Geisinger Clinic

Annual Progress Report: 2011 Nonformula Grant

Reporting Period

July 1, 2014 – August 29, 2014

Nonformula Grant Overview

The Geisinger Clinic received $1,000,000 in nonformula funds for the grant award period June 1, 2012 through August 29, 2014. Accomplishments for the reporting period are described below.

Research Project: Project Title and Purpose

Diagnostic-Prognostic Testing in Patients at High Risk for Esophageal Cancer – The purpose of this project is to clinically validate a diagnostic-prognostic test for esophageal cancer, which will accurately diagnose at a premalignant stage and predict which patients are at high risk for esophageal cancer to enable early, preventative therapy. A prototype test has been developed and proof-of-concept of the testing technology has been established in collaborative work by Geisinger and Cernostics. The project aims to perform clinical validation studies in a training cohort and two independent validation cohorts of esophageal biopsies with clinical outcome data from Geisinger, University of Pittsburgh and University of Pennsylvania to select diagnostic and prognostic classifiers and to establish the sensitivity, specificity and positive and negative predictive values of the diagnostic-prognostic test for patients at high risk for esophageal cancer.

Duration of Project

6/1/2012 – 8/29/2014

Project Overview

The broad objective of the research is to clinically validate a diagnostic and prognostic test that accurately assigns diagnosis and predicts risk of developing esophageal cancer. The test is a spatial systems biology-based approach to anatomic pathologic testing. The test employs multiplexed fluorescence labeling of tumor system biomarkers, including malignant, immune and stromal processes in anatomic pathology specimens with digital imaging and image analysis to quantify biomarker expression and spatial relationships between biomarkers in the context of tissue morphology. This is coupled to classifier software to integrate biomarker data with morphology data and clinical data to produce diagnostic and prognostic scores. These scores will be used to accurately diagnose and predict the risk of developing esophageal cancer in individual patients to enable early treatment. A prototype test has been collaboratively developed by Geisinger (lead applicant) and Cernostics, Inc. (small business collaborator) as a proof-of-

concept. As a next step, a consortium of investigators will perform retrospective clinical validation studies of the test towards the long term goal of commercializing the test via a CLIA-certified laboratory. The test will be performed first in a training cohort of formalin-fixed paraffin-embedded esophageal biopsies with clinical data from Geisinger using Cernostics' spatial systems biology technology, and diagnostic and prognostic classifiers will be developed. The test, including the classifiers, will then be performed in two independent validation patient cohorts from the University of Pittsburgh and the University of Pennsylvania to determine specificity, sensitivity and positive and negative predictive values of the diagnostic-prognostic test. The specific research aims are, 1) Determine the performance of the prototype test in stratifying patients according to diagnosis and predicting risk for esophageal cancer in a retrospective training patient cohort; and 2) Validate the diagnostic and prognostic performance of the optimized diagnostic-prognostic test in two independent retrospective patient cohorts. The training and validation cohorts represent both urban and rural populations and are designed to reach the maximum number of the underserved and will ensure a significant statewide impact on the health of Pennsylvanians. Paralleling the proposed project, Cernostics and Geisinger will perform further analytical validation studies on the test. The test will be commercialized by Cernostics and will be offered as a service to pathologists and gastroenterologists to guide individualized patient management to help prevent the development of esophageal cancer.

Principal Investigator

Jeffrey W. Prichard, DO
Director of Surgical Pathology
Geisinger Medical Laboratory
100 North Academy Avenue
Danville, PA 17822

Other Participating Researchers

Jinhong Li, MD, PhD; David L. Diehl, MD – employed by Geisinger Clinic
Rebecca J. Critchley-Thorne, PhD; Bruce Campbell, MS – employed by Cernostics, Inc.
Gary W. Falk, MD, MSc; Anil K. Rustgi, MD; Nirag Jhala, MD, PhD – employed by the University of Pennsylvania
Jon M. Davison, MD; Chakra Chennubhotla, Ph.D. – employed by the University of Pittsburgh
Blair A. Jobe, MD; Ali H. Zaidi, MD – employed by West Penn Allegheny Health System
Yi Zhang, Ph.D. – consultant statistician

Expected Research Outcomes and Benefits

The project employs a testing technology for which Geisinger Health System and Cernostics have demonstrated proof-of-concept. The investigators have selected a comprehensive panel of diagnostic and prognostic biomarkers, many of which have established significance in diagnosing the stages of Barrett's esophagus and in predicting risk for esophageal cancer. Therefore, the expected research outcomes of the project are classifiers based on optimal sets of biomarker, morphology and clinical data that can accurately assign diagnosis and predict

whether a patient will develop high grade dysplasia or esophageal cancer and also estimate the sensitivity, specificity and overall accuracy of the diagnostic-prognostic test. It is expected that the test will have high sensitivity and specificity and high positive and negative predictive values based on the known diagnostic and prognostic significance of the panel of biomarkers and based on the high stringency of feature selection for the classifiers. It is also expected that the research will identify a key set of biomarkers and related molecular pathways involved in the progression of Barrett's esophagus to esophageal cancer, which will lead to a better understanding of the biology and behavior of esophageal cancer and aid in the design of new therapeutic agents to prevent and treat esophageal cancer.

The diagnostic utility of the test will improve health status by increasing the accuracy of pathological diagnosis, thus reducing the number of repeat endoscopies and biopsies that patients with Barrett's esophagus must currently undergo, particularly for patients who are initially diagnosed as "indefinite/indeterminate" for dysplasia. The prognostic utility of the test will improve health status by identifying patients at high risk for developing esophageal cancer early in the disease progression when treatments such as endoscopic mucosal resection and radiofrequency ablation can be applied to effectively prevent development of cancer. The prognostic utility will also identify low risk patients, who will not develop esophageal cancer, and who can be spared unnecessary endoscopies, biopsies and treatments.

The expected benefits of the project include; significant improvements in diagnostic and prognostic accuracy to prevent delays in treatment of patients at high risk for esophageal cancer, and a reduction in unnecessary and costly endoscopies and biopsies. This individualized approach will benefit patients by reducing the incidence and mortality associated with esophageal adenocarcinoma and will benefit health care systems by targeting treatments and screenings to the high-risk patients who need them.

Summary of Research Completed

Specific Aim 1: Determine the performance of the prototype test in stratifying patients according to diagnosis and predicting risk for esophageal cancer in a retrospective training patient cohort.

During this project period we continued to expand the patient cohort with additional biopsies from University of Pennsylvania and University of Pittsburgh (n=7 and 54 patients, respectively). Table 1 summarizes the total number of patient cases acquired by each institution for the study thus far. During this project period the research also evaluated the diagnostic significance of TissueCypher image analysis features. The histologic diagnosis of dysplasia in Barrett's esophagus is limited by intra- and inter-observer variability. Immunohistochemical detection of biomarkers such as Ki-67, p53 and AMACR have been used to aid diagnosis, however, interpretation of diagnostic markers by light microscopy is challenging in Barrett's esophagus. In addition to evaluating the prognostic significance of the TissueCypher assay, this study evaluated whether the TissueCypher approach could objectively identify aberrations in biomarker expression and nuclear morphology in subpopulations of metaplastic cells that are correlated with grade of dysplasia. Barrett's cases with gastrointestinal subspecialist pathologist confirmed diagnoses of no dysplasia (ND, n=132 patients), low grade dysplasia (LGD, n=28

patients) and high grade dysplasia (HGD, n= 20 patients) (Figure 1A-C) from the training cohort were fluorescently immunolabeled for Ki-67 and CK-20 plus Hoechst labeling of nuclei. Whole slide four channel digital images of the biopsy sections (Figure1D-F) were analyzed by the TissueCypher platform to segment subcellular compartments and tissue compartments and measure biomarker and morphology features within the appropriate subcellular and tissue compartments. Multiple image analysis features derived from Ki-67 and CK-20 in combination with nuclear morphology showed different levels in the diagnostic subsets of BE. In the ND-LGD-HGD sequence there was an increasing proportion of CK-20+ cells proliferating (Ki-67+) (Figure1D-G). Ki-67+ CK-20+ cells showed higher Ki-67 intensity, larger nuclear area and equivalent diameter and loss of nuclear solidity in biopsies with HGD or LGD versus ND (Figure1H-I). This part of the study demonstrated that the TissueCypher quantitative, multiplexed biomarker-morphology imaging approach detects significant differences between BE with ND, LGD and HGD and may provide an adjunctive tool to conventional pathological analysis for the objective assessment of Barrett's esophagus. There is a much greater market need for a prognostic test for Barrett's than a diagnostic test. Therefore, the research has focused on the development and validation of a prognostic test than can be commercialized as a risk prediction tool.

Specific Aim 2: Validate the diagnostic and prognostic performance of the optimized diagnostic-prognostic test in two independent retrospective patient cohorts.

Specific Aim 2 was achieved during this reporting period.

A prospectively defined classifier 1 (development of which was reported on in previous annual report) was evaluated on the independent validation cohort of 193 patients with Barrett's esophagus, including 67 progressors and 126 non-progressors. Patients from four institutions were combined into a single independent validation cohort. As in the training cohort, diversity was increased in the independent validation cohort by inclusion of patient cases from Academic Medical Center (AMC), Amsterdam, Netherlands. The independent validation cohort content is summarized in Table 2 and patient characteristics are summarized in Table 3. All classifier parameters were pre-specified (30 image analysis feature/measures, coefficients, cutoffs between low-intermediate and intermediate-high risk groups). The classifier was tested on the independent validation cohort by Dr. Zhang, the outside consultant statistician for the project. The performance of the classifier in stratifying patients according to risk of progression to HGD/EAC is shown in Figure 2A-B. The classifier predicts probability of remaining HGD/EAC-free at 5 years with hazard ratios of 2.49 (intermediate vs. low risk) and 7.32 (high vs. low risk), p value <0.0001. The probability of being free of HGD/EAC at 5 years was 0.8 in the low risk class and 0.17 in the high risk group, corresponding to NPV and PPV of 0.80 and 0.83, respectively. The prevalence adjusted percentages of patients receiving low, intermediate and high risk scores with the TissueCypher test were 69.6%, 26.0%, 4.4%, respectively. In multivariate Cox models in which progression to HGD/EAC was evaluated in relation to the TissueCypher risk classes and the pathologist's Dx or Barrett's segment length, the intermediate risk and high risk classes provided significant prognostic power that was independent of the pathologist's diagnosis (Figure 2C) and the Barrett's segment length (Figure 2D). The results

demonstrated that the TissueCypher classifier outperformed the standard clinical variables and passed independent validation.

Table 1. Summary of Barrett's Cohorts from Each Clinical Institution (total acquired to date)

Institution	Patients	Non-Progressor Patients	Progressor Patients	HGD/EAC Patients
Geisinger Clinic	204	139	40	25
University of Pittsburgh	188	121	47	20
University of Pennsylvania	95	49	21	25

Table 2. Summary of Multi-Institutional Training and Validation Barrett's Cohorts that the TissueCypher Test has been Performed on to Date

Cohort	Total # Patients	Progressors	Non-Progressors	Institution			
				Geisinger	UPitt	UPenn	AMC
Training	223	80	143	99	27	24	73
Validation	193	67	126	72	36	20	65

UPitt: University of Pittsburgh, UPenn: University of Pennsylvania, AMC: Academic Medical Center, Netherlands.

Table 3. Summary of Cohort Patient Metadata

Characteristic		Training Cohort	Independent Validation Cohort
Median HGD/EAC-free surveillance time, days (non-progressor patients)		3117	2911
Median progression time to HGD/EAC, days (progressor patients)		542	636
Median Age (years)		59	60
Gender (%)	Female	16	20.3
	Male	84	79.7
Race (%)	Caucasian	93.2	84.8
	African American	0	1.5
	Hispanic	0.46	0
	Other	0.46	0
	Unknown	5.9	13.7
BE Segment Length (%)	Long	53.9	55.8
	Short	39.7	37.1
	Unknown	6.4	7.1

Figure 1.

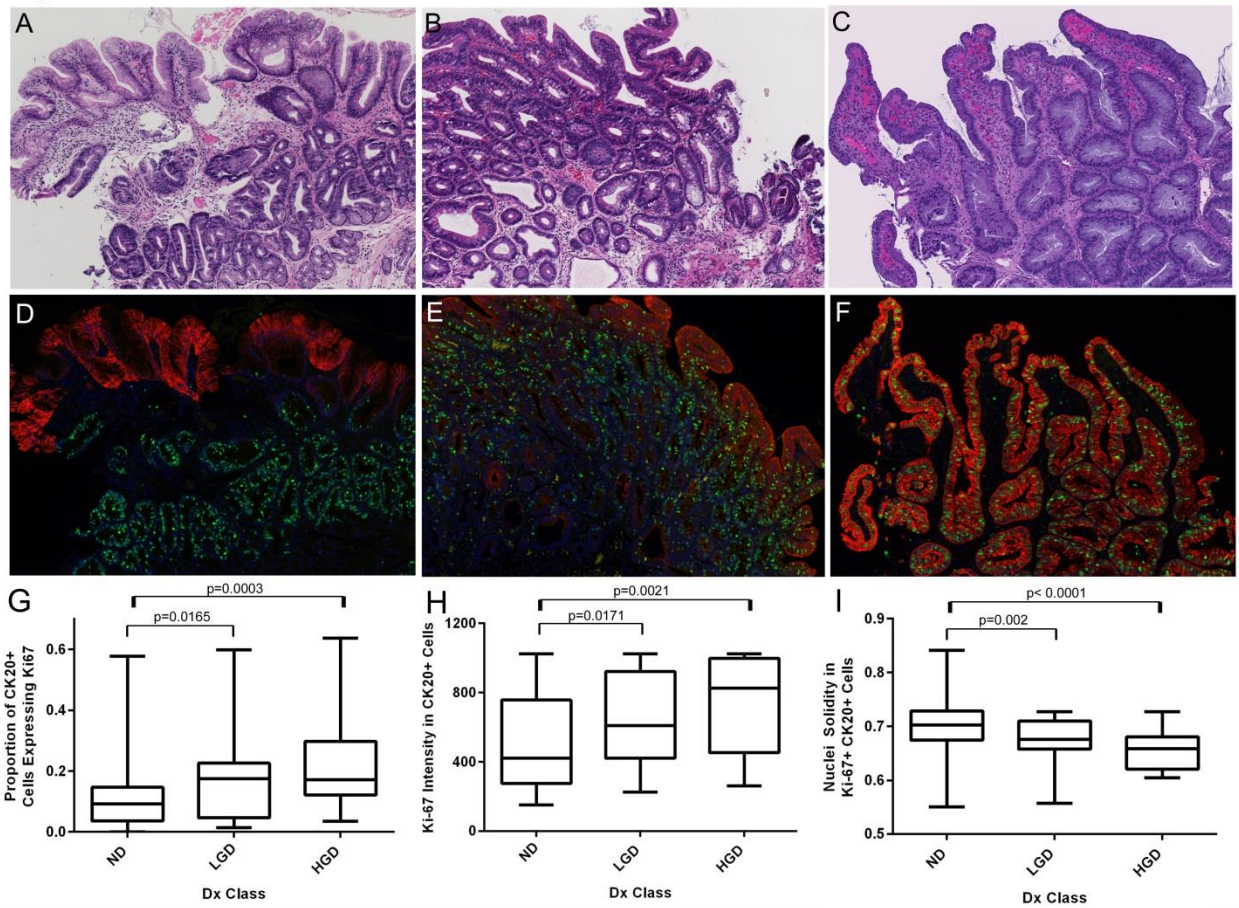
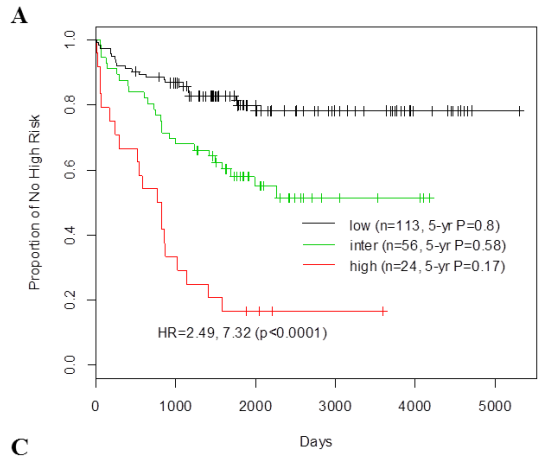
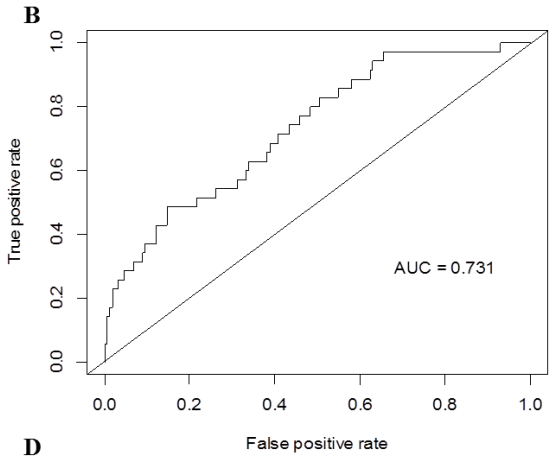


Figure 1. Hematoxylin and Eosin staining (A-C) and TissueCypher labeling of Hoechst (blue), CK-20 (red) and Ki-67 (green) (D-F) in sections of Barrett's biopsies with no dysplasia (ND), LGD, HGD, respectively. Box and whisker plots showing quantitative image analysis features in Barrett's biopsies with ND, LGD, HGD; G: Proportion of CK-20+ cells expressing Ki-67, H: Ki-67 intensity in CK-20+ cells and I: Nuclei solidity (indicator of morphology aberration) in CK-20+ Ki-67+ cells. P values shown on panels G-I are from two-tailed Mann-Whitney tests.

Figure 2.





C

Multivarate Cox Results - Analysis of TissueCypher Classifier 1 vs General Pathologist's Dx in Independent Validation Cohort		
	HR (95% CI)	P value
General Pathologist Dx		0.52
Indefinite vs Non-dysplastic	1.06 (0.32 – 3.52)	0.92
Low grade dysplasia vs Non-dysplastic	1.49 (0.76– 2.92)	0.25
TissueCypher Classifier 1		0.0001
Intermediate vs Low Risk	3.44 (1.77– 6.68)	0.0003
High vs Low Risk	5.09 (2.04 – 12.67)	0.0005

D

Multivarate Cox Results - Analysis of TissueCypher Classifier 1 Barrett's Segment Length in Independent Validation Cohort		
	HR (95% CI)	P value
Barrett's Segment Length		
Long (>3cm) vs Short (≤3cm	1.43 (0.83 – 2.45)	0.20
TissueCypher Classifier 1		4.4×10^{-9}
Intermediate vs Low Risk	2.84 (1.56 – 5.16)	0.0006
High vs Low Risk	8.19 (4.30 – 15.60)	1.6×10^{-10}

Figure 2. Performance of TissueCypher Classifier 1 in Independent Validation Cohort. A: KM survival curve for classifier 1 with probability cutoffs 0.78 and 0.32 demonstrating stratification of Barrett's patients into low, intermediate and high risk groups. B: ROC; C: Results from multivariate Cox analysis of Classifier 1 vs. General Pathologist Dx,; D. Results from multivariate Cox analysis of Classifier 1 vs. Barrett's segment length.