Course unit name: PROTEIN ANALYSIS BY CYTOMIC APPROACHES: APPLICATIONS ON CANCER BIOLOGY AND CLINICS

1.- General information

Code	303014	Plan		ECTS	
Type	Elective	Course	2024/2025	Periodicity	1st Semester
Department	Cancer Research Center				
Virtual Platform	Platform:	moodle.usal.es			
	URL de Acces:	https://studium.usal.es/			

Faculty

Professor Coordinator 1	Dr. Alberto Orfao de Matos Correia e Vale		
Department	Medicine		
Research area	Medicine		
Center	Cancer Research Center		
Office	Laboratory 11		
Tutorials	Appointment by email		
URL Web	https://www.cicancer.org/grupo?id=27		
E-mail	orfao@usal.es	Phone	+34 923294811

Professor Coordinator 1	Dra. Julia Mª Almeida Parra		
Department	Medicine		
Research area	Immunology and Cancer		
Center	Cancer Research Center		
Office	Laboratory S3		
Tutorials	Appointment by email		
URL Web	https://www.cicancer.org/grupo?id=79		
E-mail	jalmeida@usal.es	Phone	+34 923294500 5816

Professor	Dr. Manuel Fuentes García		
Department	Medicine		
Research area	Molecular biology, proteomics, nanotechnology, and immunotechnology		
Center	Cancer Research Center		
Office	Laboratory 11		
Tutorials	Appointment by email		
URL Web	https://www.cicancer.org/grupo?id=81		
E-mail	jmfuentes@usal.es	Phone	+34 923294811

Professor	Dr. Martín Pérez de Andrés		
Department	Medicine		
Research area	Immunology and Cancer		
Center	Edificio I+D+i		
Tutorials	Appointment by email		
E-mail	jmmmar@usal.es	Phone	+34 923294500 5505

Professor	Dra. Mª Aránzazu Rodríguez Caballero		
Department	Medicine		
Research area	Immunology and Cancer		
Center	Cancer Research Center		
Office	Laboratory 11		
Tutorials	Appointment by email		
E-mail	arocab@usal.es	Phone	+34 923294811

Professor	Dr. Sergio Matarraz Sudón		
Center	Cancer Research Center		
Office	Laboratory 11		
Tutorials	Appointment by email		
E-mail	smats@usal.es	Phone	+34 923294811

2.- The course in the context of the Master´s Program

Treaning Module

Second block (out of five) of master program organization.

General aim of the subject

To contribute to provide comprehensive training for students on the field of Cellular Biology -in order they can start a research career, and to facilitate them their incorporation into a PhD program-, through acquisition of transversal (general) master competences (CG1 to CG4), as well as the following specific competences:

- Students will be able to recognize the genes and proteins involved in tumor process, and their basic mechanisms.
- Students will be able to interpret basic biological data on tumor genes and proteins, and to translate it to the clinical level and in the development of diagnostic, prognostic or therapeutic applications.
- Students will be able to recognize the specific clinical and molecular characteristics of the different types of cancer, diagnostic methods and therapeutic approaches.
- Students will know, in a general way, the methods used for diagnosis and treatment of the different types of cancer.
- Students will know how to access information and data on specialized areas of research on Molecular and Cellular Cancer Biology.
- Students will be able to integrate new knowledge in the field of Molecular Cancer Biology, and develop their ability for self-learning.
- Students will be able to discriminate between cause and consequence using biological experimentation.
– Students will recognize contents and how to access to the major sources of biological resources and biomolecular databases.

Professional specialization

Master Degree on Health Sciences

3.- Previous recommendations

To meet the following general requirements, as regards admission into “Cancer Biology and Clinic University Master”: i) Have completed at least one bachelor degree on Biology, Biotechnology, Pharmacy, Medicine or any other degree on Biomedicine; ii) interest in scientific production; ii) a high English level is recommended.

4.- Aims of the subject

To know the concept of CYTOMICS, and its field of study, which is focused on the exhaustive and multiparameter analysis of the immunophenotype of individualized cells (i.e. at the single-cell level), and to understand that this phenotype results from a complex interaction between genotype and environmental influences.

To know the major cytomic techniques, mainly multiparameter flow-cytometry, laser-scanner cytometry and confocal microscope, and its applications on cancer study.

Acquisition of skills and ability to interpret laboratory results derived from cytomic approaches applied to the study of tumor cells (at the biological and clinical levels).

5.- Contents

Theory:

Lesson 1. The tumor cell and its normal cell-counterpart.

Lesson 2. Methods for cell analysis.

Lesson 3. Sample preparation for phenotypic analysis at the single-cell level.

Lesson 4. Applications of flow-cytometry in cancer analysis: immunophenotypic identification and characterization of tumor cells at the single-cell level.

Lesson 5. Functional assays. Quantification of surface membrane-cell molecules by flow-cytometry.

Lesson 6. Identification and quantification of soluble molecules by flow-cytometry.

Lesson 7. Tumor heterogeneity and clonal evolution. Cell purification for biochemical and molecular analyses.

Lesson 8.- Clonogenic tumor cell. Models of study of tumor stem cells.

Lesson 9. Proliferative assay in tumor cells: evaluation of tumor proliferative index and signaling pathways.

Lesson 10. Altered differentiation patterns in tumor cells: phenotypic analysis of maturational blockades and dysplasia.

Lesson 11. Cell survival, senescence and cell-death in tumors: flow-cytometry analysis of cell-death and its application on tumor-cell biology.

Lesson 12. Proteogenomics for the characterization of protein expression profiles, intracelular signaling and protein interactions between tumor cells and their normal counterparts.

Lesson 13. Cytomic applications to Farmacology and Toxicology.

Lab training / data analysis with specific software programs:

Lesson 1. Flow cytometer. Calibration and data acquisition.

Lesson 2. Approaches for staining surface membrane and intracellular molecules for immunophenotypic analysis.

Lesson 3. Software programs for data-analysis of flow-cytometry files.

Lesson 4. Novel strategies of phenotypic analyses applied to the study of cancer..

Lesson 5. Cell-sorting by flow cytometry.

Lesson 6. Cell-isolation by immunomagnetic approaches.

Lesson 7. Proteomics to identify differential protein-expression profiles in immune cells.

Lesson 8. Functional cytomics I.

Lesson 9. Functional cytomics II.

Seminars:

Students will individually present scientific papers either on hot / controversial aspects in the field of “Cytomic approaches in cancer study” or on other contents of the subject directly related with their master theses. After oral presentation, the presented study will be collectively discussed.

6.- Skills to be acquired

Basic skills

- To acquire a practical overview about human cancer models carrying different functional alterations.

-To acquire skills and to be able to interpret the results derived from basic cytomic approaches currently used for the analysis of the phenotype of tumor cells and their products, as well as their interaction with tumor microenvironment.

Specific skills

-To understand the applicability of cytomic analysis of (tumor and non-tumor) cells from patients with cancer in clinical settings.

Transversal skills

7.- Teaching methodology

The student must attend the theory classes, after having previously read and understood the recommended bibliography. In the first day, a general overview on how the subject is structured will be given, as well as the contents of the subject.

The student must attend all the lab and data analysis training.

The student must attend the seminars, in which each of them will individually present a recent or controversial paper already published on this field, and then will collectively discussed with the teacher and the other students.

8.- Estimated learning time

		Hours tutored by the teacher		Individual work (hours)	TOTAL HOURS
		Attendance required (hours)	Distance learning (hours)		
Lectures		16		30	**46**
Practices	Clasroom				
	Laboratory	3		1	**4**
	Computer room	1			**1**
	Countryside				
	Visualization classroom				
Seminars					
Work presentations and debates		1			**1**
Tutorials		0,5	0.5		**1**
Online activities				6	**6**
Work preparation				5	**5**
Other activities					
Exams - evaluation		1		10	**11**
TOTAL		**22.5**	**0.5**	**52**	**75**

9.- Materials

Books

Other bibliographical, electronic references or any other type of resource

- Bayguinov PO, Oakley DM, Shih CC, Geanon DJ, Joens MS, Fitzpatrick JAJ. Modern Laser Scanning Confocal Microscopy. Curr Protoc Cytom. 2018 Jul;85(1):e39.
- Cherian S, Hedley BD, Keeney M. Common flow cytometry pitfalls in diagnostic hematopathology. Cytometry B Clin Cytom. 2019;96(6):449-463. doi:10.1002/cyto.b.21854
- Cossarizza A, Chang HD, Radbruch A, et al. Guidelines for the use of flow cytometry and cell sorting in immunological studies (second edition). Eur J Immunol. 2019 Oct;49(10):1457-973.
- Dimitriadis S, Dova L, Kotsianidis I, Hatzimichael E, Kapsali E, Markopoulos GS. Imaging Flow Cytometry: Development, Present Applications, and Future Challenges. Methods Protoc. 2024;7(2):28. Published 2024 Mar 23. doi:10.3390/mps7020028
- Edwards BS, Sklar LA. Flow Cytometry: Impact on Early Drug Discovery. J Biomol Screen. 2015 Jul;20(6):689-707.
- Manohar SM, Shah P, Nair A. Flow cytometry: principles, applications and recent advances. Bioanalysis. 2021;13(3):181-198. doi:10.4155/bio-2020-0267
- Montante S, Brinkman RR. Flow cytometry data analysis: Recent tools and algorithms. Int J Lab Hematol. 2019 May;41 Suppl 1:56-62.
- Papa S, Ortolani C, Fernández P, O'Connor JE. Flow Cytometry and Its Applications to Molecular Biology and Diagnosis 2.0. Int J Mol Sci. 2023;24(22):16215. Published 2023 Nov 11. doi:10.3390/ijms242216215
- Povinelli BJ, Rodriguez-Meira A, Mead AJ. Single cell analysis of normal and leukemic hematopoiesis. Mol Aspects Med. 2018 Feb;59:85-94.
- Rieger AM. Flow Cytometry and Cell Cycle Analysis: An Overview. *Methods Mol Biol*. 2022;2579:47-57. doi:10.1007/978-1-0716-2736-5_4
- Robinson JP, Ostafe R, Iyengar SN, Rajwa B, Fischer R. Flow Cytometry: The Next

Revolution. Cells. 2023;12(14):1875. Published 2023 Jul 17. doi:10.3390/cells12141875

- Robinson JP. Flow cytometry: past and future. *Biotechniques*. 2022;72(4):159-169. doi:10.2144/btn-2022-0005
- Sanjai C, Hakkimane SS, Guru BR, Gaonkar SL. A comprehensive review on anticancer evaluation techniques. Bioorg Chem. 2024;142:106973. doi:10.1016/j.bioorg.2023.106973
- Spasic M, Ogayo ER, Parsons AM, Mittendorf EA, van Galen P, McAllister SS. Spectral Flow Cytometry Methods and Pipelines for Comprehensive Immunoprofiling of Human Peripheral Blood and Bone Marrow. Cancer Res Commun. 2024;4(3):895-910. doi:10.1158/2767-9764.CRC-23-0357
- Validation of Artificial Intelligence (AI)-Assisted Flow Cytometry Analysis for Immunological Disorders. Diagnostics (Basel). 2024;14(4):420. Published 2024 Feb 14. doi:10.3390/diagnostics14040420
- Wlodkowic D, Telford W, Skommer J, Darzynkiewicz Z. Apoptosis and beyond: cytometry in studies of programmed cell death. Methods Cell Biol. 2011;103:55-98. doi:10.1016/B978-0-12-385493-3.00004-8

Websites of interest:

https://isac-net.org/

https://www.escca.eu/

10.- Assessment

Assessments on the performance of the student

Continuous assessment system:

- Attendance to theory clases, seminars, practical sessions and tutorials.
- Active participation in all programmed activities
- Continuous evaluation

Written exam: exam consisting of multiple-choice questions.

Personal (individual) preparation and oral presentation and debate of a previously published paper in this field.

Written final exam of the contents of theory lessons (45% of the final grade).

Active participation in all the programmed activities (20% of the final grade).

Personal (individual) preparation and oral presentation and debate of a previously published paper in this field (30% of the final grade).

Evaluation of the subject by the student (5% of the final grade)

Recommendations

Students who have not passed the subject (a mark of minimum 5 out of 10) will have only to submit to a new written exam, but the grade obtained in continuous evaluation and oral presentation will be maintained.