana Dini, Sapienza Università di Roma luciana.dini@uniroma1.it

5.2 **3D structured illumination microscopy for studies on nuclear integrity and cancer invasion.** Super-resolution microscopy is now used to unravel the organization of cell macro-complexes, as a complementary method to in vitro biochemistry. We will describe the example of 3D structured illumination microscopy, which delivers ~120nm resolution. We will report on examples of intracellular structures studied with this and similar technologies, in comparison with analyses of the same structures performed with other approaches.
Isabella Saggio, Sapienza Università di Roma isabella.saggio@uniroma1.it

5.3 **Spatio-temporal organization of macromolecular complexes involved in lymphocyte signaling.** Recent developments in fluorescence microscopy provide the sensitivity and selectivity requested to explore the molecular dynamics at the plasma membrane of lymphocytes during signaling events. Within this frame, we explore the lateral diffusion of biomolecules in living cells by fluorescence fluctuation spectroscopy-related techniques to decipher the membrane reorganization of receptors at steady-state and during signaling events. We complete these dynamic approaches by picturing the instantaneous molecular distribution of membrane receptors by single-molecule localization microscopy combined with cryo-fixation technics.
Didier Marguet, CIML, Aix-Marseille Université marguet@ciml.univ-mrs.fr

5.4 **Isolation and characterization techniques for the study of exosomes.** All cell types secrete extracellular vesicles in their environment as a mean of intercellular communication. In the scientific practice their study is challenging because of their size, smaller than the resolution limit in the visible range imposed light diffraction. Thus, different techniques have to be adapted to characterize exosomes from a sample. We will use a workflow of techniques to isolate (size exclusion chromatography) and characterize (bead assisted blow cytometry, negative staining in electron microscopy and visualization by TIRFM) of these nanometric vesicles.
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Keynote speaker: Francisco Sánchez-Madrid, Universidad Autónoma de Madrid
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Intercellular communication in the immune response: transfer of genetic information during synapsis.