ImmunoTools *FlowISiAM* Award 2024

Simona Romano, PhD, Professor

Dept Medicina Molecolare e Biotecnologie Mediche, Federico II University - Via Pansini, 5 - 80131 Napoli, ITALY

FlowISiAM investigation on *in vitro* activated macrophages and *ex-vivo* PBMCs from advanced bladder cancer patients

Background The *FlowISiAM* assay is based on the concept that the immune surveillance exerted by macrophages makes these cells valuable sensors of the presence of a tumor. Thanks to their phagocytic activity on cancer, circulating macrophages can show the presence of Apo10 and TKTL1. Very recently, studies on macrophage metabolism have shown significant metabolic adaptations occurring in macrophages dependent on their polarization state. Dependence on glycolysis, like the Warburg effect described in cancer cells, is seen in classically activated M1 macrophages. Glycolysis produces most of the ATP and intermediates for biosynthetic pathways required for microbicidal and antitumor functions of M1 macrophages. Differently, M2 macrophage metabolism exploits the mitochondrial respiratory machinery that is a significant driving force in alternative macrophage polarization. There are no studies evaluating TKTL1 in activated and differentiated macrophages. As TKTL1 is a vital tumor marker, clarifying whether an endogenous protein influences the results of *FlowISiAM* assay on circulating macrophages of cancer patients is essential. Furthermore, several non-tumor diseases are associated with increased apoptosis. Apoptosis is a process that occurs physiologically and in the adult organism. For example, lymphocytes die by apoptosis following their activation and expansion during an infectious or, more generally, inflammatory disease; therefore, the sensitivity and specificity for cancer cells of *FlowISiAM* assay should be further investigated. Bladder cancer has the 5th highest incidence of all malignancies in the world. This tumor is the 3rd most common cancer in men. More than 83,000 new cases are diagnosed yearly in men and women. About 30% of bladder cancers are muscle-invasive bladder cancer (MIBC) spreading into the detrusor muscle of the bladder. This cancer is more likely to spread to other parts of the body. These patients undergo cisplatin-based chemotherapy as a neoadjuvant approach before surgical resection of the bladder. Bladder resection has a notable impact on the quality of life of affected patients. Predicting tumor progression could greatly impact the type of bladder resection (total or partial) with better results in terms of quality of life and patient survival.

Aims This project presents two main purposes: **1.** An *in vitro* study on polarized M1 and M2 macrophages will assess their ability in changing the expression of TKTL1 and Apo10 following contact with tumor and non-tumor cells undergoing apoptosis. **2.** An *ex vivo* study will investigate the *FlowISiAM* on a cohort of patients with advanced bladder cancer.

Experimental design Our study aims to investigate *FlowISiAM* assay: **1.** to assess the basal expression of TKTL1 and Apo10 in differently polarized macrophages (M0, M1, M1d, M2); **2.** to assess changes in markers' expression in different polarized macrophages cocultured with tumor cells in condition or not of induced-cell death; **3.** to assess changes in markers' expression in different polarized macrophages cocultured with non-tumor cells in condition or not of induced-cell death; **4.** to assess changes in markers' expression in 3 time-points for each oncological patient (about 30/year): 1. pre-chemotherapy; 2. pre-surgery; 3. 4-weeks after surgery follow-up.

Methods Human peripheral blood mononuclear cells (PBMCs) isolated from healthy donors using Ficoll density gradient centrifugation will be used to obtain polarized macrophages. Briefly, PBMCs will be stimulated for 6 days with ImmunoTools GM-CSF and M-CSF and then polarized into M1 macrophages with ImmunoTools IFN-γ and LPS or M2a/M2c macrophages with ImmunoTools IL-4 or IL-10, respectively. *FlowISiAM* will be used to assay polarized macrophages (co-cultured or not) and blood from the selected cohort of cancer patients.

Impact Our study will strengthen the sensitivity and specificity values of the *FlowISiAM*-assay and provide new basis for its application in advanced bladder cancer.

Cooperation partner: The group of Prof. Dr. Simona Romano will work together with ImmunoTools to adjust the experimental and instrumental set-up to conduct *FlowISiAM* analysis. Furthermore, ImmunoTools will provide antibodies and cytokines for cytometric examination and for M1 und M2 macrophage differentiation. ImmunoTools and it´s partner SME, INVIGATE, will share specific know-how for computer-aided scoring from *FlowISiAM* raw data for optimal test outcomes. Prof. Dr. Simona Romano and Dr. Sebastian Krause (INVIGATE) intend to explore possible actions on the identification of specific markers that could facilitate early detection of bladder cancer by *FlowISiAM* testing, along with setting-up a tentative action plan and initial experimental evaluation. They envisage to create good preconditions for a joint research grant application.

ImmunoTools *FlowISiAM* AWARD for **Simona Romano,** 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.