Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-231-2825.

1. **Grantee Institution:** Geisinger Clinic

2. **Reporting Period (start and end date of grant award period):** July 1, 2012–August 29, 2014

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Jeffrey W. Prichard, D.O.

4. **Grant Contact Person's Telephone Number:** 570-214-6795

5. **Grant SAP Number:** 4100059193

6. **Project Number and Title of Research Project:** 01- Diagnostic-Prognostic Testing in Patients at High Risk for Esophageal Cancer

5. **Start and End Date of Research Project:** July 1, 2012 – August 29, 2014

7. **Name of Principal Investigator for the Research Project:** Jeffrey Prichard, D.O.

8. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 1,002,408.33

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project	Cost
Prichard, Jeffrey	Principal Investigator, Geisinger	10%	$43,436
Diehl, David	Co-Investigator, Geisinger	1%	$3,744
Li, Jinghong	Co-Investigator, Geisinger	5%	$19,078
Barley, Matthew	Data Support Analyst, Geisinger	100%	$111,898
Brown, Adam	Program Director, Geisinger	<1%	$679
Critchley-Thorne, Rebecca	Director, Biomarker and Diagnostics Development-Cernostics	50%	$107,094.40
Campbell, Bruce	Director, Imaging Informatics-Cernostics	50%	$130,292.24
Repa, Kathy	Senior Associate Scientist-Cernostics	25%, Y2	$18,039.36
Falk, Gary	Principal Investigator-Penn	10%	$30,273
DeMarshall, Maureen	Clinical Research Coordinator-Penn	45%	$35,170
Price, Carly	Clinical Research Assistant=Penn	38%	$17,483
Davison, Jon	Assistant Professor- Pitt	10%	$16,795
Foxwell, Tyler	Research Specialist-Pitt	46%	$37,702
Samanthapudi, Keerthana	Student Worker-Pitt	100%	$1,047

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project
Lia Reese	Senior Associate Scientist-Cernostics	50% Yr 2
Mai Nguyen	Senior Associate Scientist-Cernostics	25% Yr 2
Varughese, Alicia Susan	Masters Student Intern at Cernostics	25% Yr 1
Virginia Burger	PhD Student Intern at Cernostics	25% Yr 1

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost

of the equipment.

Type of Scientific Equipment	Value Derived	Cost
Leica Whole Slide Digital Scanners	Scanned 3,500 whole slides mounted with tissue sections from esophageal biopsies	$39,141.46
HP Computers	Data storage and automated image analysis of whole slide digital images	$43,272.33

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No___√_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes____√_____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds awarded:
TissueCypher Testing for Risk Assessment in Barrett's Esophagus	☑NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	April, 2014	$1,185,637	Council review completed, pending funding decision.

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes____√_____ No_____

If yes, please describe your plans:

We plan to apply for NIH grant funding to support additional independent validation studies of the TissueCypher test for risk prediction in Barrett's, such as blinded testing in cohorts of Barrett's patients from institutions not involved in the initial development and validation of the test. We also plan to apply for NIH and Patient-Centered Outcomes Research Institute (PCORI) grant funding to support health economics research to model the potential cost savings/losses, changes in use of care and impact on patient outcomes associated with the use of the TissueCypher test for risk prediction in patients with Barrett's esophagus.

12. Future of Research Project. What are the future plans for this research project?

The future plan is to launch the TissueCypher test as a laboratory-developed test (LDT). Cernostics, Inc will sell the test as a service to gastroenterologists and pathologists. The test will be performed at Cernostics' laboratory that is in the process of being set up to be CLIA-certified. Research on the test will be ongoing to build additional levels of evidence to support clinical adoption of the test. Ongoing research will include evaluation of the test in independent Barrett's esophagus cohorts, clinical utility studies and cost-effectiveness studies.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes___✓_____ No_____

A Masters student (Masters in Biomedical Engineering at Drexel University, Philadelphia, PA) participated in the research for 6 months as an unpaid internship for credit towards her degree. A Graduate student (PhD in Computational Biology at University of Pittsburgh, PA) participated in the research as a summer intern. The students did not receive salary support from the grant.

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				
Female		1	1	
Unknown				
Total		**1**	**1**	

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic		1	1	
Unknown				
Total		**1**	**1**	

	Undergraduate	Masters	Pre-doc	Post-doc
White			1	
Black				
Asian		1		
Other				
Unknown				
Total		**1**	**1**	

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No__√_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes_√_____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

At each of the three academic institutions the pathology databases were mined (in a de-identified format) to develop registries of Barrett's esophagus patients who have been in endoscopic surveillance and have outcome data demonstrating the course of their disease. In addition to being utilized in this research to develop and validate the TissueCypher test, the registries and the characterized cohorts within them will be a valuable resource for additional research in this disease area.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes__√_____ No_____

If yes, please describe the collaborations:

The research project aided in establishing a research collaboration with Dr Jacques Bergman, M.D., PhD. at the Academic Medical Center (AMC), Amsterdam, Netherlands, which is one of the world's leading institutions for research on endoscopic treatment and detection of early neoplasia in Barrett's esophagus. Dr Bergman and colleagues at the AMC have developed a Barrett's registry and database containing 5,000 Barrett's patients at 16 hospitals in the Amsterdam region. The research collaboration enables access to a nested case-control cohort of 500 Barrett's patient samples and de-identified clinical and pathological data as well as expertise from gastroenterologists and academic researchers. A subset of this cohort has been used to further increase diversity of the patient cases in the cohort used to develop and validate the TissueCypher test. The remaining subset will be used as a separate cohort for additional independent validation of the TissueCypher test in future studies.

16(B) Did the research project result in commercial development of any research products?

Yes_____ No__√_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No___√_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

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.

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.

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.

Progress in Achieving Research Goals, Objectives and Aims.

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

Objectives

Specific Aim 1 was achieved during the project. The primary objective was to determine whether a multivariable TissueCypher classifier could stratify patients with Barrett's esophagus according to risk of progression to high grade dysplasia (HGD) or esophageal adenocarcinoma (EAC). The secondary objective was to determine whether the TissueCypher risk classes (associated with risk of progression) add independent prognostic information beyond that of the current standard clinical variables, specifically the pathologist's histologic diagnosis and Barrett's segment length. TissueCypher is a spatial systems biology-based approach to anatomic pathologic testing. The technology 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 biomarkers in the context of tissue morphology. This is coupled to a multivariable classifier to integrate biomarker and morphology data into scores. In Barrett's these scores will be used to predict risk of malignant progression in Barrett's esophagus in individual patients. The progression score will aid in the individualized management of patient with Barrett's esophagus. High risk patients can be treated endoscopically with radiofrequency ablation (RFA) or endoscopic mucosal resection (EMR) to prevent HGD/EAC, whereas low risk patients can potentially reduce their surveillance frequency and avoid unnecessary interventions and costs.

Patient Cohorts for Retrospective Training and Validation Studies

Nested case-control cohorts were constructed to develop and validate a TissueCypher assay to predict risk of malignant progression in patients with Barrett's esophagus. Geisinger, University of Pittsburgh and University of Pennsylvania provided formalin-fixed paraffin-embedded (FFPE)

esophageal specimens and clinicopathological data from 487 patients with Barrett's esophagus during the course of the study. Patient cases were assigned to either the training or independent validation cohort. The multi-institutional training cohort enabled training of multivariate prognostic classifiers on a diverse set of patients, which increases the likelihood that the classifiers will generalize to patient cases outside the training population. To further increase diversity patient cases from Academic Medical Center (AMC), Amsterdam, Netherlands, have been included. The use of AMC's patient biospecimens and de-identified patient data for this study was approved by AMC's IRB. Cernostics has a separate collaboration (funded by Cernostics) with Jacques Bergman, M.D., Ph.D., at AMC to utilize Barrett's cohorts for validation studies of Cernostics' tests. The case-control cohorts are summarized in Table 1. The total number of patient cases acquired by each institution for the study with funding from the research grant is summarized in Table 2. 223 patients were assigned to the cohort, including 80 progressor patients (cases) and 142 non-progressor patients (controls).

Each institution has provided de-identified clinical and pathological data for each patient case included in the study. The data elements include the following: patient key, case key, case collection date (time-shifted for de-identification), original diagnosis, expert review diagnosis, outcome (progressor/non-progressor), progression endpoint diagnosis, high grade dysplasia (HGD)/ esophageal adenocarcinoma (EAC)-free surveillance time, total surveillance time, time-shifted date and diagnosis of every surveillance biopsy, age, gender, race, Barrett's segment length (cm), Barrett's segment length class (short/long), hiatal hernia. Patient metadata is summarized in Table 3.

Development and Evaluation of Multivariable Prognostic Classification Algorithm

TissueCypher Data Generation: Cernostics performed its 15-marker TissueCypher assay on Barrett's biopsies from a total of 416 patients during the study. 223 patients were assigned to the training cohort and 193 to the validation cohort (see Table 1). Data from the validation cohort was quarantined for later use in independent validation. Sections from the 233 biopsies from 223 unique patients were labeled with Cernostics' assay and imaged by whole slide 4-channel fluorescence scanning at 20x magnification, resulting in 1,631 whole slide images for analysis. Slides were analyzed using Cernostics' TissueCypher image analysis software. 1,970 image analysis features were extracted from the 15 biomarkers, including morphology. Each feature was summarized as multiple measures, resulting in 26,939 feature/measures per patient case. The feature/measure data from biopsies in the training cohort was transferred to Yi Zhang, Ph.D. (consultant biostatistician).

Statistical Analyses: The goal of the statistical analyses performed by Dr Zhang was to build multivariable prognostic classifiers based on a subset of the 26,939 feature/measures that can predict risk of progression to HGD/EAC in individual Barrett's patients. There were 2 patient risk groups in the training cohort: 1. Progressors, i.e. patients who progressed from no dysplasia (ND), indefinite for dysplasia (IND) or low grade dysplasia (LGD) to HGD or EAC, and 2. Non-Progressors (patients who did not progress to HGD/EAC). Two feature selection methods were evaluated: 1. Univariate Conditional Logistic Regression (CLR) with all 26,939 feature/measures in progressors vs non-progressors (using 84 case-control sets); 2. Univariate Cox Proportional Hazard (Cox) with all 26,939 feature/measures in progressors vs non-

progressors (using progressor group as the event, and non-progressors as censoring). The univariate results from both the CLR and Cox were reviewed and two subsets of 50 feature/measures (CLR-selected set and a Cox-selected set) were manually selected based on the performance in the univariate ranking (AUC and p-value) and on ability to capture known mechanisms of neoplastic progression while minimizing feature redundancy.

The top 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 features/measures for the CLR-selected set and the Cox-selected set were combined into prognostic classifiers by multivariate Cox model. Survival time was defined as the time between the tested and the diagnosis of HGD/ EAC for progressors or the last HGD/EAC-free follow-up for non-progressors. Leave-one-out cross validation was performed by setting 1 testing biopsy aside and using all other biopsies as the training set to select features/measures. The final prediction model was built using the Cox model that uses the selected features/measures on the training set of patients, then this model was applied on the 1 testing biopsy to calculate the probability of remaining high risk free at 2, 3, 4, and 5 years. This process was repeated until each biopsy was treated as the testing biopsy once. At the end of this process, each biopsy had a calculated probability of remaining HGD/EAC-free at 2, 3, 4, and 5 years. C-indices were calculated and Receiver Operating Characteristic (ROC) curves were plotted. Kaplan-Meier curves were plotted with 2 probability cutoffs. Area under the ROC curve (AUROC), C-indices, hazard ratios, positive predictive values (PPV) and negative predictive values (NPV) were used to select the top performing classifier from the CLR-selected features (classifier 1) and from the Cox-selected features (classifier 2) (see Table 4). The probability cutoffs were optimized to maximize NPV, PPV and hazard ratios for each classifier. Both classifiers stratified Barrett's patients into low, intermediate and high risk groups, identified progressor patients who are missed by the current standard pathology and showed similar performance across the 4 institutions. Results from the two prognostic classifiers are shown in Figures 1 and 2.

TissueCypher Classifier 1: Classifier 1 is based on 30 image analysis feature/measures derived from 12 biomarkers and nuclear morphology. The optimal probability cutoffs were 0.78 (low-int risk), 0.32 (int-high risk). Classifier 1 predicts probability of remaining HGD/EAC-free at 5 years with AUROC of 0.875 and hazard ratios of 6.3 (intermediate vs. low risk) and 21.9 (high vs. low risk), p value <0.0001 (Figure 1A-B). The PPV and NPV were 0.911 and 0.903, respectively, i.e. 91.1% of the high risk group are progressors and 90.3% of the low risk group are non-progressors. Classifier 1 showed similar performance across the diagnostic categories (Figure 1C, D, E) and across the four institutions with AUROC 0.923, 0.781, 0.875 and 0.836 for 5 year prediction in patients from Geisinger, University of Pennsylvania, University of Pittsburgh and AMC, Netherlands, respectively. In multivariate Cox models in which progression to HGD/EAC was evaluated in relation to the classifier 1 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 and the Barrett's segment length (Figure 1F-G). Progressor patients constitute ~30% of the training cohort, however, the progression rate in the general population of patients esophagus is very low. Published estimates of progression to HGD/EAC in patients with Barrett's no dysplasia or low grade dysplasia [1, 2] were used to estimate HGD/EAC prevalence at 4.25% at 5 years. Prevalence adjusted NPV and PPV for the TissueCypher test were 0.991 and 0.45. Prevalence adjusted estimates of patients receiving low, intermediate and high risk TissueCypher scores if the test were performed in the general US Barrett's population were 65.2%, 30.2%, 4.6%, respectively.

Classifier 2: Classifier 2 is based on 40 image analysis feature/measures derived from 8 biomarkers and nuclear morphology. Classifier 2 predicts probability of remaining HGD/EAC-free at 5 years with AUROC of 0.83 and hazard ratios of 3.7 (intermediate vs. low risk) and 17.4 (high vs. low risk), p value <0.0001 (Figure 2 A-B). The PPV and NPV were 0.803 and 0.909, respectively. Classifier 2 showed similar performance across the diagnostic categories (Figure 2C, D, E). Multivariate Cox analysis of Classifier 2 versus the pathologist's diagnosis and Barrett's segment length showed that Classifier 2 adds independent prognostic information and has stronger prognostic power than either diagnosis or Barrett's segment length (Figure 2F-G).

Evaluation of TissueCypher to Aid Diagnosis of Barrett's Esophagus: 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 also 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 3A-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 (Fig3ure D-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+) (Figure 3D-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 (Figure 3H-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 esophagus 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 the study. The primary objective was to evaluate the performance of a pre-specified classifier to predict risk of progression to HGD/EAC in an independent, multi-institutional cohort of patients with Barrett's esophagus.

In the training phase of the study (described above in specific aim 1), classifier 1 demonstrated the highest performance in predicting risk of malignant progression in Barrett's patients. The

prospectively defined classifier 1 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 1. 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 4A-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 4C) and the Barrett's segment length (Figure 4D). The results demonstrated that the TissueCypher classifier outperformed the standard clinical variables and passed independent validation.

References

1. Wani S, Falk G, Hall M, et al. Patients with nondysplastic Barrett's esophagus have low risks for developing dysplasia or esophageal adenocarcinoma. Clin Gastroenterol Hepatol 2011;9:220-7; quiz e26.
2. Wani S, Falk GW, Post J, et al. Risk factors for progression of low-grade dysplasia in patients with Barrett's esophagus. Gastroenterology;141:1179-86, 86 e1.

Table 1. 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

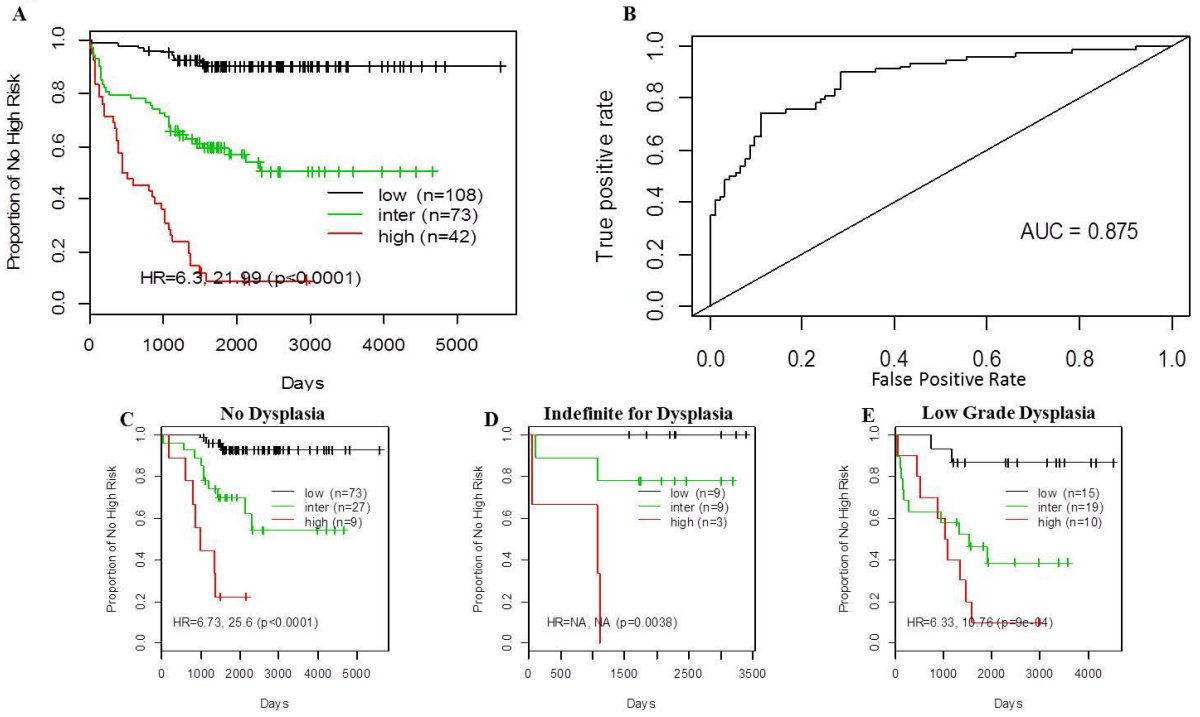
UPitt: University of Pittsburgh, UPenn: University of Pennsylvania, AMC: Academic Medical Center, Netherlands.

Table 2. Summary of Barrett's Cohorts from Each Clinical Institution (total acquired during research project)				
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 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

Table 4. Statistical Methods and Biomarker/Morphology Features used in the Development of TissueCypher Classifiers 1 and 2		
	TissueCypher Classifier 1	**TissueCypher Classifier 2**
Feature Selection	Univariate Conditional Logistic Regression	Univariate Cox Proportional Hazard
Model Building & Prediction	Cox Proportional Hazard	Cox Proportional Hazard
Probability Cutoffs in 3-Tier Classification	0.78 (low-int), 0.32 (int-high)	0.89 (low-int), 0.38 (int-high)
# Feature/measures	30	40
# Biomarkers	12	8
Biomarkers	p53, p16, HER2, CK-20, Ki-67, NF-kappaB, HIF1alpha, CD45RO, Beta-catenin, COX2, CD68, AMACR (plus morphology)	p53, HER2, CK-20, Ki-67, HIF1alpha, CD45RO, COX2, CD68 (plus morphology)

Figure 1.



F

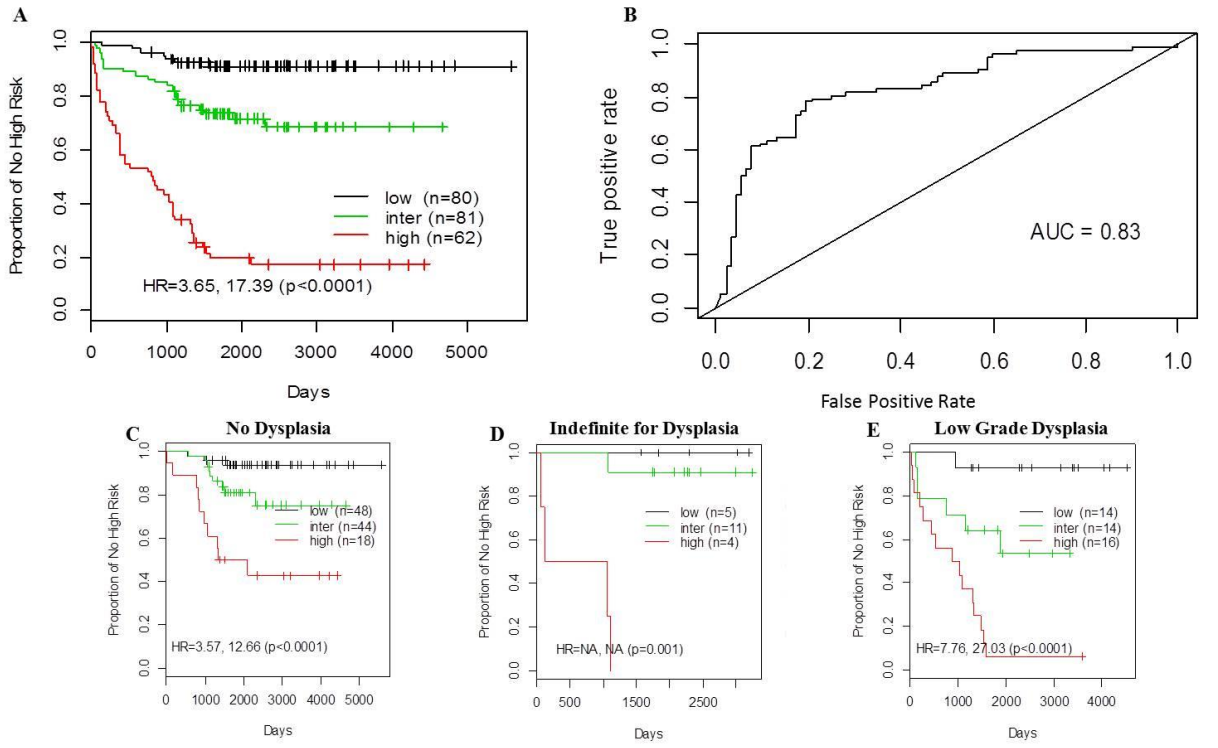
Multivarate Cox Results - Analysis of Classifier 1 vs General Pathologist's Dx		
	HR (95% CI)	**P value**
General Pathologist Dx		0.24
IND vs ND+RA	0.83 (0.31 – 2.22)	0.72
LGD vs ND+RA	1.58 (0.86 – 2.92)	0.14
TissueCypher Classifier 1		3.2×10^{-12}
Int vs Low Risk	7.20 (3.05– 16.98)	6.6×10^{-6}
High vs Low Risk	20.11 (8.08– 50.05)	1.1×10^{-10}

G

Multivarate Cox Results - Analysis of Classifier 1 vs Barrett's segment length		
	HR (95% CI)	**P value**
Barrett's Segment Length		
Long (>3cm) vs Short (≤3cm	1.06 (0.66 – 1.71)	0.81
TissueCypher Classifier 1		$<1.0 \times 10^{-16}$
Int vs Low Risk	6.60 (3.12– 13.98)	8.1×10^{-7}
High vs Low Risk	27.58 (12.96 – 58.66)	$<1.0 \times 10^{-16}$

Figure 1. Performance of Classifier 1 in Barrett's Training Cohort. A: KM survival curve for classifier 1 with 2 probability cutoffs (0.78 and 0.32) demonstrating stratification of Barrett's patients into low, intermediate and high risk groups. B: ROC; C, D &E: KM curves showing performance in cases with diagnosis of Barrett's no dysplasia, Barrett's indefinite for dysplasia, Barrett's low grade dysplasia, respectively; F. Results from multivariate Cox analysis of Classifier 1 vs. General Pathologist Dx, N= 182 patients with general pathologist review (note than a subset of UPenn , UPitt and GML have only expert GI pathologist Dx available); G. Results from multivariate Cox analysis of Classifier 1 vs. Barrett's segment length, N = 217 patients from all 4 institutions (note that 16 patients had unknown Barrett's segment class were not excluded).

Figure 2.



F

Multivarate Cox Results - Analysis of Classifier 2 vs General Pathologist's Dx		
	HR (95% CI)	**P value**
General Pathologist Dx		0.025
IND vs ND+RA	1.41 (0.53 – 3.78)	0.50
LGD vs ND+RA	2.34 (1.28–4.27)	0.006
TissueCypher Classifier 2 F40		2.2×10^{-12}
Int vs Low	4.36 (1.45– 13.10)	0.009
High vs Low	20.11 (6.99– 57.81)	2.6×10^{-8}

G

Multivarate Cox Results - Analysis of Classifier 2 vs Barrett's segment length		
	HR (95% CI)	**P value**
Barrett's Segment Length		
Long (>3cm) vs Short (≤3cm	1.41 (0.87 – 2.26)	0.16
TissueCypher Classifier 2 F40		$<1.0 \times 10^{-16}$
Int vs Low Risk	3.81 (1.54– 9.45)	0.003
High vs Low Risk	20.29 (8.62 – 47.76)	5.5×10^{-12}

Figure 2. Performance of Classifier 2 in Barrett's Training Cohort. A: KM survival curve for classifier 2 with 2 probability cutoffs (0.89 and 0.38) demonstrating stratification of Barrett's patients into low, intermediate and high risk groups. B: ROC curve; C, D &E: KM curves showing performance in cases with diagnosis of Barrett's no dysplasia, Barrett's indefinite for dysplasia, Barrett's low grade dysplasia, respectively. F. Results from multivariate Cox analysis of Classifier 2 vs. General Pathologist Dx, N= 182 patients with general pathologist review (note than a subset of UPenn , UPitt and GML have only expert GI pathologist Dx available); G. Results from multivariate Cox analysis of Classifier 2 vs. Barrett's segment length, N = 217 patients from all 4 institutions (note that 16 patients had unknown Barrett's segment class were not excluded).

Figure 3.

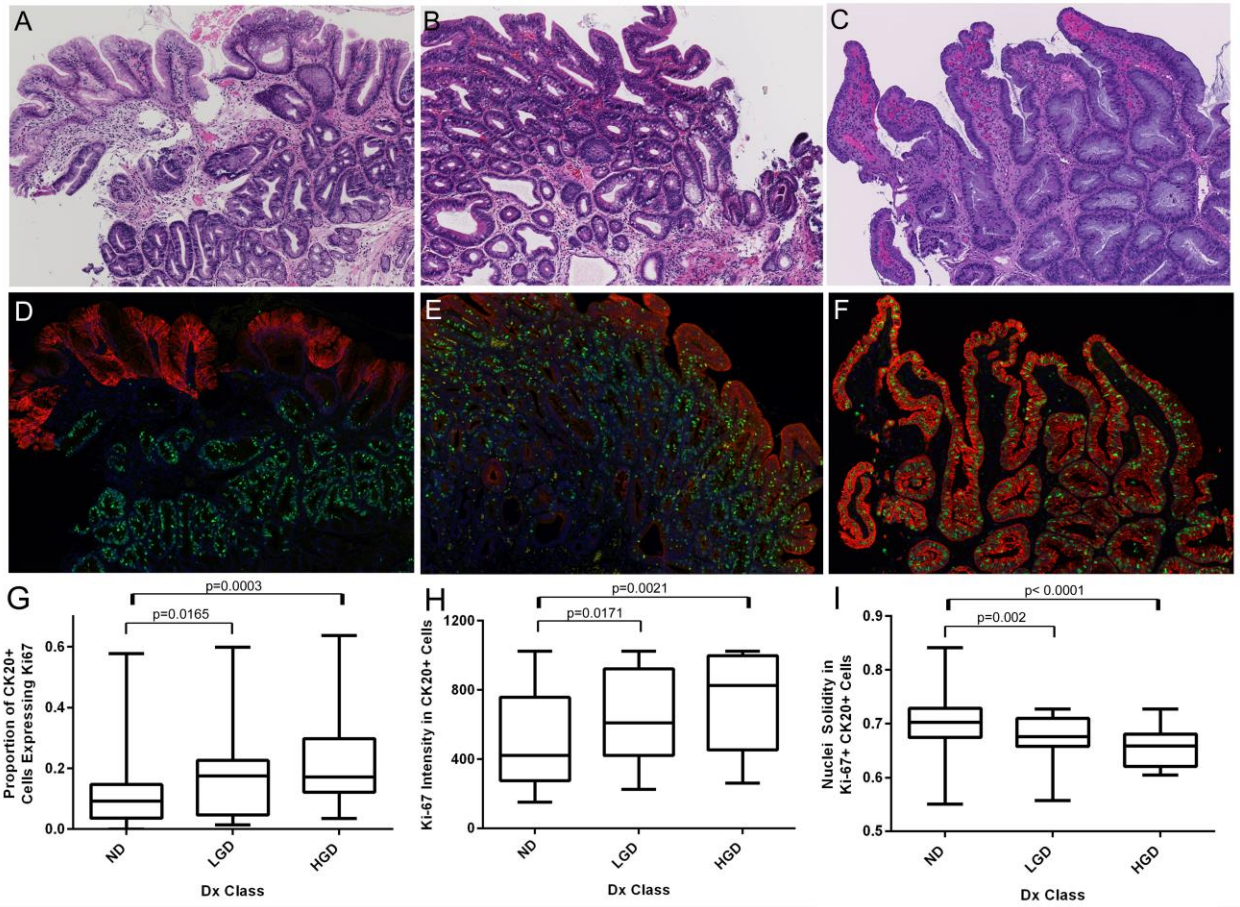
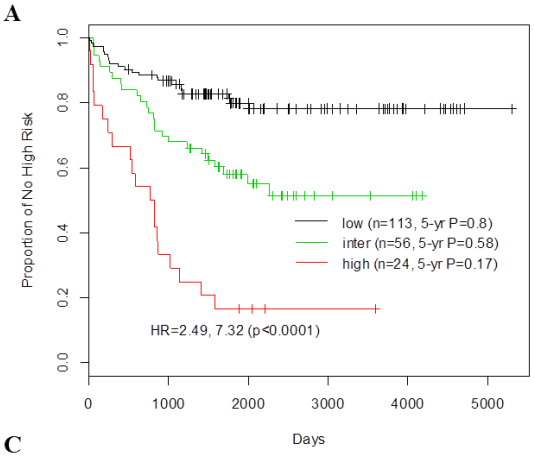


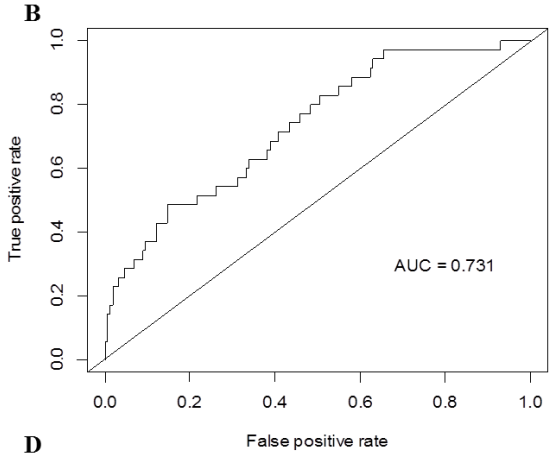
Figure 3. Hematoxylin and Eosin staining (A-C) and TissueCypher labeling of Hoechst (blue), CK-20 (red) and Ki-67 (green) (D-F) in sections of Barrett's biopsies with no dysplasia (ND), LGD, HGD, respectively. Box and whisker plots showing quantitative image analysis features in Barrett's biopsies with 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 4.

A



B



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 4. 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.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__√___No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
_√_____No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
___√__ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. None				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes___√_____ No_____

If yes, please describe your plans:

The following manuscripts are in preparation:

1. Draft title: A Tissue Systems Pathology Approach Predicts Risk of Malignant Progression in Patients with Early Barrett's Esophagus: A Multi-Center Validation Study. This manuscript describes the development of the TissueCypher test (i.e. the case-control training study to select the optimal prognostic classifier to predict risk of progression to HGD/EAC in Barrett's patients) and independent validation of the TissueCypher test (i.e. evaluation of the performance of the pre-specified test in an independent validation cohort of Barrett's patients). An abstract on this study will be submitted in December 2014 for presentation at Digestive Diseases Week in May 2015.
2. Draft title: Quantitative Multiplexed Biomarker and Morphology Analysis to Aid Diagnosis of Dysplasia in Barrett's Esophagus. This manuscript describes the use of TissueCypher software to extract quantitative biomarker and morphology measurements from whole slide images of Barrett's esophagus biopsies and the diagnostic performance of such measurements in distinguishing between non-dysplastic, low grade dysplasia and high grade dysplasia in Barrett's esophagus. An abstract on this study was submitted in October 2014 for presentation at the USCAP annual meeting in March 2015.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project.

Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

The TissueCypher test is still at the pre-commercialization stage, however, the research project demonstrated that the test can identify Barrett's patients who are at high risk of malignant progression who would be candidates for therapeutic interventions such as radiofrequency ablation and/or endoscopic mucosal resection to prevent cancer development. The test also identifies patients who are at very low risk of malignant progression and could

potentially reduce their endoscopic surveillance frequency. Once commercialized the test could be used to provide individualized risk prediction to patients who are undergoing endoscopic surveillance for Barrett's esophagus. Patients identified as high risk could be treated early, which would impact patient outcomes by reducing the incidence of esophageal cancer. Patients identified as low risk could extend their surveillance intervals to 5 years, which would result in cost savings to the healthcare system and would also reduce patient anxiety about developing esophageal cancer. A cost-effectiveness study has been performed in parallel with the research project. In collaboration with health economics investigators at Geisinger Health System, a Markov model has been built that evaluates the cost savings/losses, use of care/procedures and patient outcomes in 10,000 patients with Barrett's who follow the standard of care and 10,000 patients with Barrett's who take the TissueCypher test and use the risk score to determine surveillance intervals and therapeutic interventions. Preliminary results indicate that the TissueCypher test will cost less and be more effective than the standard of care by reducing the number of endoscopies in the vast majority of low risk patients and by preventing cancer development in the subset of patients at high risk for malignant progression.

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

Despite extensive screening programs aimed at preventing HGD/EAC in patients with Barrett's, the incidence of this cancer continues to rapidly increase and survival rates remain extremely poor. The vast majority of patients with Barrett's will not develop HGD/EAC yet they unnecessarily undergo frequent endoscopies with biopsies and experience severe anxiety about developing EAC. Furthermore, most of the patients who do progress to HGD/EAC are missed by the current surveillance paradigm. The research project that was undertaken addressed this clinical need by developing and independently validating a novel prognostic test termed TissueCypher to predict risk of malignant progression in individual patients with Barrett's esophagus. The test added independent prognostic information beyond that provided by the pathologist's diagnosis and the Barrett's segment length, which are the current clinical variables used to assess stage and risk in Barrett's esophagus. Cernostics will commercialize the test as a laboratory-developed test (LDT) offered via a CLIA-certified lab. The test would be available to both gastroenterologists and pathologists to order. The test will be an addition to, not a replacement to, the analysis of H&E-stained Barrett's esophagus biopsy slides by pathologists. Ordering physicians will send Barrett's biopsies (whole blocks or unstained sections) to Cernostics where the test will be performed. Cernostics will provide a report back to the physicians with a progression score and risk class. Ordering physicians can use the risk score in the context of all the other information they have on each patient to determine the appropriate frequency of endoscopic surveillance and whether therapeutic interventions such as RFA or EMR are indicated to prevent HGD/EAC. Once commercialized the TissueCypher test has the potential to shift the current clinical paradigm

from mass surveillance for all Barrett's patients, to targeted surveillance and early preventative interventions for high risk patients, and minimized surveillance for low risk patients. The progression rate to HGD/EAC is only 0.5% per year, therefore, intensive surveillance and treatments need only be targeted to a very small subset of patients to reduce the incidence of HGD/EAC. There are safe and effective treatments, particularly RFA and EMR, which can be used to eradicate Barrett's in high risk patients. This new paradigm of targeted screening and interventions based on accurate risk assessment will not only improve outcomes for high risk patients but will also be cost effective due to reduced surveillance frequencies in low risk patients, who constitute the vast majority of Barrett's patients.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___√___

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No___

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No___
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No___

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes____√_____ No_____

If yes, please describe your plans:

Cernostics will commercialize the TissueCypher Barrett's assay as a laboratory-developed test offered via a CLIA-certified laboratory. Cernostics currently owns three issued patents, two in the US and one in Japan, covering the company's foundational technology, the TissueCypher technology platform. The scope of claims in these patents relate to the company's ability to evaluate tumor, immune, and stromal biomarkers simultaneously on a single slice of tissue within the discipline of anatomic pathology and tissue diagnostics. Additionally Cernostics filed a global PCT patent application with priority date March 17, 2011 that covers compositions of matter of the TissueCypher Barrett's test and methods for completing the test using our foundational technology. This patent application entered examination in February 2014.

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

Biosketches for all key investigators were included in the original grant application.