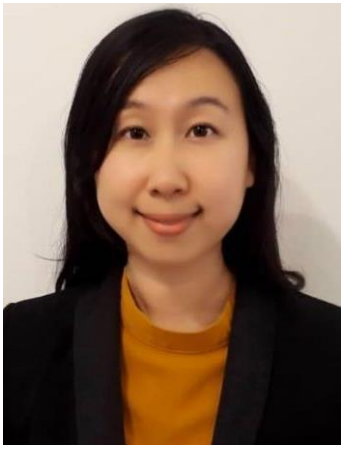
ImmunoTools *FlowISiAM* Award 2026



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Identification of Blood Biomarkers for Lung Cancer Early Screening

Background: Lung cancer remains a leading cause of cancer-related mortality worldwide, largely due to late-stage diagnosis and limited tools for early detection and disease monitoring. **Problem statement:** Current diagnostic approaches lack reliable, minimally invasive biomarkers capable of reflecting tumour dynamics and guiding clinical decision-making. Liquid biopsy has emerged as a promising strategy to address this unmet need by enabling the detection of circulating biomarkers associated with tumour biology. **Objective:** This study aims to identify and validate circulating biomarkers for lung cancer using peripheral blood samples. Specifically, three candidate biomarkers previously identified through liquid chromatography–mass spectrometry (LC-MS) will be validated to assess their utility in early detection. **Methodology:** Blood samples will be collected in EDTA tubes at the point of diagnosis, prior to the initiation of any therapeutic intervention. In addition to LC-MS-based validation, integrative immune profiling will be conducted using the *FlowISiAM* and ImmunoTools *AMANDUS* platform. This approach enables the assessment of complementary circulating biomarkers, including TKTL1, associated with tumor glycolytic metabolism, and DNaseX (Apo10), indicative of impaired apoptosis regulation, particularly in circulating monocytes and macrophages. **Expected outcome:** We expect the successful validation of a small panel of minimally invasive biomarkers capable of distinguishing lung cancer patients at initial diagnosis. This framework has the potential to improve early detection, support risk stratification at baseline, and lay the foundation for future personalized therapeutic strategies.

Background

Lung cancer remains one of the leading causes of cancer-related mortality worldwide (Bray et al. 2024). Despite advances in surgery, radiotherapy, and chemotherapy, patient survival remains poor, mainly due to late-stage diagnosis and high recurrence rates (Tang et al. 2025). Early detection is critical for improving outcomes, but current diagnostic approaches, including imaging and tissue biopsy, are often invasive or insufficiently sensitive to detect early disease (Zafar et al. 2025). Liquid biopsy has emerged as a promising minimally invasive approach, enabling the detection of circulating biomarkers that reflect tumour biology (Abreu et al.).

Research Gap

However, there is still a lack of reliable and validated circulating biomarkers that can accurately detect lung cancer at an early stage and monitor disease progression. Existing biomarkers often lack sensitivity, specificity, or clinical validation, limiting their utility in routine practice (Qi et al. 2026).

Problem Statement

The absence of robust blood-based biomarkers hinders early detection, effective patient stratification, and real-time disease monitoring in lung cancer. Therefore, there is a critical need to identify and validate minimally invasive biomarkers that can reliably distinguish lung cancer patients from healthy individuals and reflect tumour dynamics for improved clinical decision-making.

General Objective

To identify and validate minimally invasive circulating biomarkers for lung cancer using peripheral blood, with the aim of improving early detection and disease monitoring.

Specific Objectives

1. To validate three candidate protein biomarkers identified from LC-MS analysis in lung cancer patients compared to healthy controls.
2. To determine the presence and expression levels of these biomarkers in circulating exosomes/extracellular vesicles derived from patient blood samples.
3. To evaluate the association between these biomarkers and clinical characteristics at initial diagnosis, including their ability to distinguish cancer from non-cancer cases.
4. To perform immune profiling using *FlowISiAM* and ImmunoTools *AMANDUS*, focusing on markers of metabolism (TKTL1), apoptosis (DNaseX/Apo10), and redox status (CD14, CD16, CD95, CD38, CD71, CD56).
5. To integrate exosomal biomarker data with immune profiling results to identify a combined biomarker signature for lung cancer detection and monitoring.

Methodology

1. Study Design and Sample Collection

This study will employ a case–control design involving lung cancer patients and healthy controls. Peripheral blood (3–5 mL) will be collected in EDTA tubes at the point of diagnosis, prior to any therapeutic intervention. Plasma will be separated by centrifugation and stored under appropriate conditions until analysis.

2. Exosome / Extracellular Vesicle Isolation

Plasma-derived exosomes/extracellular vesicles (EVs) will be isolated using standard methods such as ultracentrifugation or commercially available isolation kits. The presence of EVs will be confirmed using established markers (e.g., CD63, CD81).

3. Validation of Candidate Biomarkers

Three candidate proteins identified from LC-MS analysis will be validated in plasma-derived EVs. Protein expression levels will be assessed using antibody-based detection methods (e.g., flow cytometry or immunoassays) with validated reagents. Comparisons will be made between lung cancer patients and healthy controls to determine differential expression.

4. Immune Profiling Using *FlowISiAM* and ImmunoTools *AMANDUS*

Peripheral blood mononuclear cells (PBMCs) will be isolated from whole blood and analyzed using the ***FlowISiAM*** **platform** in combination with **ImmunoTools** *AMANDUS* **antibodies**. Immune profiling will focus on:

- **Monocyte and NK cell subsets:** CD14, CD16, CD56
- **Apoptosis marker:** CD95 (Fas)
- **Metabolic and redox markers:** CD38, CD71
- **Tumour markers:** TKTL1 (glycolysis) and DNaseX/Apo10 (apoptosis dysregulation)

Multiparametric flow cytometry will be used to quantify marker expression.

5. Data Analysis

Protein expression levels in EVs and immune cell profiles will be statistically analysed to:

- Compare lung cancer patients and healthy controls
- Assess diagnostic performance (e.g., sensitivity, specificity)
- Identify correlations between exosomal biomarkers and immune cell functional changes

Integrated analysis will be performed to identify a combined biomarker signature for lung cancer detection.

Expected Outcomes

This study is expected to successfully validate a panel of circulating biomarkers in plasma-derived exosomes from lung cancer patients. These biomarkers are anticipated to show

differential expression compared to healthy controls, supporting their potential utility in early detection.

In addition, immune profiling using *FlowISiAM* and *AMANDUS* is expected to reveal distinct functional changes in circulating immune cells, particularly in monocytes and NK cells, reflected by alterations in metabolic (TKTL1), apoptotic (DNaseX/Apo10, CD95), and redox-related markers (CD38, CD71).

The integration of exosomal protein data with immune profiling is expected to generate a combined biomarker signature that improves diagnostic accuracy, supports patient stratification at baseline, and provides insights into tumour–immune interactions. Ultimately, this approach may contribute to the development of a minimally invasive liquid biopsy framework for lung cancer detection and monitoring.

Collaboration Plan

This study will be conducted in collaboration with ImmunoTools and its partner INVIGATE. The collaboration will provide access to high-quality antibodies, reagents, and technical expertise required for implementing the *FlowISiAM* platform and ImmunoTools *AMANDUS* based immune profiling.

The partners will cooperate regarding:

- **Selection and optimization of antibodies** for detecting target biomarkers (e.g., LGALS3BP, PIGR, FN1, TKTL1, DNaseX/Apo10)
- **Protocol development and technical training** for multiparametric flow cytometry (FACS)
- **Data analysis and interpretation**, including integration of immune and exosomal biomarker datasets
- **Standardization of assays** in line with *FlowISiAM* and *AMANDUS* frameworks

This collaboration will facilitate knowledge transfer, assay validation, and integration into an international research network, enhancing the translational potential of the study and supporting future development of clinically applicable lung cancer biomarker panels

References

Abreu R da S, Ferreira DDP, de Araujo NS, et al Liquid biopsy in cancer diagnosis and prognosis: a paradigm shift in precision oncology. Front Mol Biosci 12:1708518. https://doi.org/10.3389/fmolb.2025.1708518

Bray F, Laversanne M, Sung H, et al (2024) Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians 74:229–263. https://doi.org/10.3322/caac.21834

Qi H, Li F, Peng L, et al (2026) The progress of biomarkers detection to lung cancer. Discov Onc 17:519. https://doi.org/10.1007/s12672-026-04625-w

Tang FH, Wong HYT, Tsang PSW, et al (2025) Recent advancements in lung cancer research: a narrative review. Transl Lung Cancer Res 14:975–990. https://doi.org/10.21037/tlcr-24-979

Zafar S, Hafeez A, Shah H, et al (2025) Emerging biomarkers for early cancer detection and diagnosis: challenges, innovations, and clinical perspectives. Eur J Med Res 30:760. https://doi.org/10.1186/s40001-025-03003-6