ImmunoTools *FlowISiAM* Award 2025

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Non-Invasive Immune Profiling for Early Detection and Risk Stratification of HSIL and Operable Cervical Cancer Using *FlowISiAM*

Project Description

Cervical cancer ranks as the fourth most common cancer in women worldwide, causing over 600,000 new cases and 340,000 deaths annually (WHO, 2023). It typically develops from high-grade squamous intraepithelial lesions (HSIL, CIN2/3) arising after persistent infection with high-risk HPV types. HSIL is detected in approximately 0.3–0.8% of screened women, and up to 25% progress to invasive cervical carcinoma if untreated. Current triage methods—including cytology, HPV testing, and biopsies—are often invasive, subjective, and limited in predicting which HSIL lesions will progress. This limitation is particularly consequential for women in their reproductive years: surgical management such as conization or LEEP carries significant obstetric risks, including a ~2-fold increased risk of preterm birth and late miscarriage, particularly with deeper or repeated excisions (Berghella 2013). As more women delay childbirth, avoiding unnecessary invasive treatments has become a critical unmet clinical need.

FlowISiAM (Epitope Detection in Activated Monocytes/Macrophages) offers a promising non-invasive alternative. Across cancer types (breast, lung, oral), *FlowISiAM* has demonstrated nearly 95–97% sensitivity and specificity, detecting apoptotic dysregulation (Apo10) and tumor-specific metabolic reprogramming (TKTL1) inside circulating

monocytes/macrophages (Coy 2017; Grimm 2013; Xie 2023). In a cervical cancer cohort (152 cases vs. healthy controls), Wang et al. (2025) reported that Apo10, TKTL1, and their combined APT score achieved an AUC of 0.905, significantly outperforming traditional serum markers (CEA: 0.690, CA125: 0.594, SCC A: 0.806), which are non-specific but used as benchmarks in cervical cancer studies. Remarkably, *FlowISiAM* maintained high accuracy even in HPV-negative (AUC 0.967) and cytology-negative (AUC 0.958) patients, indicating its ability to detect early molecular changes invisible to current screening tools. Recent evidence also highlights the vaginal microbiome as a key modulator of HPV persistence and HSIL progression. Dysbiosis—characterized by depletion of protective Lactobacillus species and overgrowth of anaerobes such as Gardnerella, Prevotella, and Sneathia—is associated with chronic inflammation, elevated cytokines (IL 1β, IL 6, TNF α), and impaired mucosal immunity, creating a microenvironment conducive to neoplastic transformation (Mitra 2020; Audirac-Chalifour 2016). These inflammatory signals activate local macrophages and may enhance uptake of apoptotic and hypoxic tumor antigens (Apo10, TKTL1, CAIX), potentially amplifying the *FlowISiAM*-detectable signal. Based on these insights, we hypothesize that tumor antigens and hypoxia markers are already phagocytosed by circulating monocytes during HSIL, and that vaginal microbiome dysbiosis influences this immune response. Early detection of these immune signatures via *FlowISiAM* could enable risk stratification of HSIL lesions, identifying those likely to regress versus those requiring surgical intervention. This strategy may reduce unnecessary excisions, lowering the risk of obstetric complications while ensuring timely treatment for high-risk patients.

Objective

Primary Objective

To evaluate the performance of *FlowISiAM* (intracellular detection of Apo10, TKTL1, and CAIX in circulating monocytes/macrophages) in distinguishing HSIL (CIN2/3), early-stage operable cervical cancer (FIGO IA–IB2), and healthy controls, aiming to provide a non-invasive, blood-based diagnostic alternative to invasive biopsies.

Secondary Objectives

I. To correlate soluble CAIX levels in plasma and CAIX mRNA expression in cervical lavage fluid with intracellular macrophage antigen profiles.

II. To investigate how the vaginal microbiome composition influences macrophage activation and antigen uptake, potentially modulating *FlowISiAM* signals.

III. To develop an integrated biomarker panel combining immune profiling, CAIX measurements, and microbiome signatures for risk stratification of HSIL lesions, guiding personalized treatment and reducing unnecessary excisional procedures that increase preterm birth and miscarriage risk.

Methodology

Patient Cohorts and Access

20 healthy controls (HPV-negative, normal cytology)

30 HSIL patients (CIN2/3)

20 early-stage cervical cancer patients (FIGO IA–IB2)

Patients will be enrolled during routine colposcopy visits and pre-surgical evaluations at our collaborating gynecology department at Hospital of Lithuanian University of Health Sciences. Ethical approval will be obtained, and written informed consent will cover blood and cervical lavage fluid collection.

FlowISiAM Immune Profiling

Whole blood or PBMC isolation

Multicolor flow cytometry staining for CD14/CD16 monocytes/macrophages

Intracellular markers: Apo10, TKTL1, CAIX

Immune activation markers: HLA-DR, CD86, CD163, CD206

Quantification of tumor epitope-positive macrophages as a percentage of total circulating monocytes.

Complementary Biomarker Analyses

Soluble CAIX: Plasma levels assessed via ELISA

CAIX mRNA: Cervical lavage fluid analyzed using ddPCR/qPCR

Vaginal Microbiome:

DNA extracted from lavage samples

16S rRNA sequencing performed to characterize bacterial diversity and abundance

Bioinformatics analyses to identify microbial signatures associated with HPV persistence, HSIL severity, and immune activation profiles

Data Analysis

Statistical comparisons across healthy, HSIL, and cancer groups

ROC analysis to assess diagnostic accuracy

Multivariate modeling to integrate *FlowISiAM* immune data, CAIX levels, and microbiome composition into a unified risk stratification tool

Exploratory analyses to examine associations between specific microbiome taxa and macrophage intracellular antigen load

Collaboration with ImmunoTools and INVIGATE

ImmunoTools and INVIGATE will provide during this project monoclonal antibodies targeting Apo10, TKTL1, anti-CD14, and anti-CD16, along with some additional antibodies against immune markers. Both companies will actively support the establishment of the *FlowISiAM* protocol, including antibody panel optimization, troubleshooting, and expert guidance on data analysis and interpretation. Furthermore, ImmunoTools and INVIGATE are

engaged in the development and validation of antibodies against CAIX that are compatible with the *FlowISiAM* platform. This study aims to initiate collaborative investigations into novel epitopes specific to cervical lesions, with the objective of informing the development of future *FlowISiAM*-based diagnostic panels.

Project Timeline

- Months 1–2: Kick-off, technical training, antibody delivery
- Months 3–4: Pilot optimization of staining panel using healthy donor samples
- Month 6 onward: Recruitment and full-scale patient cohort analysis, ongoing SME collaboration for troubleshooting and data evaluation

Expected Impact

FlowISiAM will enable non-invasive immune profiling of cervical neoplasia, potentially detecting HSIL lesions with high risk of progression earlier than cytology or histology. Integration with soluble CAIX, local CAIX mRNA, and microbiome analysis will enhance diagnostic specificity and provide mechanistic insight into tumor–immune–microbiome interactions. The resulting multi-modal biomarker panel could potentially reduce unnecessary surgical excisions, preserving fertility and minimizing obstetric complications.

This project will expand *FlowISiAM* applications into gynecologic oncology and establish a foundation for long-term collaboration with ImmunoTools and INVIGATE, including co-development of novel antibodies and joint funding initiatives.

ImmunoTools *FlowISiAM* AWARD for

Daumantas Matulis, Agnė Petrošiūtė and **Švitrigailė Grincevičienė** includes

antibodies for *FlowISiAM*, know how transfer and protocol, support regarding selection of specific antibodies against some specific biomarkers from INVIGATE, engagment regarding development and validation of antibodies against CAIX that are compatible with the *FlowISiAM* platform, and expert assistance in evaluating the results obtained, and integration into the ImmunoTools *FlowISiAM* network.

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