Multi-Institutional Study Assessing STLA101 Assay Performance for the Detection of Cancerous Progression in Barrett's Esophagus

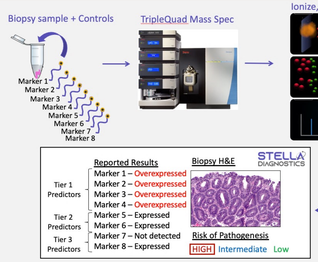
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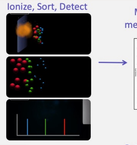
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Introduction

Pre-malignant metaplastic tissue, or Barrett's Esophagus (BE), is the only known precursor for esophageal adenocarcinoma (EAC). Periodic histological assessment of endoscopic forceps biopsy along dysplasia-carcinoma sequence is used to assess progression risk. Current surveillance protocols have clinical limitations as 4 out of 5 patients still present in late stages of EAC. The aim of this study is to compare novel proteomic expression patterns to distinguish stable BE patients from those who developed EAC using the STLA101 assay. The STLA101 panel was designed to work seamlessly into the current standard screening protocols for BE patients.

STLA101 Diagnostic Workflow (Biopsy to Results)





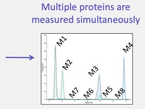




Figure 1: Microdissected, formalin-fixed esophageal tissue was covered in 40 µl of buffer and underwent a series of heating and digestion stages to be synthesized into a mass spec-friendly lysate. The STLA101 early cancer detection assay utilizes mass spectrometry to offer high-throughput, multiplexable, and confident data, assessing the presence and amount of multiple disease-specific biomarkers simultaneously. To ensure highly accurate quantification, we spike in a known quantity of our synthesized peptides which represent the proteins in the STLA101 panel. The control markers are isotopically labeled, so they can be detected, quantified and subtracted from the endogenous signal. This method increases accuracy and precision in the quantification of all biomarkers of interest. The results of the expression trends of the entire panel will translate into a risk score - revealing low, intermediate, or high risk of disease progression based on the molecular expression trends of the patient's BE biopsy.

Barrett's Esophagus Monitoring - Current Standard of Care


Biopsy Retrieval and Gross Inspection (Endoscopy)


Biopsy Fixation, Mounting and Staining


Microscopic Inspection

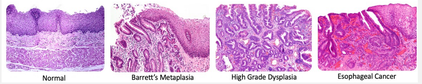


Figure 2: The current pathology standard of prognosis for these diseases is gross and microscopic inspection of esophageal biopsies. Little to no molecular information is assessed with current standard screening protocols for this pre-malignant condition.

Methods

1. After Institutional Review Board approval patients with BE/GERD were identified.
2. Two cohorts were selected: progressors (progressed to EAC at subsequent interval of 0.5-3 years) and non–progressors (~10 years of f/u surveillance without progression along dysplasia/carcinoma path).
3. Formalin fixed paraffin embedded (FFPE) tissue was cut at 10 microns with one 5µm section stained with H&E.
4. The serially sectioned unstained slides were microdissected based upon the guidance of a board-certified pathologist (Figure 1).
5. Dissected cells were then heated to break formalin cross-links, trypsinized, and reduced with DTT.
6. Isotopically labeled control peptides representing the STLA101 panel were spiked in with digested tissue samples to quantify all of the markers simultaneously using a triple-quad mass spectrometer.
7. Expression patterns were confirmed in a selected group of biomarkers via immunofluorescence.

Clinical Design of STLA101 Assay



❖ Gastroenterologist/ Surgical Endoscopist Friendly
- No extra biopsies are needed to run Stella Assay
- Quick turn around time (1-2 weeks)
- No changes required for endoscopic procedure
- Compatible with other tissue retrieval techniques
- Molecular analysis now available for patient's tissue



❖ Pathologist Friendly
- Antibody-free detection and quantification avoids:
  - Non-specific binding and overstaining
  - Subjective interpretation
- Very little tissue requirements (8mm²)



Pinch biopsies for Mass Spec



❖ Patient Friendly
- No additional biopsies are required from patient (no extra risk of bleed)
- No additional time under anesthesia
- Peace of mind knowing if molecular hallmarks of cancer are present or not

Results

- A total of 136 tissues were analyzed. 45 BE biopsies progressed to EAC within a mean of 380 days, while the remaining 36 BE specimens did not progress over a 10-year period.
- All 8 STLA101 biomarkers were expressed at a higher level in BE tissue that progressed to cancer when compared to BE/GERD tissue that remained stable over 10 years (Figure 3).
- In the 55 EAC tissues analyzed, all 8 markers of the STLA101 panel were nearly universally overexpressed and had significantly greater expression quantities compared to traditional biomarkers like HER2, EGFR and PD-L1 (Figure 5).
- Immunofluorescence confirmed overexpression of STLA101 markers in Progressive BE.

STLA101 Carcinogenesis Expression Patterns in BE

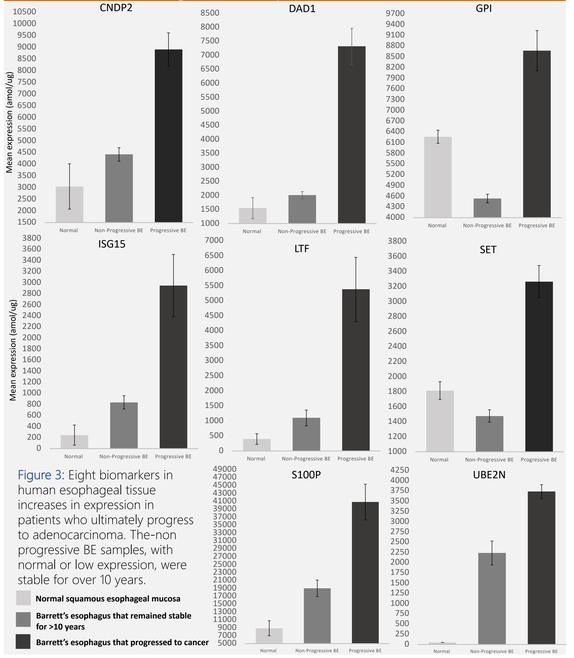


Figure 3: Eight biomarkers in human esophageal tissue increases in expression in patients who ultimately progress to adenocarcinoma. The-non progressive BE samples, with normal or low expression, were stable for over 10 years.

Case Study: High Risk Detected 2.4 years Before Cancer Diagnosis

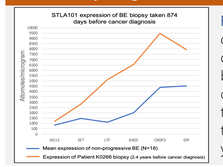


Figure 4: A biopsy retrieved from the Mayo Clinic 874 days before the patient was diagnosed with esophageal cancer was analyzed. The STLA101 assay of the patient's biopsy demonstrated consistent overexpression compared to the mean expression of Barrett's parents that never developed cancer. The patient's expression trends yielded a HIGH RISK for cancer score roughly 2.5 years before esophageal cancer diagnosis.

STLA101 Expression in EAC Tumors Compared to Standard Markers

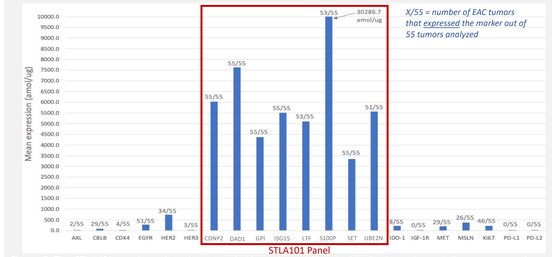
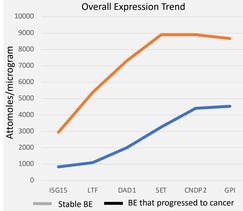


Figure 5: The STLA101 panel consists of proteomic biomarkers affiliated with distal esophageal disease progression and are expressed at much higher quantities compared to markers routinely screened for EAC. At present, HER-2 and anti-PD-L1 therapies are approved by the FDA for EAC. Additionally, off-label use of EGFR directed therapy has been reported. 55 EAC tissues were analyzed. HER2, PD-L1 and EGFR were overexpressed only in 16.3%, 0%, and 0% of tumors, respectively. New oncoproteins DAD1 and S100P were noted to have high (7,634.0 and 30286.7 attomoles/ug) and consistent (98.1% and 94.5%) overexpression in EAC samples.

Conclusions



The overexpression of the STLA101 panel in dysplastic BE tissue that progressed to cancer demonstrates the potential predictive power of this assay when assessing molecular hallmarks of carcinogenesis in precancerous tissue. The expression quantities of the STLA101 panel in non-progressive BE tissues were closer to normal mucosa and may warrant patients with this profile type to be screened less. Patients with high expression quantities of the STLA101 panel may be candidates for a more frequent screening cadence or therapeutic intervention. The mass spectrometry assay also revealed low prevalence of target proteins in currently approved EAC therapies and high levels of the STLA101 panel, potentially demonstrating that these disease drivers in BE carry over into EAC to promote cell proliferation and invasion and inhibit cell death.

Disclosures

Dr. Abdo is a full-time employee and Dr. Mittal is a clinical advisor for Stella Diagnostics, Inc. The other authors have nothing to disclose. Stella Diagnostics assisted in the assembly of this presentation. The data is original and has not been previously published.

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