ImmunoTools *FlowISiAM* Award 2026

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The Potential of Activated Circulating Monocytes/Macrophages as a Liquid Biopsy for the Diagnosis and Therapeutic Monitoring of Head and Neck Cancer

Background and Rationale

Head and neck squamous cell carcinoma (HNSCC) represents the sixth most common cancer worldwide and remains a major cause of cancer-related morbidity and mortality.[1] Despite advances in surgery, radiotherapy, chemotherapy, and immune checkpoint inhibition, the prognosis of patients with recurrent or metastatic disease remains poor. Furthermore, approximately 50% of patients develop locoregional recurrence or distant metastases following curative treatment.[2] Current surveillance strategies rely predominantly on clinical examination and imaging, while robust blood-based biomarkers for early diagnosis, treatment monitoring, and detection of minimal residual disease remain limited.

HNSCC develops through distinct molecular pathways driven by environmental carcinogens and oncogenic viruses. Chronic exposure to tobacco and alcohol induces oxidative stress, DNA damage, and genomic instability, whereas persistent infection with high-risk human papillomavirus (HPV) drives a biologically distinct subgroup of tumours.[1] Consequently tumour-derived cytokines, extracellular vesicles, damage-associated molecular patterns and inflammation contribute to systemic immune reprogramming that can be detected in circulating myeloid cells.

Circulating non-classical monocytes/macrophages represent an attractive liquid biopsy compartment due to their continuous sampling of tumour microenvironment through phagocytosis and integration of tumour-derived proteins, metabolites and inflammatory mediators.[3] Unlike circulating tumour DNA, which primarily reflects tumour burden, intracellular profiling of circulating monocytes provides functional information on the dynamic interaction between the tumour and the host immune system. This offers the

opportunity to detect tumour-associated immune reprogramming before overt clinical progression becomes apparent.

Among the intracellular biomarkers associated with malignant transformation, transketolase-like protein 1 (TKTL1) reflects enhanced aerobic glycolysis and metabolic reprogramming characteristic of the Warburg phenotype, whereas DNaseX (Apo10) indicates impaired apoptosis and resistance to programmed cell death.[4] Both markers have been detected within phagocytosing monocytes and macrophages and are considered surrogate markers of tumour-associated metabolic and apoptotic dysregulation.

The *FlowISiAM* platform enables standardized intracellular multiparameter flow cytometry directly from whole blood without prior cell isolation, preserving physiological immune cell states while allowing simultaneous quantification of metabolic, apoptotic, and immune biomarkers at single-cell resolution. By integrating *FlowISiAM*-based intracellular monocyte profiling with established and novel HNSCC-associated biomarkers, this study aims to establish a comprehensive liquid biopsy strategy for early diagnosis, prognostic stratification, therapeutic monitoring, and detection of disease recurrence in patients with HNSCC.

Experimental Design:
Peripheral blood samples from patients with HNSCC will be collected and analysed using the *FlowISiAM* technique as well as *AMANDUS* (an enhanced immunoprofiling platform). Ethical approval will be obtained from the appropriate institutional review board and all participants will provide written informed consent prior to enrolment and blood collection.

Approximately 3 ml of peripheral whole blood will be collected into EDTA tubes and processed within max. 4 hours using the standardized *FlowISiAM* whole-blood workflow, thereby avoiding prior cell isolation and preserving the physiological immune cell composition. Flow cytometric acquisition, gating, and data analysis will be performed in a blinded manner. Clinical information and outcome data will only be revealed after completion of the predefined analyses. Patients will be recruited into 3 longitudinal cohorts: (I) a pre-treatment cohort diagnosed with de novo HNSCC, sampled before initiation of therapy, (II) a healthy control cohort and (III) a treatment cohort, with serial blood collections performed before treatment, during therapy, after completion of therapy, and, where applicable, at the time of disease recurrence. This longitudinal design will enable evaluation of biomarker dynamics throughout the clinical course and assessment of their potential for treatment monitoring and early detection of relapse.

Circulating monocytes will be identified by forward- and side-scatter characteristics together with CD14 and CD16 surface expression, allowing discrimination between classical and non-classical monocyte subsets. Following surface staining, cells will be fixed and permeabilised using the *FlowISiAM* intracellular staining protocol.

The *FlowISiAM* technique will quantify intracellular expression of TKTL1, a marker of altered glucose metabolism, and DNaseX (Apo10), a marker of impaired apoptosis, within circulating monocytes. These biomarkers have been associated with tumour-related metabolic reprogramming and apoptosis resistance and will be quantified as median fluorescence intensity (MFI) and the proportion of biomarker-positive cells using multiparameter flow cytometry. Fluorescence-minus-one (FMO) controls will be included to ensure accurate discrimination of positive and negative cell populations.

The combined biomarker approach will be evaluated for its ability to improve blood-based diagnosis, therapeutic monitoring, and early detection of disease recurrence in patients with HNSCC.

References:

[1] D. E. Johnson, B. Burtness, C. R. Leemans, V. W. Y. Lui, J. E. Bauman, and J. R. Grandis, "Head and neck squamous cell carcinoma," (in eng), *Nat Rev Dis Primers*, vol. 6, no. 1, p. 92, Nov 26 2020, doi: 10.1038/s41572-020-00224-3.

[2] C. R. Leemans, P. J. F. Snijders, and R. H. Brakenhoff, "The molecular landscape of head and neck cancer," (in eng), *Nat Rev Cancer*, vol. 18, no. 5, pp. 269-282, May 2018, doi: 10.1038/nrc.2018.11.

[3] X. Wang, S. Zhang, D. Xue, D. Neculai, and J. Zhang, "Metabolic reprogramming of macrophages in cancer therapy," (in eng), *Trends Endocrinol Metab*, vol. 36, no. 7, pp. 660-676, Jul 2025, doi: 10.1016/j.tem.2024.08.009.

[4] J. F. Coy, "EDIM-TKTL1/Apo10 Blood Test: An Innate Immune System Based Liquid Biopsy for the Early Detection, Characterization and Targeted Treatment of Cancer," (in eng), *Int J Mol Sci*, vol. 18, no. 4, Apr 20 2017, doi: 10.3390/ijms18040878.

ImmunoTools *FlowISiAM* AWARD for

Christoph Lange includes

antibodies for *FlowISiAM*, know how transfer and protocol, support regarding selection of specific antibodies against some specific biomarkers from INVIGATE, engagment regarding development of specific *AMANDUS*, and expert assistance in evaluating the results obtained, and integration into the international ImmunoTools *FlowISiAM* network.

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