# Predicting Lymph Node Metastasis in Endometrial Cancer by multi-modal mass spectrometry imaging


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## Overview

- Determine lymph node metastasis (LNM) in endometrial cancer (EC) using MSI
- Complementary MALDI MSI MS platforms used to generate metabolomic + proteomic profiles, providing more signals to evaluate as markers for EC with LNM.

## Introduction

- One reliable prognostic factor in EC is the presence of LNM.
- Clinicians currently face challenge that radiological imaging and conventional surgical-pathological variables are unreliable in determining if the EC has metastasized**.**
- Molecular markers could provide insight into the tumour biology and the process that leads to metastasis and may immediately serve as diagnostic markers to guide surgeons

## Methods

- Single sections (from one patient – 60B) or formalin-fixed paraffin embedded EC tissue microarrays (TMAs) containing 2 cores from 43 EC patients (16 classified as LNM being present, 27 classified as no LNM) were sectioned for analysis.
- For peptide MSI, antigen retrieval and matrix deposition was carried out using two different described methods (Ref. 1,2).
- For small molecule MSI, sample preparation and matrix was carried out as previously described (Ref. 3).
- Prepared TMA's were analysed using two different MALDI MS platforms:
  - Bruker UltrafleXtreme MALDI-ToF MS
  - Bruker MALDI-tims-Qq-ToF MS platform
- Peptide imaging measured in positive ion mode, small molecule imaging in negative ion mode. Data assessed using SCiLS lab software

## Results

- It was observed in Fig. 1 that MALDI-tims-Qq-TOF Skyline Spectra exhibited more peaks, with higher intensity, compared to MALDI-TOF MS Skyline Spectra

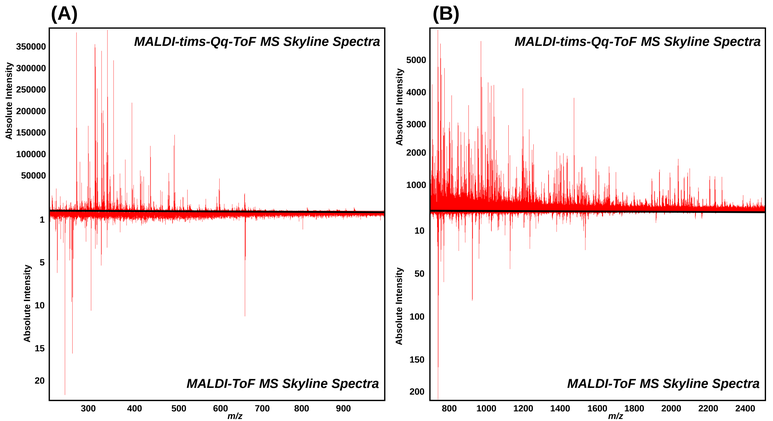



**Figure 1:** Skyline spectra obtained for metabolite (A) and peptide (B) MSI using the two MALDI MS platforms

- Fig. 2A shows distribution of a Phosphatidylinositol (PI) lipid, PI(18:0/0:0), in EC tumour region of a single section, also previously shown to be higher intensity in colon cancer tumour regions (Ref. 3)
- ROC assessment identified 29 signals from MALDI-tims-Qq-ToF data showing potential to be used as markers for LNM
- Fig. 2B shows PI(18:0/0:0) in EC tumour region of TMAs, which may be a potential marker for LNM. A comparison of the MALDI-tims-Qq-TOF measured signal against simulated PI(18:0/0:0) signal demonstrates that isotopic fidelity is preserved.
- PI(18:0/0:0) was observed using both MALDI-tims-Qq-TOF and MALDI-TOF, but more prominent in MALDI-tims-Qq-TOF (Fig. 2C)

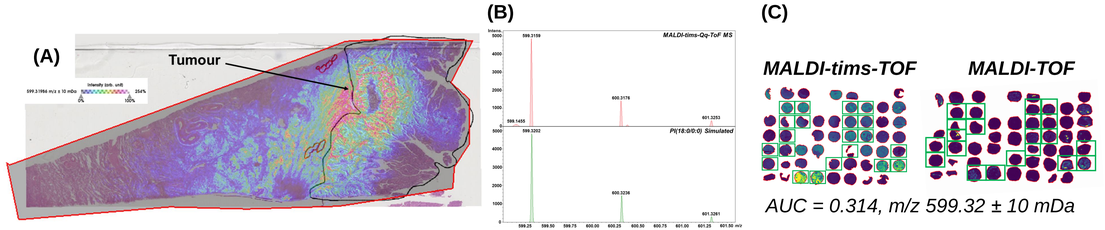
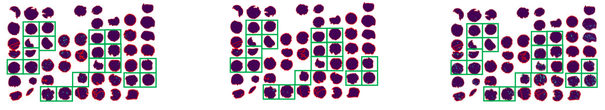



**Figure 2:** Distribution of *m/z* 599.32 in EC in single section (A), comparison of signal to simulated PI (B) and (C) ROC assessment to discriminate LNM presence (Green boxes) and absence in representative EC TMA using MALDI-tims-Qq-TOF

- Previous MSI analysis combined with IHC has identified peptides as markers for LNM in EC (Ref. 2, 4)
- Previous MALDI-TOF MS analysis (Ref. 4) observed Annexin A2 (*m/z* 1542.83), alpha Actinin 4 (*m/z* 1429.76 Da) and Annexin A1 (*m/z* 1099.29 Da), but not observed in MALDI-tims-Qq-TOF MS analysis
- A possible reason could be the use of different antigen retrieval and matrix deposition methods for each of the measurements (Ref. 1 for MALDI-tims-Qq-ToF MS, Ref. 2 for MALDI-ToF MS)



*AUC = 0.507, m/z 1542.83 ± 15 mDa* | *AUC = 0.515, m/z 1429.76 ± 15 mDa* | *AUC = 0.523, m/z 1099.29 ± 15 mDa*

**Figure 3:** No distribution of Annexin A2 (*m/z* 1542.83), alpha actinin 4 peptide (*m/z* 1429.76 Da) and Annexin A1 peptide (*m/z* 1099.29 Da) in representative EC TMA using MALDI-tims-Qq-TOF MS

## Conclusions

- MALDI-tims-Qq-TOF MS provided new potential small molecule markers for LNM
- Peptides from previous MSI analysis (Ref. 4) were not observed using MALDI-tims-Qq-TOF MS, but were observed using MALDI-TOF MS. This may be due to different sample preparation methods measured on each system, but also indicates high complementary nature between systems
- Future work aims to combine datasets using canonical correlation analysis (Ref. 2) and neural networks of signals from small molecule and peptide MSI

## References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