ImmunoTools *FlowISiAM* Award 2024

Giorgio Mangino, PhD, Associate Professor

Department of Medical-Surgical Sciences and Biotechnologies, Sapienza University of Rome, C.so della Repubblica 79, 04100 Latina, ITALY

Use of $CD14^{high}/CD16^{+}$ intermediate inflammatory monocytes as potential markers in the evaluation and follow-up of psoriasis patients

Background: Psoriasis (pso) is a chronic, systemic, inflammatory, and multigenic disease characterized by red and scaly skin plaques. Its pathogenesis is characterized by aberrant keratinocyte proliferation and differentiation, development of new blood vessels, and infiltration of T lymphocytes, dendritic cells, neutrophils, and other elements of innate immunity *[Nestle, PMID 19641206]*. Pso is nowadays, regarded as part of a systemic inflammatory disease complex associated with other chronic inflammatory conditions, such as diabetes mellitus type 2, cardiovascular disease, obesity, arthritis, and Crohn disease. Although the mechanism underlying the development of pso has been almost completely clarified, the patient evaluation and follow-up is still based on clinical criteria, mainly the Psoriasis Area and Severity Index (PASI) and the Dermatology Life Quality Index (DLQI). Hence, the need to identify markers for the patient management, possibly through a liquid biopsy approach.

Several evidences correlated the $CD14^{high}/CD16^{+}$ intermediate monocytes (iMo) with pso and its association with comorbidities. iMo are expanded in the patients' peripheral blood and produced TNFα *[Brunner, PMID: 23890755; Kouris, PMID: 25679113]*; their expansion is induced by the cathelicidin LL-37 and by peptidoglycan *[Qian, PMID: 26279752]*. iMo also display increased adhesion and aggregation proprieties probably contributing to the increased risk of development of cardiovascular diseases *[Golden, PMID: 26223654]*. It has also been reported that iMo express CD86 and that they can be retrieved in the pso lesions *[Nguyen, PMID: 29395574]*. Some reports correlate anti-pso therapies by biological agents (anti-TNFα-Infliximab and anti-IL12/23-Ustekinumab) *[Yamanaka, PMID: 25099154]* or by granulocyte and monocyte adsorption apheresis (GMA), with a reduction of circulating iMo and an amelioration of clinical symptoms *[Fujisawa, PMID: 23046368]*. These assumptions suggest iMo as a promising marker candidate for the management and the follow-up of pso patients through a liquid biopsy approach using *FlowISiAM* technology. An initial GEO analysis we performed on dataset obtained from lesional compared to non-lesional skin suggest that both TKTL1 and DNAse1L1 are deregulated in pso. In addition, we and others

identified cyto-/chemokines and antimicrobial peptides overexpressed in keratinocytes treated with IL-17 the driven cytokine for the development of pso *[Capriotti, PMID 36147689; Mangino PMID 31373041; Nograles, PMID: 18684158]*; we hypothesize that these mediators might be exploited by *FlowISiAM* technology as inflammatory, pso-related markers.

Experimental design and expected outcomes: the proposed study will be developed in three steps. Firstly, we will evaluate if inflammatory iMo could be detected by *FlowISiAM* assay and if difference exist between pso patients and sex/age matched healthy volunteers (fifty subjects for both group). Patients with moderate to severe pso before the administration of topical and/or systemic treatments will be enrolled. The obtained data will be correlated to PASI and/or DLQI. ROC analysis will be performed to allow sensitive and specific discrimination between pso patients and healthy individuals and to determine the best cutoff range for healthy group compared with the pso group *[Zweig, PMID: 8472349]*.

Then we will compare the data from pso, the prototypical Th1/Th17 inflammatory disease, with those obtained from atopic dermatitis (AD) patients. AD is a typical Th2 disease and it has been also correlated with increased levels of $CD14^+/CD16^+$ cells in peripheral blood *[Novak, PMID: 12269940]*. Our hypothesis is that *FlowISiAM* might discriminate between iMo expanded in pso and AD.

Finally, we will follow up the pso patients after the administration of biological agents therapy, testing the hypothesis that inflammatory iMo levels or certain iMo phenotypes in the blood detected by *FlowISiAM* might correlate with clinical improvements and/or therapy failure wherever they occur.

Cooperation partner: The group of Prof. Dr. Giorgio Mangino will work together with *ImmunoTools* to adjust the experimental and instrumental set-up to perform *FlowISiAM* assay. Furthermore, *ImmunoTools* will support the project by providing antibodies and reagents for cytometric immunophenotyping of iMo using previously identified markers (i.e. CD86). The group of Prof. Dr. Giorgio Mangino and *ImmunoTools* will also collaborate to possibly develop new reagents for the detection of inflammatory markers (i.e. antimicrobic peptides; inflammatory cyto-/chemokines; other overexpressed markers in lesional skin) by *FlowISiAM* technology and for intracellular staining for TNFα and/or inflammatory cyto-/chemokines. *ImmunoTools* and its partner SME, INVIGATE, will share specific know-how for computer-aided scoring from *FlowISiAM* raw data for optimal test outcomes. Prof. Dr. Giorgio Mangino and Dr. Sebastian Krause (INVIGATE) intend to explore possible actions on the identification of specific inflammatory markers that could correlate *FlowISiAM* assay results with both pso assessment made by PASI and DQLI indexes as well as with therapeutic outcomes. They eventually envisage to create good preconditions for a joint research grant application.

ImmunoTools *FlowISiAM* AWARD for **Giorgio Mangino,** 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.