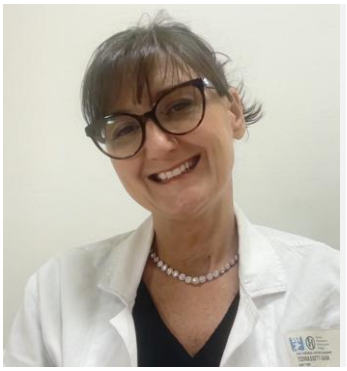
ImmunoTools *FlowISiAM* Award 2025



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FlowISiAM in Idiopathic Pulmonary Fibrosis (IPF) early diagnosis and disease progression assessment

Introduction

Idiopathic pulmonary fibrosis (IPF) is the most lethal form of progressive fibrosing Interstitial Lung Diseases (PF-ILD), a group of chronic conditions characterized by diffuse parenchymal lung fibrosis with significant morbidity and mortality [1-2].

The exact pathophysiological mechanisms involved in IPF development and progression remain elusive. The most shared hypothesis is that IPF lesion is the result of repeated subclinical injuries to a dysfunctional pulmonary epithelium, destroying the alveolar-capillary basement membrane and the progressive deposition of extracellular matrix (ECM) components, leading to tissue loss of function. The progression of IPF is inexorable, though the rate of worsening is unpredictable and varies among patients [3-6].

Objective

The focus of the project is to identifying and quantifying circulating macrophages using flow cytometry exploiting *FlowISiAM* technology.

Experimental Design & Methods:

Circulating macrophages from ILDs patients will be analysed and compared (n=30) from the Interventional Pulmonology Unit according to the study nr. AMBI 26414_bio/2024 (Comitato Etico Regione Toscana AREA VASTA CENTRO, Careggi Hospital).

1. Patient Cohort: Recruitment of subjects with ILDs suspected of having IPF.
2. Blood Collection: Peripheral blood samples will be used to isolate mononuclear cells.
3. Flow Cytometry: Implementation of the *FlowISiAM* protocol for intracellular markers of activated macrophages.
4. Data Analysis: Comparison of circulating activated macrophages (frequencies between groups and statistical evaluation of diagnostic significance).

Expected impact

The project is aimed to optimize early diagnosis of IPF patients among ILD subjects who exhibit clinical overlap with IPF and require a multidisciplinary diagnostic approach. Moreover, the project is aimed to improve the prediction of which IPF patients are more likely to develop acute exacerbations, enabling earlier intervention and improved patient outcomes.

Collaboration with ImmunoTools and INVIGATE

ImmunoTools and INVIGATE will play a central role in this project by providing some specific antibodies for flow cytometry and supporting the establishment of the extended *FlowISiAM* protocol. This collaboration will enable the development of customized solutions for detecting circulating IPF-positive macrophages and the continuous refinement of the methodology.

Strengths:

- IPF diagnosis is complex and often delayed – a novel immunophenotyping approach could significantly improve early detection.
- Our project with *FlowISiAM* aims to search for a non-invasive method with the potential to complement current diagnostic standards
- Potential future clinical application may lead to a better stratification of ILD patients
- Ethics application in progress and access to a well-defined patient cohort, will provide the potential for a quick project start.
- Existing collaboration with another group investigating ILD/IPF within the network, allowing valuable exchange of knowledge and strengthening the overall research effort. Additionally, the partnership with another *FlowISiAM* user group in Florence will further enhance the *FlowISiAM* network in the region, encouraging closer collaboration and shared progress.

Challenges and limitations

- Specific intracellular markers to be used are not clearly defined.
- While promising, *FlowISiAM* is still a new approach, and there's limited experience regarding its diagnostic sensitivity and specificity for IPF.
- Factors like systemic inflammation or comorbidities might impact macrophage phenotypes.

References

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Francesca Bianchini and **Sara Tomassetti** includes antibodies for *FlowISiAM*, know how transfer and protocol, support regarding selection of specific antibodies against specific biomarkers from INVIGATE, expert assistance in evaluating the results obtained, and integration into the ImmunoTools *FlowISiAM* network.

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