Development and Prospective Validation of A Serum microRNA Assay for Gastric Cancer Early Detection in High Risk Population

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ASCO ANNUAL MEETING

Abstract Number: 4065

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1. BACKGROUND

High mortality from gastric cancer is related to the late manifestation of its symptoms. In high-incidence countries such as Japan and Korea, mass screening for gastric cancer is practiced using photofluorography or more recently, endoscopy. In these settings, over 50% of gastric cancer patients are diagnosed at early stages and their survival are excellent. However, in most countries, mass screening is not feasible or cost-effective. Endoscopy is costly and invasive with poor compliance. A blood-based biomarker with the ability to detect all stages of gastric cancer could significantly improve patient outcomes. We aimed to develop a novel serum miRNA assay for early detection of gastric cancer.

We conducted a 3-phase, multi-center study involving 5248 cancer and control subjects from Singapore and Korea to develop and validate the multi-target miRNA assay. Using RT-qPCR, we quantified the expressions of 578 serum miRNAs in 682 gastric cancer and control subjects and developed a 12-miR biomarker panel through multi-variant data analysis. The test results were generated with the use of a logistic-regression algorithm, with the value of 40 or more considered to be positive. We subsequently validated this multi-miR assay in a large prospective cohort involving 4566 high risk subjects and compared its performance with traditional markers such as *H.Pylori* and Pepsinogen. All participants underwent gastroscopy independent of the assay results.

2. STUDY DESIGN

578 candidate miRNAs
(miRbase annotated high confidence miRNA)

↓

1. Discovery Phase
Case-Control Cohort (Singaporean)
N=472 (236 cancers)

↓

75 candidate biomarker miRNAs
(Differentially expressed in cancer vs control)

↓

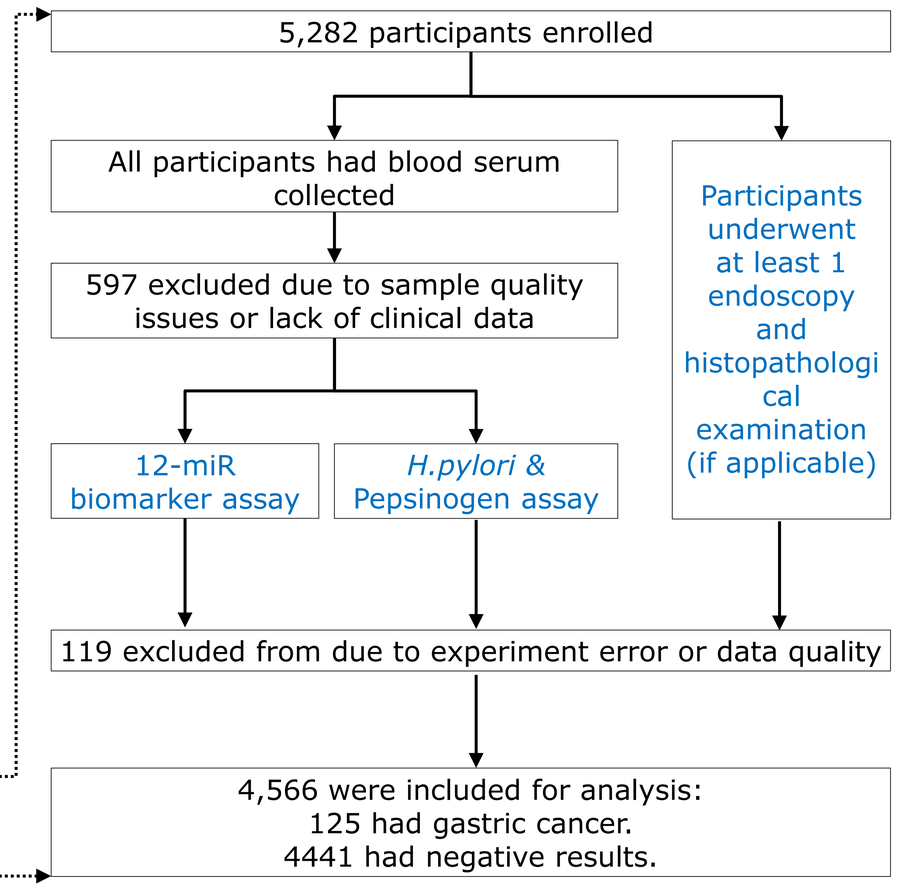
2. Verification Phase
Case-Control Cohort
(Singaporean & Korean)
N=210 (94 cancers)

↓

12-miR multi-target miRNA assay
(With prespecified logistic-regression algorithm and cut-off)

↓

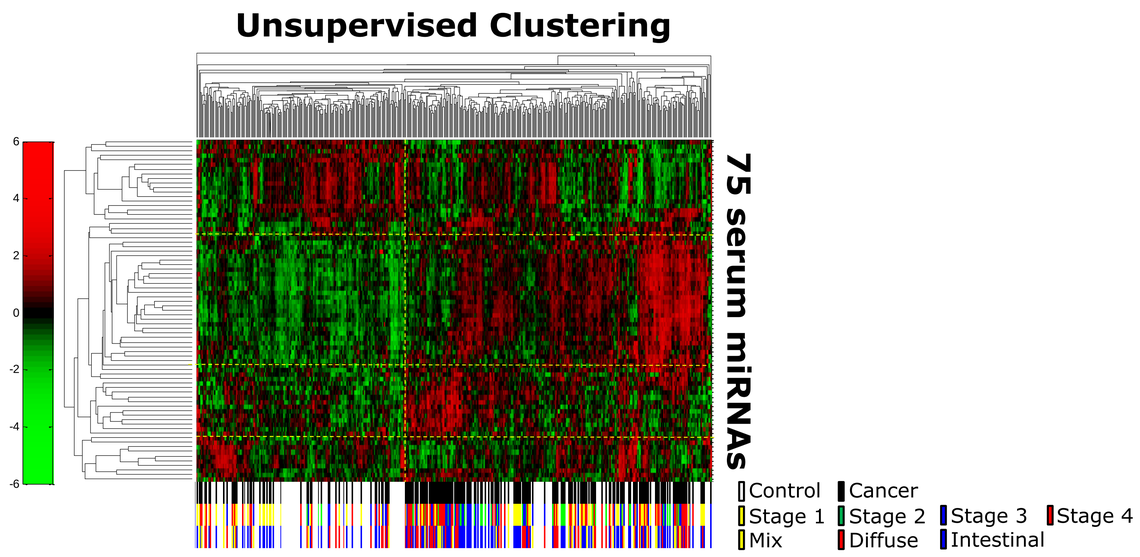
3. Validation Phase
Prospective Cohort (Singaporean)
N=4566 (125 cancers)



3. RESULTS

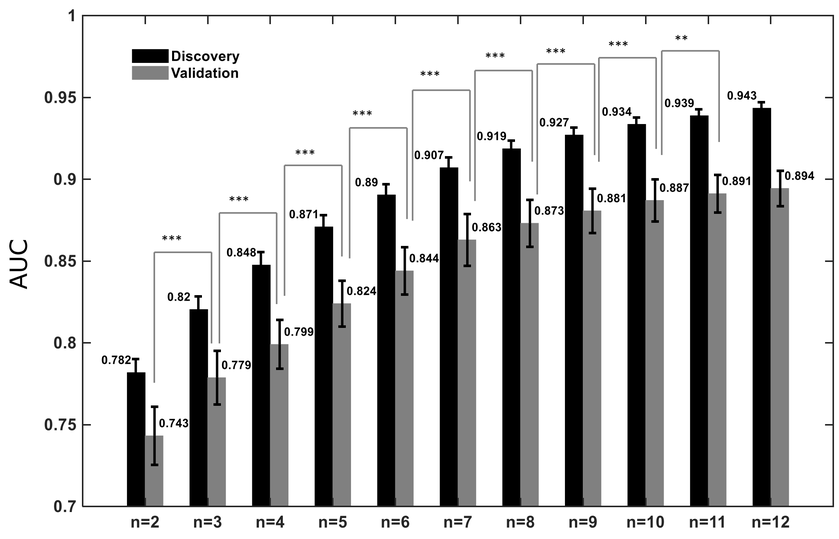
Identification of gastric cancer associated miRNA biomarkers

The absolute expression copy numbers of 578 candidate miRNAs were quantified in 682 cancer and control biospecimen using analytically validated miRNA-specific RT-qPCR assays (MiRXES, Singapore) via a highly-controlled workflow. Seventy-five miRNAs were found to be regulated between cancer and matched controls.



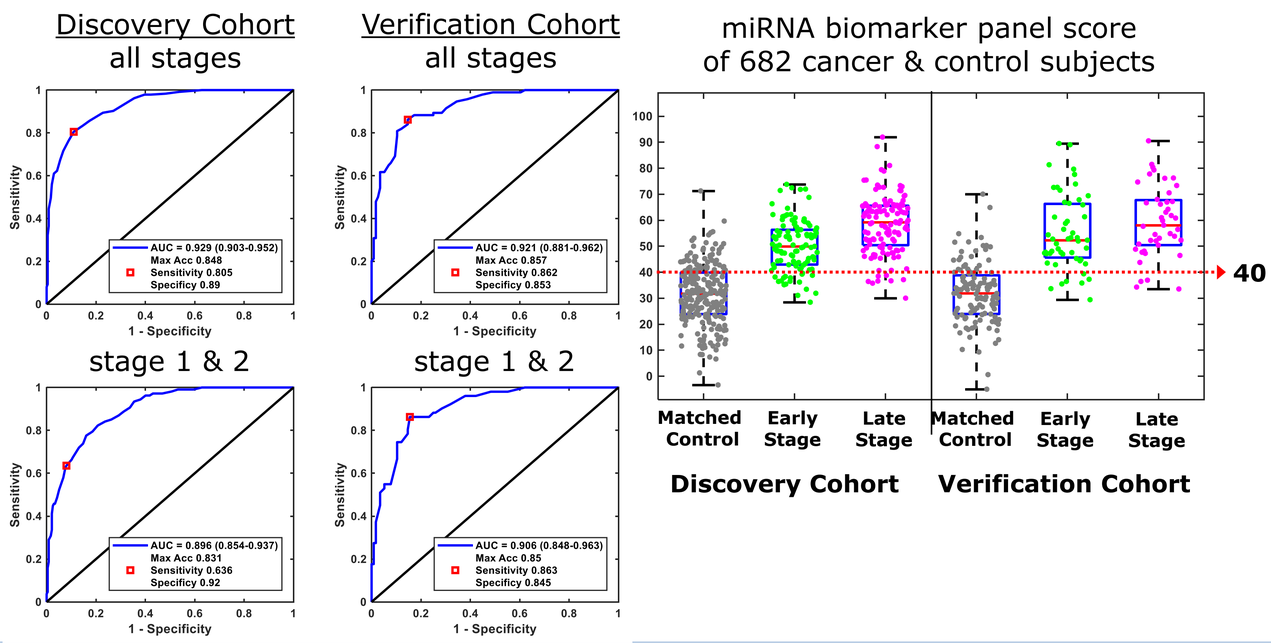
12-miR biomarker panel optimization & cross validation

Combinations of distinctively-regulated miRNAs enhanced diagnostic accuracy. We identified and tested several miRNA panels (3-12 miRNAs) with high AUC in distinguishing gastric cancer subjects from controls using four-fold cross-validation (matched by gender, cancer subtype and stage)



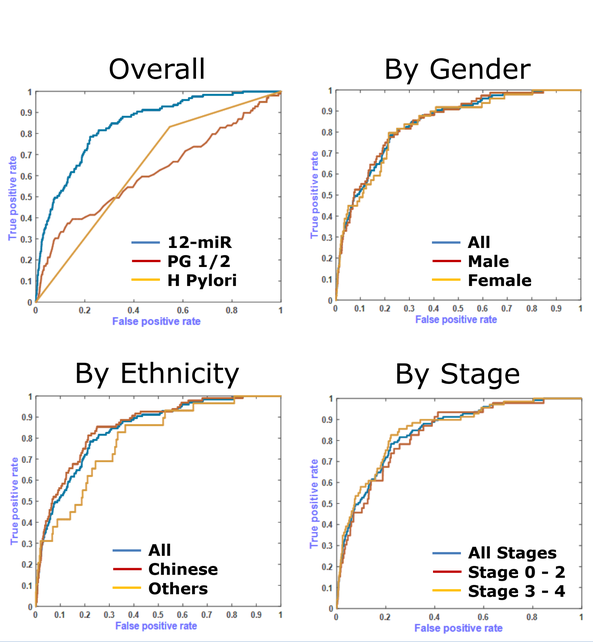
Verification of miRNA biomarker expression

We subsequently verified the expression of the identified candidate biomarkers and normalizers in 2 retrospective cohorts including 210 Singaporean and Korean subjects. We observed good correlations in miRNA expression fold-changes between the Discovery cohort and Verification cohorts.



Prospective clinical validation of 12-miR panel

The 12-miR assay, with a prespecified algorithm, was validated in a prospective cohort of Singaporean patients. Measured by AUC, the discrimination between cancer and controls was significantly higher with the 12-miR assay than with H. Pylori and PG1/2 ratio (0.84 vs 0.64 and 0.62 respectively)



Conventional Markers	AUC	Sen	PPV	NPV
PG1	0.41	38.40%	1.50%	96.80%
PG1/2 ratio	0.62	23.20%	8.01%	98.20%
PG_index	0.57	18.20%	7.69%	98.10%
H.Pylori	0.64	83.20%	4.11%	99.00%
12-miR Panel	**AUC**	**Sen**	**PPV**	**NPV**
1. Overall	0.84	84.80%	7.03%	99.38%
2. By Gender				
Male	0.85	85.53%	7.90%	99.31%
Female	0.84	83.67%	5.99%	99.45%
3. By Ethnicity				
Chinese	0.86	85.42%	7.28%	99.41%
Others	0.79	82.76%	6.28%	99.28%
4. by Stage				
Stage 0 – 2	0.83	82.61%	3.18%	99.77%
Stage 3 – 4	0.85	88.41%	0.85%	99.93%

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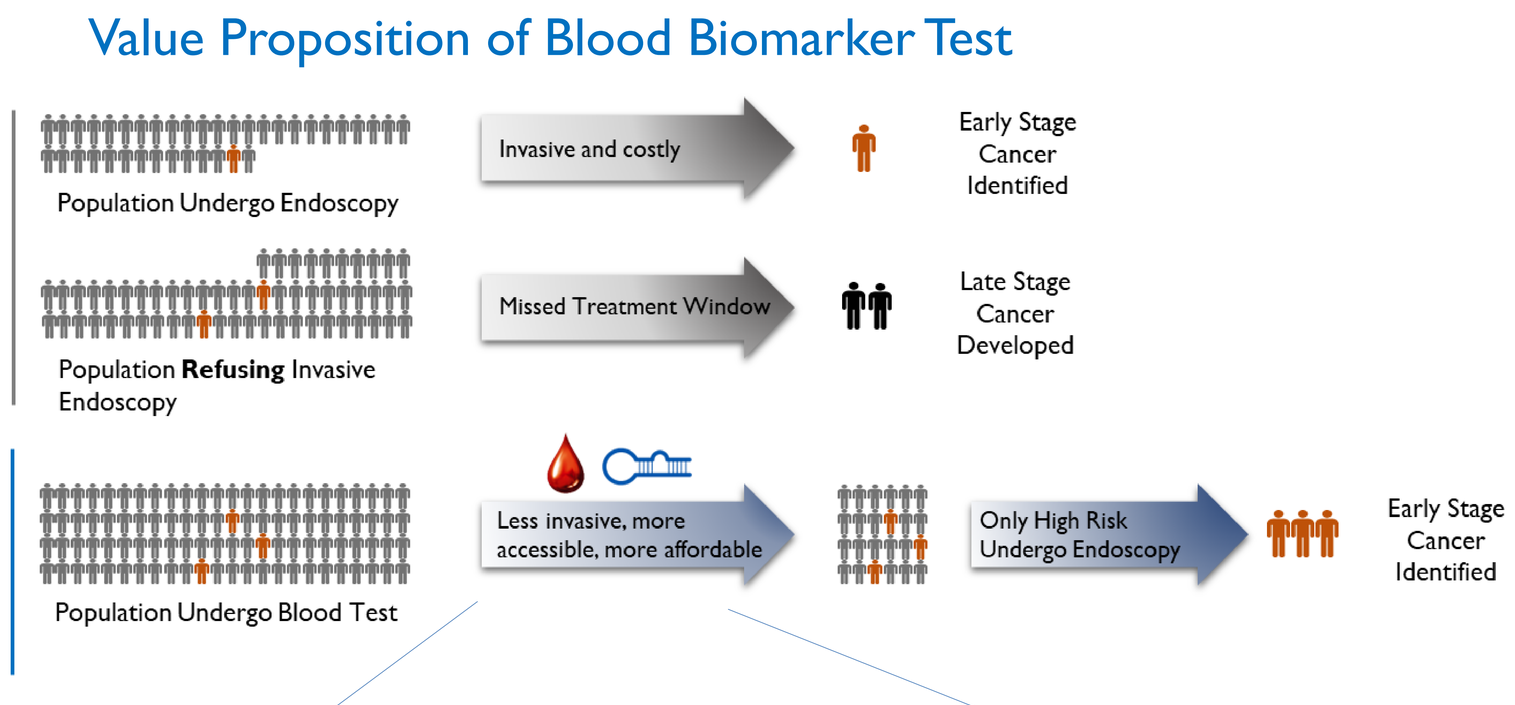
Value Proposition

Greater Coverage

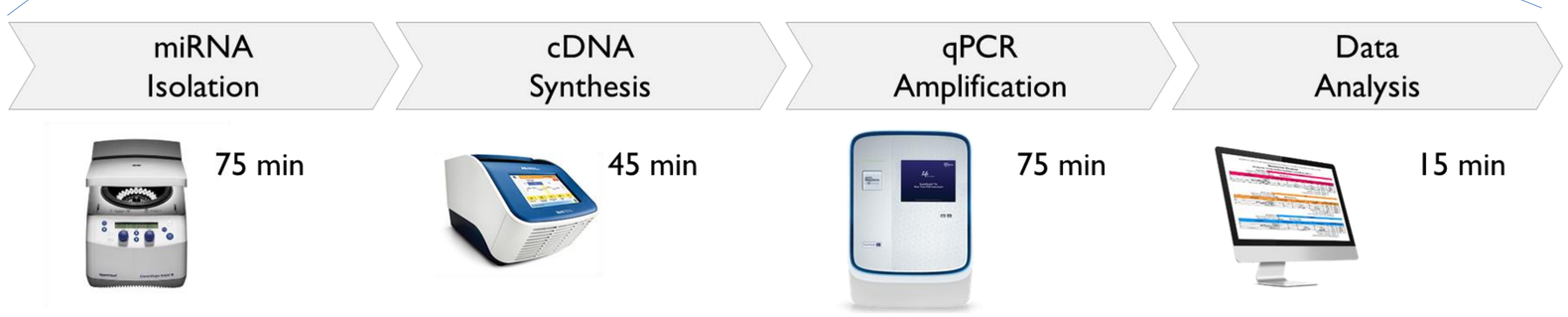
Better Prognosis

Cost Reduction

Value Proposition of Blood Biomarker Test



Laboratory procedures for 12-miR multi-target assay



The 12-miR multi-target qPCR assay was developed and manufactured in accordance with ISO13485 medical devices quality management systems. Extraction of RNA is performed by combining phenol/guanidine-based lysis of serum sample and silica-membrane-based purification of total RNA. During cDNA synthesis, miRNA targets from each specimen are converted into cDNAs using corresponding miRNA specific reverse transcription primers in a single reaction. At the qPCR step, each miRNA target is amplified by a sequence-specific forward PCR primer and a hemi-nested sequence specific reverse PCR primer and detected using SYBR Green in single-plex reactions. Ct values of the biomarker and reference miRNAs were incorporated into a validated, prespecified logistic-regression algorithm, with a single numerical score to indicate test positivity or negativity.

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