ImmunoTools *FlowISiAM* Award 2025

Konstantinos Tsamis
MD, Professor

Foivos S. Kanellos
PhD

Georgios S Markopoulos
PhD

Physiology Lab, Faculty of Medicine, University of Ioannina, 45 110 Ioannina, GREECE

Monocyte Expression Markers in Patients with Parkinson Disease

Background

Parkinsons disease (PD) is the second most prevalent neurodegenerative disorder after Alzheimer's disease [1]. Recent advances in biomarker and genetic research have significantly deepened our understanding of PD, presenting critical opportunities for earlier diagnosis, refined patient stratification, and personalized therapeutic strategies. Emerging evidence highlights the critical role of innate immune activation—particularly monocytes and macrophages—in the pathophysiology of Parkinson's disease (PD). Recent studies have identified immunological biomarkers reflecting systemic and central inflammation, which are increasingly recognized as key contributors to disease onset and progression. Monocytes and macrophages from PD patients exhibit altered expression of pro-inflammatory cytokines and increased surface expression of markers [2,3].

There is preliminary evidence suggesting that there is altered expression of specific markers in the surface of macrophages like CD14, CD16 and CD163 [4-6]. There is however a panel of **candidate markers** expressed in monocytes, that could characterize patients with PD and disease severity like CD9, CD11b, CD36, CD63, CD64 (FCγRI), CD81, CD86, CD274 (PD-L1) CD319 (SLAMF7), HLA-DR, CCR2, S100A9/S100A8, TLR2, TLR4, CX3CR1.

Monocytes show a distinct gene expression signature already in the early course of PD [4]. There are evidence indicating altered expression levels of multiple proteins, particularly those involved in mitochondrial function, proteo-lysosomal function, immune activation and directly

to PD pathology. Proteins like **a-synuclein, ApoE ε4, LRRK2, dynamin-related protein 1 (Drp1), caspase-1, Syntaxin-4, MTIF3 and TOMM7.**

Objectives

FlowISiAM methodology enables detection and quantification of internalized material within monocytes/macrophages, providing a readout of innate immune cell activation, phagocytic antigen uptake, and processing dynamics. In the context of PD, altered clearance of neuronal debris or misfolded proteins and impaired mitochondrial function are directly associated to innate immune dysregulation and the appearance of main pathological hallmarks of the disease. Through the use of *FlowISiAM* method, **we aim** to:

- Quantify the intracellular levels of a-syn, LRRK2, Drp1, caspase-1 and ApoE at baseline and after one year.
- Assess whether changes in expression levels are associated with subtypes of PD progression (e.g., cognitive decline, motor fluctuations).
- Correlate functional readouts with clinical measures of disease severity (UPDRS, Hoehn & Yahr), inflammatory marker profiles, and genetic background.

This initiative is a part of a broader project, the **PD Biomarkers project,** initiated at the University Hospital of Ioannina, in collaboration with the Department of Physiology at the Medical School of University of Ioannina. This project comprises a prospective study aiming to identify biomarkers in patients with Parkinson's disease. The primary objective of the study is to define a distinct **patient phenotype** characterized by a specific genetic, imaging, clinical, and hematological profile. We believe this approach is highly synergistic with our surface marker profiling and aligns with your team's expertise and support around *FlowISiAM* reagents and protocols. Integrating this functional intracellular assay into our study design will significantly strengthen the immunological characterization of PD and may uncover novel disease-related immune phenotypes.

Methods

Blood samples from PD patients were collected at two distinct time points (baseline-12 months) and stored at -80 degree as part of PD Biomarkers study procedures. The samples will undergo *FlowISiAM* analysis of a-syn, LRRK2, Drp1, caspase-1 and ApoE for each patient at baseline and at one year follow up.

Impact

The results will hopefully be correlated with other data obtained throughout the study (demographics, genetic profile, clinical progression, digital biomarkers and other serum-derived vesicles), providing a complementary biomarker in PD. This collaboration makes a

significant contribution towards the improvement of understanding of monocyte and macrophage activation states that lead to improved disease progression monitoring and the identification of novel immunomodulatory interventions. Targeting these immune pathways could offer a novel approach towards **personalized interventions** that can **alter the trajectory of PD** and inform the development of disease-modifying therapies.

Cooperation Partners: The consortium will collaborate closely with ImmunoTools to optimize both the experimental and instrumental set-up required for *FlowISiAM* analysis. ImmunoTools and its partner SME, INVIGATE, will contribute specific expertise in computer-aided scoring of FlowISiAM raw data to ensure reliable and reproducible results. In addition, INVIGATE will engage in the development of some selected monoclonal antibodies and support the initial evaluation phase. Through this collaboration, we aim to generate preliminary results that will enhance the prospects for a joint research grant application and subsequent funding.

References

[1] Tysnes OB, Storstein A. Epidemiology of Parkinson's disease. J Neural Transm (Vienna). 2017 Aug;124(8):901-905. doi: 10.1007/s00702-017-1686-y

[2] Grozdanov V, Bliederhaeuser C, Ruf WP, Roth V, Fundel-Clemens K, Zondler L, Brenner D, Martin-Villalba A, Hengerer B, Kassubek J, Ludolph AC, Weishaupt JH, Danzer KM. Inflammatory dysregulation of blood monocytes in Parkinson's disease patients. Acta Neuropathol. 2014 Nov;128(5):651-63. doi: 10.1007/s00401-014-1345-4.

[3] Wijeyekoon RS, Kronenberg-Versteeg D, Scott KM, Hayat S, Jones JL, Clatworthy MR, Floto RA, Barker RA, Williams-Gray CH. Monocyte Function in Parkinson's Disease and the Impact of Autologous Serum on Phagocytosis. Front Neurol. 2018 Oct 16;9:870. doi: 10.3389/fneur.2018.00870.

[4] Grozdanov V, Bliederhaeuser C, Ruf WP, Roth V, Fundel-Clemens K, Zondler L, Brenner D, Martin-Villalba A, Hengerer B, Kassubek J, Ludolph AC, Weishaupt JH, Danzer KM. Inflammatory dysregulation of blood monocytes in Parkinson's disease patients. Acta Neuropathol. 2014 Nov;128(5):651-63. doi: 10.1007/s00401-014-1345-4.

[5] Smith, A.M., Depp, C., Ryan, B.J., Johnston, G.I., Alegre-Abarrategui, J., Evetts, S., Rolinski, M., Baig, F., Ruffmann, C., Simon, A.K., Hu, M.T.M. and Wade-Martins, R. (2018), Mitochondrial dysfunction and increased glycolysis in prodromal and early Parkinson's blood cells. Mov Disord., 33: 1580-1590. https://doi.org/10.1002/mds.104

[6] Nissen, S.K., Ferreira, S.A., Nielsen, M.C., Schulte, C., Shrivastava, K., Hennig, D., Etzerodt, A., Graversen, J.H., Berg, D., Maetzler, W., Panhelainen, A., Møller, H.J., Brockmann, K. and Romero-Ramos, M. (2021), Soluble CD163 Changes Indicate Monocyte Association With Cognitive Deficits in Parkinson's Disease. Mov Disord, 36: 963-976. https://doi.org/10.1002/mds.28424

[7] Schlachetzki, J.C.M., Prots, I., Tao, J. et al. A monocyte gene expression signature in the early clinical course of Parkinson's disease. Sci Rep 8, 10757 (2018). https://doi.org/10.1038/s41598-018-28986-7

ImmunoTools *FlowISiAM* AWARD for

Konstantinos Tsamis, Foivos S. Kanellos, and Georgios S Markopoulos includes antibodies for *FlowISiAM*, few antibodies against surface markers (if they are available as own products), 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.

more AWARDS DETAILS about ImmunoTools