UNIVERSITÀ DEGLI STUDI DI MILANO

SELEZIONE PUBBLICA, PER TITOLI ED ESAMI, PER IL RECLUTAMENTO DI N. 1 UNITÀ DI TECNOLOGO DI SECONDO LIVELLO CON RAPPORTO DI LAVORO SUBORDINATO A TEMPO DETERMINATO DELLA DURATA DI 36 MESI, PRESSO L'UNIVERSITÀ DEGLI STUDI DI MILANO - DIPARTIMENTO DI BIOTECNOLOGIE MEDICHE E MEDICINA TRASLAZIONALE, PER L'ATTUAZIONE DEL PROGRAMMA DI RICERCA DAL TITOLO "NATIONAL CENTER FOR GENE THERAPY AND DRUGS BASED ON RNA TECHNOLOGY" DEL CENTRO NAZIONALE "CN3 - NATIONAL CENTER FOR GENE THERAPY AND DRUGS BASED ON RNA TECHNOLOGY", TEMATICA "SVILUPPO DI TERAPIA GENICA E FARMACI CON TECNOLOGIA A RNA" (CUP G43C22001320007) NELL'AMBITO DEL PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – CODICE 22253

La Commissione giudicatrice della selezione, nominata con Determina Direttoriale n. 6234 del 20.4.2023, composta da:

Prof.ssa Silvia Angela Maria Della Bella	Presidente
Prof.ssa Elena Monica Borroni	Componente
Dott.ssa Paola Contini	Componente
Dott.ssa Annalisa Brengola	Segretaria

comunica i quesiti relativi alla prova orale:

GRUPPO DI QUESITI N. 1

1 L'impiego della citofluorimetria per eseguire test funzionali
2 Strategie di analisi per l'identificazione di una popolazione cellulare specifica in un campione eterogeneo
3 L'impiego di metodi analitici computazionali in citofluorimetria

Brano in inglese:

Multicolor flow cytometry is a technology of choice for phenotyping of immune cells, and it can be used routinely for the follow up of patients in clinical trials. But it is challenging to define combinations of conjugated antibodies that efficiently allow the detailed analysis of major immune cell subsets and the identification of rare cell populations. In a collaborative work among the Immunology, Immunopathology, Immunotherapy (I^3) laboratory, and the laboratory of immunomonitoring in oncology (L.I.O), we developed and validated 12 different 10-color flow cytometry panels that allow the deep immunophenotyping of cells from whole blood for the follow up of autoimmune and cancer patients. Here, we describe these optimized flow cytometry panels, showing that they provide the advanced analysis of T cells (including regulatory T cells), B cells, NK cells, MAIT cells, myeloid cells, monocytes, and dendritic cells. Most of the panels have been dried to improve standardization of the labeling and the entire procedure can be performed on less than 2 ml of whole blood. These deep immunophenotyping flow cytometry panels constitute a powerful tool for the monitoring of immune blood cells and will hopefully lead to the discovery of new biomarkers and potential therapeutic targets in autoimmune and cancer clinical trials.

GRUPPO DI QUESITI N. 2

1 Panel design per citofluorimetria multicolore
2 La gestione dello spillover in citofluorimetria
3 Strategie per l'analisi di eventi rari

Brano in inglese:

Technological advances in fluorescence flow cytometry and an ever-expanding understanding of the complexity of the immune system have led to the development of large (20+ parameters) flow cytometry panels. However, as panel complexity and size increase, so does the difficulty involved in designing a high-quality panel, accessing the instrumentation capable of accommodating large numbers of parameters, and analyzing such high-dimensional data. A recent advancement is spectral flow cytometry, which in contrast to conventional flow cytometry distinguishes the full emission spectrum of each fluorophore across all lasers,

rather than identifying only the peak of emission. Fluorophores with a similar emission maximum but distinct off-peak signatures can therefore be accommodated within the same flow cytometry panel, allowing greater flexibility in terms of panel design and fluorophore detection. Here, we highlight the specific characteristics of spectral flow cytometry and aim to guide users through the process of building, designing, and optimizing high-dimensional spectral flow cytometry panels using a comprehensive step-by-step protocol. Special considerations are also given for using highly overlapping dyes, and a logical selection process for optimal marker-fluorophore assignment is provided.

Milano, 8 maggio 2023

La Commissione

Prof.ssa Silvia Angela Maria Della Bella - Presidente

Prof.ssa Elena Monica Borroni - Componente

Dott.ssa Paola Contini - Componente

Dott.ssa Annalisa Brengola - Segretaria