Translation of a Plasma RNA Molecular Profile for Colorectal Tumors to the Clinical Laboratory

[Ouriel Faktor[1], Tamar Peretz[2], Dan Keret[3], Dana Cohen[1], and Eran Israeli[4], Susan Belzer[1]
[1]MD Biosciences, Inc. CLIA Biomarker Laboratories, Oakdale, MN, USA, [2]Sharett Institute of Oncology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel[3]Gastroenterology and Hepatology Department, Clalit Health Services, Jerusalem, Israel[4]Institute of Gastroenterology and Liver Diseases, Hadassah-Hebrew University Medical Center, Jerusalem, Israel

ABSTRACT

- A molecular profile for early colorectal cancer (CRC) detection has recently been developed. The clinical research study determined a profile of early stage CRC and advanced adenomas (AA) by combination of current molecular knowledge with microarray technology, using efficient circulating free plasma RNA purification from blood and RNA amplification technologies.
- A literature search was joined with Affymetrix gene chip experimental procedure to draw the circulating free plasma RNA profile of colorectal cancer disease reflected in blood. The RNA panel was tested by two datasets comparing CRC patients with healthy subjects and patients with AA to healthy subjects.
- For the CRC patient cohort (28 CRC cases vs. 41 healthy controls), the ROC analysis of the selected biomarker panel generated a sensitivity of 75% and a specificity of 93% for the detection of CRC using 8-gene classification model.
- For the AA patient cohort (28 subjects vs. 46 healthy controls), a sensitivity of 60% and a specificity of 87% were calculated using a 2-gene classification model.
- A panel of 8 plasma RNA markers was identified as a preliminary panel for CRC detection and subset markers were deemed suitable for AA detection.
- The translation of the existing research assay to the clinical laboratory is being undertaken. In advance of an extensive clinical validation, the needed requirements for assay performance and reagent requirements for the clinical laboratory have been determined and are presented.
- **Keywords:** colorectal cancer; advanced colonic adenoma; RNA profiling.

BACKGROUND

- Current standard of care for colorectal cancer suggests a screening by colonoscopy procedure every 10 years
- In the case of the detection and removal of polyps with pathologic confirmation of Adenoma, the frequency of the colonoscopy can be increased to every 3-5 years
- The colonoscopy is an effective means of detection of adenomas and CRC, however compliance and ineffective patient preparation result in significant numbers of individuals not being screened.
- Compliance with usual care varies in the US with an average of 50%, however in 1 large population study the rate was less than 30% (1) for individuals in a safety-net health system.
- Fecal immunochemical test and fecal mutation test are options for patients resistant to colonoscopy procedures
- Compliance with the FIT was less than 60% (1) for individuals who were actively recruited to participate by outreach in the safety-net health care system. Colonoscopy compliance with outreach was 42%.
- A blood test for markers of CRC and AA has the advantage of increased patient participation and decreased time to diagnosis of pre-cancerous and cancerous lesions.

OBJECTIVES

- Identification of a gene signature from blood for CRC and AA
  - Concluded at Gastroenterology Departments of Hadassah Medical Center, Israel; Humanitas Clinical and Research Center, Italy and Clalit Health Services, Israel.
- Confirm the performance of the assay as transferred
  - MD Biosciences, MN, USA
- SIMPLE, ROBUST, REPRODUCIBLE, ACCURATE clinical laboratory assay
  - Pre-analytic specimen collection, processing and transport
  - Decrease specimen extraction time
  - Clinical Grade Reagents for Extraction, Reverse Transcription, Amplification, Primers
- Clinical Study using SRRA Clinical Assay

METHODS

- Gene Identification in CRC, AA and Health Subjects
  - Affymetrix Array (6577 genes) and Bioinformatics Gene List
  - 72 gene qPCR
  - 17 gene qPCR
  - 8 gene final set
  - Training set 144 Plasma samples
- Research Method
  - 10 mL EDTA whole blood collection
  - TRIzol added to plasma prior to freezing
  - Frozen plasma shipped from clinic
  - 2 Day Extraction
  - 2 Step RT-qPCR
- Clinical Lab Method (Optimal)
  - 10 mL EDTA whole blood collection
  - Stable plasma transported from clinic
  - >0.5 Day extraction
  - 1 Step RT-qPCR
  - TAT <2 days
- Pre Analytics
  - Collection, proper separation and storage of clinical samples that can be maintained in the routine clinical setting.

RESULTS

Figure 1. Receiving operating characteristic (ROC) curve analysis
Discrimination between Cancer and Health Subjects

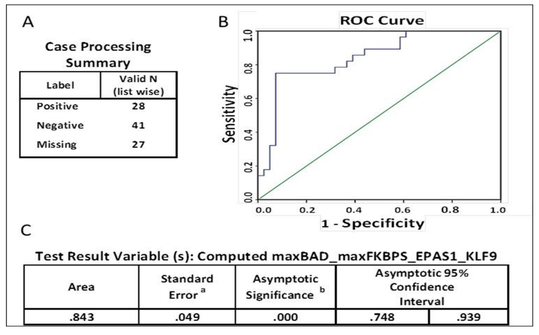
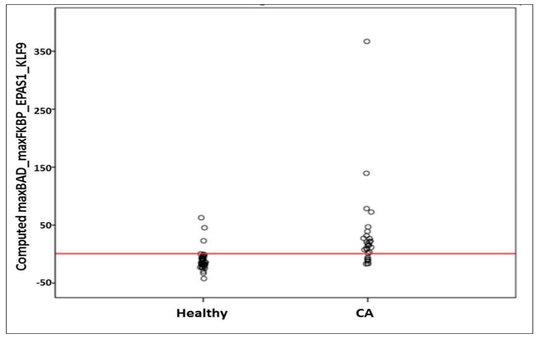


Figure 2. Maximum Youden Index Point (sensitivity + specificity -1)
Discrimination between Cancer and Health Subjects



RESULTS

Figure 3. Receiving operating characteristic (ROC) curve analysis
Discrimination between Advanced Adenoma and Health Subjects

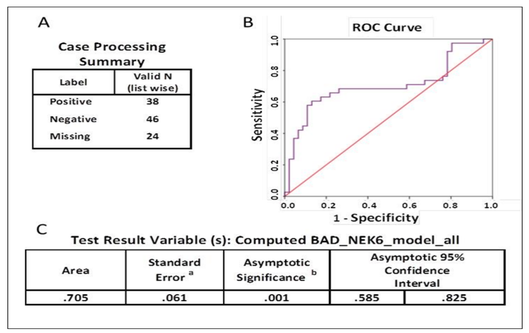
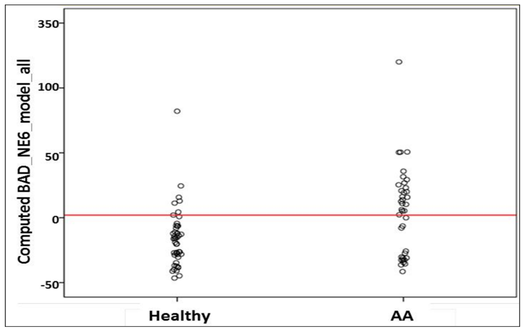


Figure 4. Maximum Youden Index Point (sensitivity + specificity -1)
Discrimination between Advanced Adenoma and Health Subjects



NEXT STEPS

- SIMPLE, ROBUST, REPRODUCIBLE, ACCURATE clinical laboratory assay
  - Pre-analytic specimen collection, processing and transport
  - Decrease specimen extraction time
  - Clinical Grade Reagents for Extraction, Reverse Transcription, Amplification, Primers
- Clinical Study using SRRA Clinical Assay

REFERENCES

Feasibility of **Unbiased RNA Profiling** of Colorectal Tumors: A Proof of Principle.
Moshayoff V, Faktor O, Laghi L, Celesti G, Peretz T, Keret D, Cohen D, Israeli E.
PLoS One. 2016 Jul 21;11(7):e0159522. doi: 10.1371/journal.pone.0159522.
PMID: 27441409

Comparative effectiveness of fecal immunochemical test outreach, colonoscopy outreach, and usual care for boosting **colorectal cancer screening** among the underserved: a randomized clinical trial. Gupta S, Halm EA, Rockey DC, Hammons M, Koch M, Carter E, Valdez L, Tong L, Ahn C, Kashner M, Argenbright K, Tiro J, Geng Z, Pruitt S, Skinner CS. JAMA Intern Med. 2013 Oct 14;173(18):1725-32. PMID: 23921906