# **ImmunoTools** *FlowISiAM* Award 2024



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## Phagocytosed Tumor Antigens in Monocytes: Pioneering Non-Invasive Bladder Cancer Diagnostics

**Background**

Bladder cancer (BLCA), particularly urothelial carcinomas, is a significant health concern in Europe, boasting some of the highest incidence rates worldwide[1]. These rates are projected to escalate further due to population growth and ageing[2]. Current diagnostic methods for BLCA are invasive and can cause discomfort for patients. Therefore, there is an urgent need to enhance the accuracy and reliability of bladder cancer diagnosis, monitor disease progression, and predict treatment outcomes through non-invasive methods.

One promising approach is the detection of tumor cells or tumor biomarkers (DNA, RNAs, proteins, peptides, metabolites, extracellular vesicles) in biological fluids like blood or urine[3]. Urothelial cancer cells express specific proteins, such as Nectin-4, a cancer-associated antigen highly expressed in high-risk non-muscle-invasive bladder cancer (HG NMIBC)[4]. A study using Protein Pathway Array technology identified proteins that were differentially expressed between non-tumor tissues and tumors in bladder urothelial cell carcinoma[5]. Of these, EGFR and cdc2p34 were correlated with muscle invasion and histological grade, while ten proteins (ß-catenin, HSP70, autotaxin, Notch4, PSTPIP1, DPYD, ODC, cyclinB1, calretinin, and EPO) were able to classify muscle invasive BCa (MIBC) into distinct groups with different survival rates.

Our research aims to explore a novel diagnostic avenue - the detection and quantification of tumor antigens within circulating monocytes[6]. Monocytes play a crucial role in the immune system, with phagocytosis being one of their key functions. Phagocytosis, a process by which monocytes engulf and digest cellular debris and foreign substances, influences the differentiation potential and functional characteristics of monocytes[7]. In the context of phagocytosis, monocytes can express markers that render them competent for phagocytosis, such as CD14, CD206, and CD163. Moreover, CD209 has been identified as a marker associated with phagocytosis capacity and directly correlates with the amount of particle uptake. In addition to phagocytosis, contact with tumor cells can induce phenotypic changes in monocytes[8-9]. These changes can serve as diagnostic or prognostic markers in cancer patients. Thus, the expansion of $CCR8^{+}$ inflammatory myeloid cells has been observed in cancer patients with urothelial and renal carcinomas, contributing to cancer-related inflammation in bladder cancer[10].

Our proposed research is a continuation of our field of research, searching for biomarkers for diagnosis in urogenital cancer[11-12]. Here, we will investigate the potential of these phagocyted tumor cells as a novel biomarker for bladder cancer, aiming to enhance the diagnostic process and improve patient outcomes. This approach could revolutionize bladder cancer diagnostics, providing a non-invasive, accurate, and reliable method for early detection and monitoring. Specifically, we aim to detect tumor antigens in circulating monocytes that originate from phagocyted tumor cells by tissue macrophages. This could provide a more detailed understanding of the tumor microenvironment and potentially lead to the development of new therapeutic strategies.

**Aim**

In this project we will investigate: the phenotype of monocytes and the extent to which monocytes that have phagocytosed urothelial carcinoma cells recirculate into the bloodstream and urine. As a next step, we will investigate whether a monocyte-based diagnostic approach can be developed.

**Experimental Design & Methods**

1. Sample Collection: Collect peripheral blood samples and urine from bladder cancer patients. Ensure that the patients have been diagnosed with different stages of bladder cancer to have a diverse sample set. Most of the cellular determinations will be performed in blood samples. Determinations in urine samples will be also explored by cytometry as a proof of concept despite the compromised cell viability and low cellular count. An alternative option for urine samples will be by immunohistochemistry with microscopic analysis using 2 - 3 simultaneous staining.
2. Mononuclear cell isolation and cytometry phenotyping from peripheral blood and urine.
3. Phagocytosis Verification: Verify the phagocytosis process in monocytes by specific markers (CD163, CD206, CD209).

4. Tumor Antigen Detection: Detect peptides derived from tumor cells within the monocytes using *FlowISiAM*. Peptides could be obtained from: Nectin-4, EGFR, ß-catenin, autotaxin, Notch4, PSTPIP1, DPYD, ODC, cyclinB1, calretinin, and EPO. Malignancy markers such as TKTL-1 and Apo-10 will be also considered for antigen detection.
5. Data Analysis: Analyze the data collected from the antigen detection step. Look for patterns or correlations between the presence/absence of specific antigens and the stage or type of bladder cancer.

**Cooperation partner**

The group of collaborators presenting this proposal will work together with ImmunoTools to adjust the experimental and instrumental set-up to conduct *FlowISiAM* analysis at the IIB-Sant Pau (Barcelona). ImmunoTools and its partner SME, INVIGATE, will share specific know-how for computer-aided scoring from *FlowISiAM* raw data for optimal test results. ImmunoTools' partner SME, INVIGATE, will take on the task of developing peptide specific monoclonal antibodies and will support the initial evaluation. With our collaborators we will explore the identification of specific markers that could facilitate early detection of peptides from bladder cancer in circulating cells by *FlowISiAM* testing. They envisage to create good preconditions for a joint research grant application.

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**ImmunoTools** *FlowISiAM* AWARD for

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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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