ImmunoTools *FlowISiAM* Award 2025

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Evaluation of tumor-associated Macrophages and TKTL1 Expression in Invasive and *In Situ* Breast Carcinomas: A Prospective Study correlating immunohistochemical results with the *FlowISiAM* concept.

Introduction

Breast cancer remains the leading cause of cancer-related mortality among females worldwide, with an increasing incidence and over 400 new cases diagnosed locally on an annual basis. A critical aspect of breast tumor biology involves the immune microenvironment, particularly the role of tumor-associated macrophages (TAMs). These immune cells can be classified into pro-inflammatory (M1) and immunosuppressive (M2) phenotypes, with significant implications in tumor progression, immune evasion, and therapeutic resistance.

The presence of TAMs in ductal carcinoma in situ (DCIS) and invasive breast carcinoma raises important questions regarding their role in tumor invasion, particularly concerning the expression of the transketolase-like 1 (TKTL1) antigen, a biomarker linked to tumor metabolic reprogramming and immune modulation. Given that DCIS and lobular carcinoma in situ (LCIS) maintain an intact basement membrane and myoepithelial barrier, TAMs should theoretically not interact with neoplastic epithelial cells in these cases. In contrast, in invasive carcinomas, TAMs actively infiltrate the tumor stroma and are expected to exhibit TKTL1 positivity. This differential expression may have significant implications for early detection, disease monitoring, and clinical management.

Objective

This project aims to expand the *FlowISiAM* concept by directly characterising in a head-to-head manner with immunohistochemistry the presence and distribution of tissue-associated macrophages (TAMs) in patients with in situ (ISCAMs) and invasive (ICAMs) breast carcinoma. Furthermore, it aims to determine the phenotypic activation status (M1/M2 polarization) of TAMs in both DCIS/LCIS and

invasive carcinoma cohorts. And finally, it aspires to evaluate the expression of TKTL1 in TAMs and assess its association with disease invasiveness.

A key hypothesis is that TAMs in DCIS and LCIS should be TKTL1-negative, given the intact basement membrane. However, if TAMs in in situ carcinoma cases exhibit TKTL1 positivity, this may indicate early immune-tumor interactions, suggesting the presence of undetected invasive disease. This could potentially allow for peripheral blood detection of pre-invasive disease using TKTL1-expressing macrophages as a biomarker.

Methodology

Study Design and Patient Cohort

This study will include biopsy specimens from 100 patients undergoing tissue core biopsies for the primary diagnosis of breast carcinoma, encompassing:

- Invasive carcinoma: No Special Type (NST), invasive lobular carcinoma, and other histologic subtypes,
- In situ carcinomas: DCIS and LCIS [1],

Immunohistochemical (IHC) Analysis

Biopsy specimens will undergo IHC staining to assess macrophage presence, polarization status, and TKTL1 expression. The following antibodies will be used:

- **Standard diagnostic panel:** ER, PR, HER2, Ki-67, E-Cadherin, CK14, P63, PD-L1.
- **Macrophage markers:** CD68 (pan-macrophage), M1 (HLA-DR, CD80), M2 (CD163, CD206).
- **Target biomarker:** TKTL1 (to assess metabolic activity and tumor-macrophage interaction) [2].

Analysis of Macrophage-Tumor Interactions

- Quantitative assessment of TAM density and spatial distribution in DCIS, LCIS, and invasive carcinoma specimens [3].
- Evaluation of M1/M2 polarization to determine whether macrophage functional state correlates with disease invasiveness [4].
- Assessment of TKTL1 positivity in TAMs and its association with histopathological features of invasion.

Potential Clinical and Translational Implications

Diagnostic Utility of TKTL1 in Macrophage Profiling

If TKTL1-positive macrophages are present in pre-invasive breast carcinoma (DCIS/LCIS), it could suggest occult invasive disease not evident in the current biopsy material. This finding could lead to modifications in clinical practice, including:

- **Surgical decision-making:** If TKTL1-positive macrophages are detected in pre-invasive breast carcinoma biopsies, a more extensive sampling procedure (e.g., vacuum-assisted breast biopsy, VABB) may be recommended to detect possible invasion in other parts of the breast.
- **Sentinel lymph node biopsy (SLNB) considerations:** Currently, SLNB is not routinely performed in low-grade DCIS. However, the presence of TKTL1-positive TAMs may indicate an increased risk of invasion, warranting reconsideration of SLNB in these patients.[5, 6]

- **Enhanced diagnostic accuracy:** Routine use of TKTL1 IHC in breast pathology to stratify patients at risk for invasion.

Peripheral Blood Detection of TKTL1-Positive Macrophages

If both in situ and invasive carcinoma-associated macrophages exhibit TKTL1 positivity, this could pave the way for a non-invasive diagnostic tool utilizing **flow-cytometry-based analysis of circulating TKTL1-positive macrophages** (*FlowISiAM*). This approach could enable:

- **Early detection of in situ carcinoma before progression to an invasive phenotype.**
- **Disease monitoring post-surgery for minimal residual disease and recurrence risk assessment.**
- **Potential therapeutic stratification based on macrophage-TKTL1 interactions in the tumor microenvironment.**

This study aims to refine our understanding of TAM biology in breast cancer, with a focus on TKTL1 as a potential biomarker for invasive transition and therapeutic monitoring. If validated, the incorporation of TKTL1 screening into standard pathology practice could significantly enhance breast cancer diagnostics and inform clinical decision-making, ultimately improving patient outcomes.

References

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