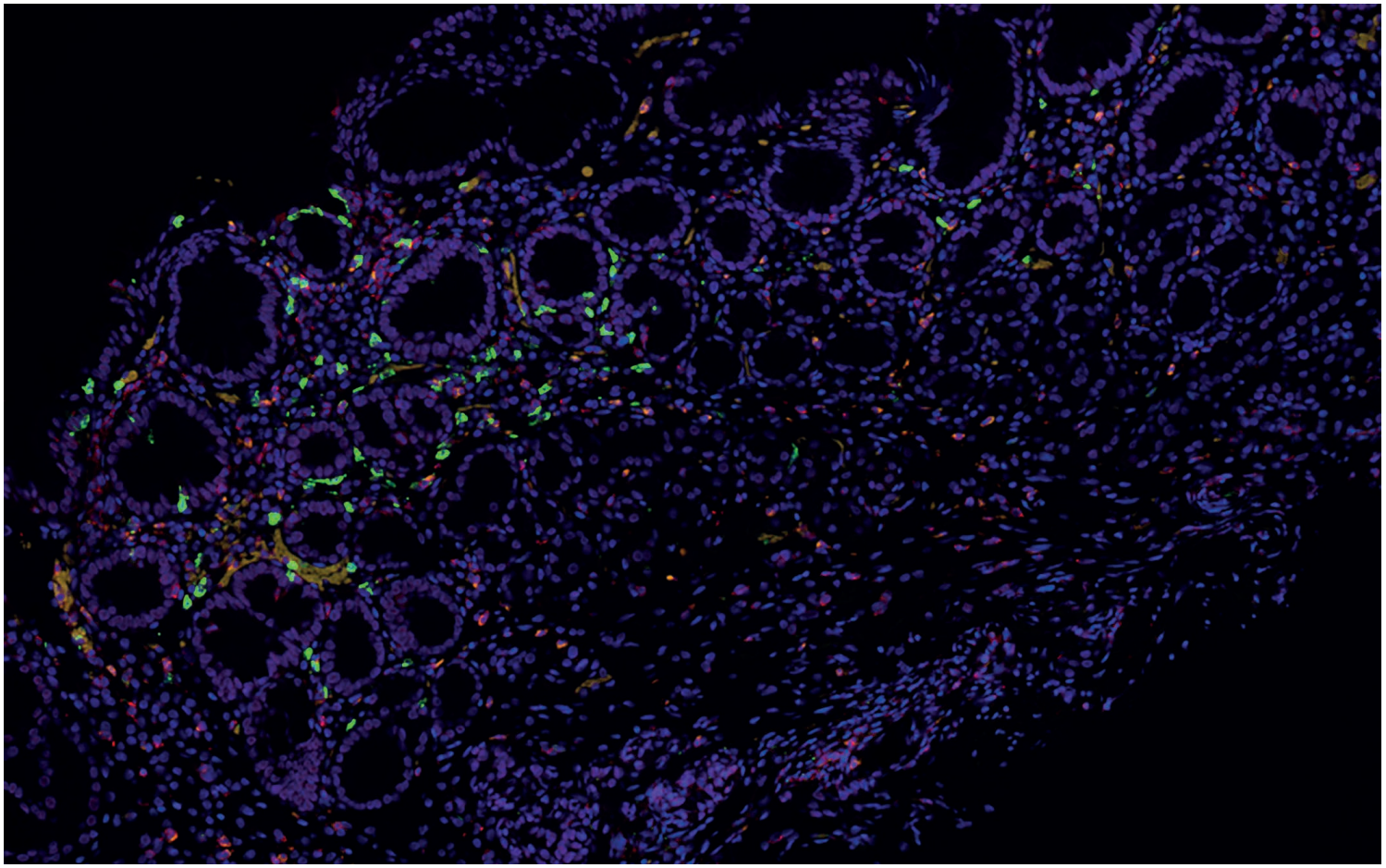


Spatialomics delivered to the clinic for improved health outcomes in Barrett's esophagus

By Craig Munroe, Mary A. Hall, and Rebecca J. Critchley-Thorne,
Castle Biosciences, Inc., 235 William Pitt Way, Pittsburgh, PA 15238

Introduction and Background: Spatialomics and the TissueCypher Approach to Barrett's Esophagus

Emerging spatialomics techniques in cancer biology are enabling the spatial context of cells and tissue systems to be preserved while investigating the morphology; multiple cellular measures; expression of multiple biomarkers; and spatial relationships within various cell populations and tissues.[1–3] Complex molecular and cellular signatures that are characteristic of normal and pathologic states can be analyzed in the context of cellular and tissue architecture to obtain a better understanding of hallmarks of disease and progression, as well as the heterogeneity of the tissue microenvironment.

In recent years, there has been a dramatic increase in research utilizing highly multiplexed fluorescence imaging and artificial intelligence (AI)-driven platforms to analyze digital slides of patient specimens.[1–3] Few of these approaches,

however, have made it into clinical practice. Nevertheless, with on-going efforts and the clinical relevance of spatialomics research, novel analytical approaches are expected to help drive future development of more advanced diagnostics and effective treatment strategies in cancer, which may even extend to other diseases and various therapeutic areas. Ultimately, the goal of emerging spatialomics techniques in cancer, including the approach described herein, is to move towards a more personalized approach for management of patients with cancer and preneoplastic conditions.[1,2]

The TissueCypher Test and Platform

The TissueCypher® Barrett's Esophagus Test (TissueCypher, Castle Biosciences, Inc., Pittsburgh, PA) is a tissue systems pathology (TSP-9) test that utilizes a spatialomics-based approach to risk-stratify patients with Barrett's esophagus (BE), which is the only known precursor to esophageal adenocarcinoma (EAC).[4,5] The TissueCypher test objectively identifies patients with BE at high risk for progression to EAC, and who may benefit either from therapeutic intervention to prevent EAC or close surveillance to detect actionable neoplasia at early, treatable stages. This aligns with better health outcomes for high-risk BE patients, as effective therapies are available to prevent progression and treat early EAC.[6–9] TissueCypher also identifies low-risk patients who can safely avoid unnecessary treatment and be effectively managed by long-interval surveillance without therapeutic intervention.

The TissueCypher test for BE patients utilizes a computational pathology platform to rapidly analyze multiple tissue system biomarkers, including nuclear morphology in whole-slide images of formalin-fixed paraffin-embedded (FFPE) tissue biopsies collected from patients during endoscopy.[10] The TissueCypher computational pathology software first identifies tissue fragments within the slide image, and then cellular and sub-cellular objects within the tissue structures.

Finally, the technology extracts high-dimensional feature data from biomarkers within the detected objects and structures. This approach enables automated quantification of key tissue system biomarkers in the spatial context of cellular and tissue architecture. The test quantifies nine protein-based biomarkers (p16, alpha-methylacyl-CoA-racemase (AMACR), p53, HER2/neu, cytokeratin-20 (K20), CD68, cyclo-oxygenase-2 (COX-2), CD45RO, and hypoxia-inducible factor 1-alpha (HIF-1a)) in order to evaluate multiple molecular and cellular indicators of malignant progression.

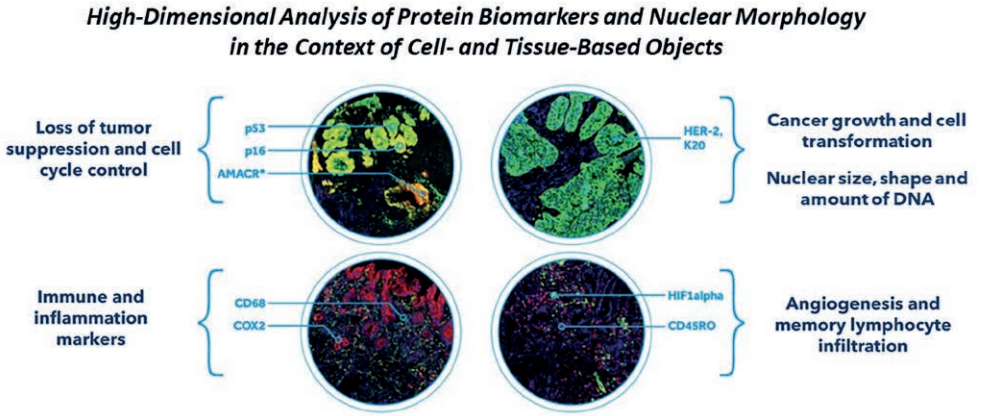


Figure 1: TissueCypher software performs automated, high-dimensional analysis of protein expression of nine protein-based biomarkers and nuclear morphology in the context of cell- and tissue structure-based objects (spatialomics) to objectively quantify 15 features from endoscopic biopsies. The nine biomarkers evaluated (p53, p16, AMACR, HER-2, K20, CD68, COX2, HIF-1α, and CD45RO) provide information about multiple pathways of potential neoplastic progression (i.e., molecular changes involved in tumor suppression, cell cycle control, cancer growth, and cell transformation; expression of immune system, inflammatory, and angiogenesis markers).

"TissueCypher (TSP-9) is the first and only commercially available precision medicine test that is indicated for patients with BE."

This includes loss of tumor suppression, loss of cell cycle control, stromal angiogenesis, and inflammation (**Figure 1**).

Nuclei are labeled using Hoechst to enable quantification of nuclear morphology and DNA content. The protein-based biomarkers and nuclei are labeled in sections from FFPE biopsy blocks using automated multiplexed immunofluorescence labeling. Labeled slides are imaged to produce whole-slide fluorescence images that are analyzed by the TissueCypher computational pathology software. Quantitative feature data from evaluation of the nine protein-based biomarkers and nuclear morphology are integrated to produce a risk score that ranges from 0-10, and a risk class of low, intermediate, or high risk for development of high-grade dysplasia (HGD) or EAC within 5 years (**Figure 2**).[11]

Barrett's Esophagus and Risk of Malignant Progression

Development of BE occurs when normal stratified squamous epithelium in the distal esophagus is replaced with columnar-lined intestinal epithelium due to long-term exposure of the esophageal lining to stomach acid.[4,5] Patients with BE can progress from non-dysplastic Barrett's esophagus (NDBE) to low-grade dysplasia (LGD), high-grade dysplasia (HGD), intramucosal carcinoma (IMC), and invasive EAC.[4,5,12,13]

Over 90% of BE patients are diagnosed with

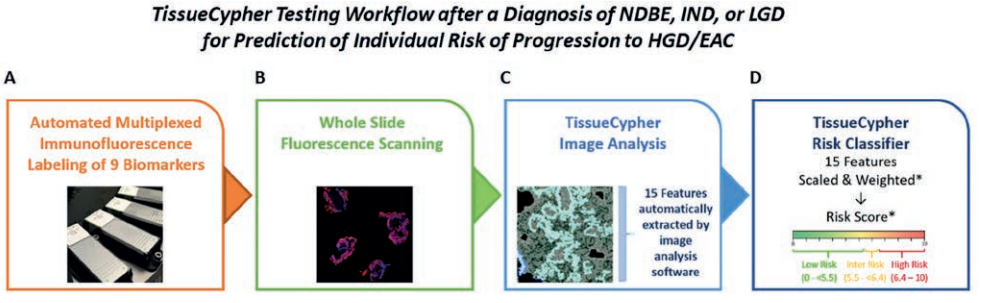


Figure 2: TissueCypher testing workflow (adapted from Frei et al., 2020[11]). The tissue systems pathology (TSP-9) test known as TissueCypher uses a fully automated, spatialomics-based platform to predict risk of progression to HGD/EAC, as well as missed prevalent HGD/EAC in patients with BE. Following a diagnosis of NDBE, IND, or LGD and an order for TissueCypher, **A)** sections from FFPE biopsy blocks undergo multiplexed immunofluorescence labeling, **B)** which is followed by whole-slide fluorescence scanning, and **C)** extraction of 15 quantitative features by automated image analysis software. **D)** A risk score based on analysis of the 15 features, scaled and weighted, is derived via the software and corresponds to risk of progression to HGD/EAC within five years of initial diagnosis. A score of 0 to <5.5 corresponds to low risk, 5.5 to <6.4 corresponds to intermediate risk, and a score of 6.4 to 10 indicates the patient has a high risk for progression.

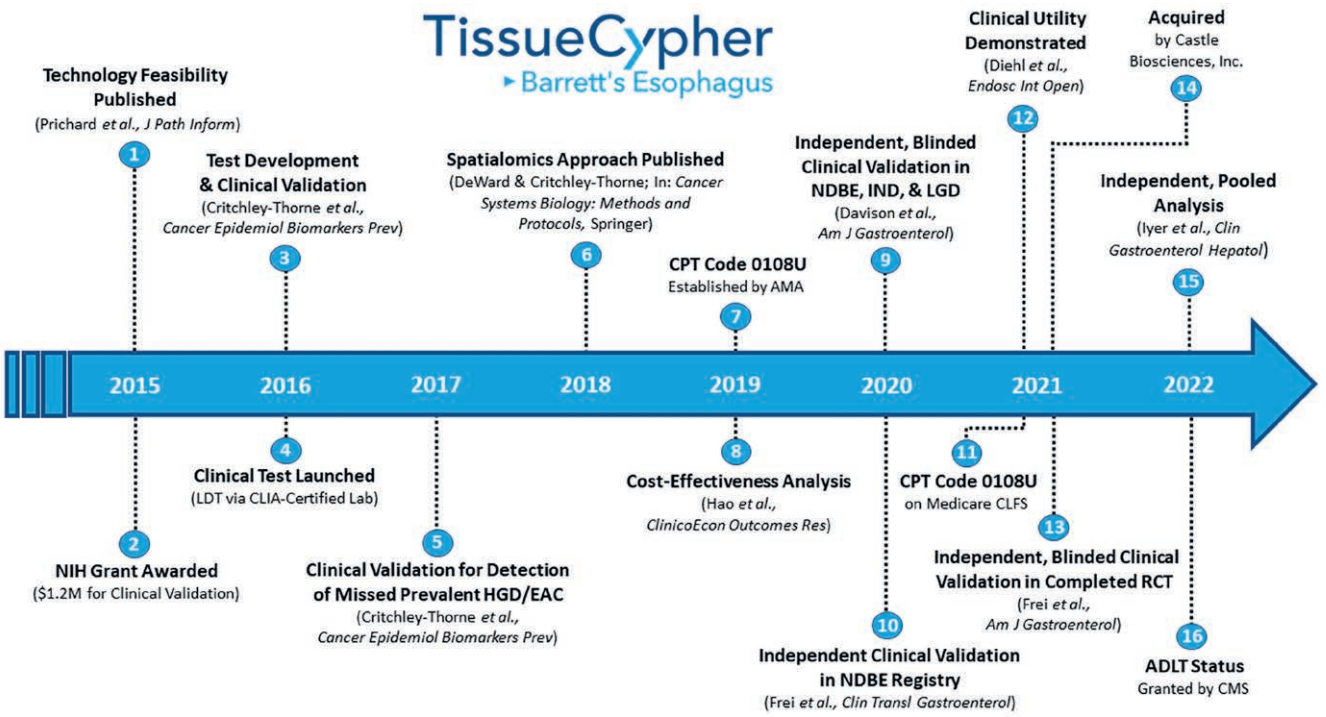


Figure 3: Major milestones for TissueCypher, including technology development, test development and validation, clinical test launch, acquisition by Castle Biosciences, Inc., and granting of Advanced Diagnostic Laboratory Test (ADLT) status. NIH, National Institutes of Health; LDT, Laboratory-Developed Test; CLIA, Clinical Laboratory Improvement Amendments; HGD/EAC, high-grade dysplasia/esophageal adenocarcinoma; CPT, Current Procedural Terminology; AMA, American Medical Association; NDBE, non-dysplastic Barrett's esophagus; IND, indefinite for dysplasia; LGD, low-grade dysplasia; CLFS, Clinical Laboratory Fee Schedule; RCT, randomized controlled trial; CMS, Centers for Medicare and Medicaid Services.

NDBE, which is highly heterogeneous at the genomic level and has been described to have a low rate of progression to HGD/EAC (approximately 0.63%/year); 3-5% of BE patients are diagnosed as indefinite for dysplasia (IND) or LGD, which is associated with rates of progression to HGD/EAC of 1.5-1.7%/year.[4,14–20] A diagnosis of IND may be given when abnormalities observed in biopsied epithelial tissue cannot be distinguished from atypia due to inflammation or are insufficient for a diagnosis of dysplasia.[16] Although the overall risk of progression from NDBE, IND, and LGD to HGD/EAC is relatively low in BE, these patients have >10-times the risk of developing EAC relative to the general population.[18,21,22] HGD is detected in a smaller subset (1-2%) of patients with BE at initial diagnosis and can progress to EAC at a rate of >10%/year.[15,23,24]

Gastroenterology and gastrointestinal (GI) societies have established guidelines for management of patients with BE, including endoscopic surveillance with biopsies that are evaluated by pathologists, with the goal of detecting and treating dysplasia and EAC at the earliest possible stages.[4,6,12,25] However, traditional standard-of-care practices have limitations that impede accurate risk stratification and, thus, optimum management of patients with BE.

TissueCypher Risk Stratification of BE Patients with NDBE, IND, or LGD

TissueCypher (TSP-9) is the first and only commercially available precision medicine test that is indicated for patients with BE. The test is intended for BE patients with NDBE, IND, or LGD, and was designed and developed to predict risk of developing HGD/EAC within 5 years.[10,11,26–29] Identifying patients with BE who have a high risk for progression to HGD/EAC is critically important, as these patients can benefit from endoscopic eradication therapy (EET), which is minimally invasive, safe, and highly effective at preventing progression in patients with BE. Patients at high risk for progression can also benefit from short-interval surveillance to detect the onset of advanced dysplasia and EAC at early stages where EET can be applied, helping patients to avoid esophagectomy procedures or need for systemic chemotherapy or radiation therapy due to extra-esophageal disease. There is also a clinical need to identify patients who have a low risk for progression to HGD/EAC, as these patients may safely avoid unnecessary and costly procedures.

TissueCypher has a history of being extensively validated (see Timeline in **Figure 3**) in international, multi-site studies, and has consistently demonstrated robust performance and improvements in risk stratification of patients with BE relative to clinical variables and histopathology.[11,26–29] In addition, TissueCypher has demonstrated clinical utility for impacting disease management decisions. Based on a personalized risk-stratification score from TissueCypher, 55% of a cohort of patients diagnosed with BE had risk-aligned escalation or de-escalation of management approaches within current guidelines.[30] Incorporation of TissueCypher with traditional pathology approaches in the clinic refines risk prediction in BE, which is imperative for more risk-aligned management and improved patient health outcomes.

Benefits of the TissueCypher Technology Approach

The development of TissueCypher was aimed at addressing several limitations of surveillance programs for patients with BE. Within society guidelines, clinicians primarily use the level of dysplasia in endoscopic biopsies to guide decisions on BE patient management, including frequency of surveillance and use of EET (such as radiofrequency ablation and cryoablation).[17] Pathology review of endoscopic biopsies, however, has significant limitations, such as observer variability and subjectivity.[4,31,32] These limitations

can lead to over-diagnosis of LGD in BE. In fact, studies have shown that 73-85% of initial diagnoses of LGD are down-staged to IND or NDBE following secondary pathology review.[31,33–36] Also, under-staging can occur due to random sampling during biopsy, which can provide a misrepresentation of the tissue microenvironment in BE.[4,31,37] More accurate prediction of risk for progression to HGD/EAC in patients with BE is necessary to prevent overuse of endoscopic surveillance and unnecessary treatment in patients at low risk of progression as well as to provide timely, risk-appropriate management strategies for patients at high risk for progression to HGD/EAC.

TissueCypher technology evaluates protein expression levels of multiple biomarkers in pinch biopsies taken during standard upper endoscopy procedures by extracting and integrating quantitative molecular and cellular expression data in the context of spatial relationships within the tissue system. This provides for improved objectivity for the evaluation of risk for malignant progression in BE patients.

BE develops in a background of chronic inflammation (driven by bile and acid reflux), which is an early driver of malignant progression. Measurement of epithelial and stromal changes by TissueCypher enables a more comprehensive evaluation of risk by quantifying both genetic and non-genetic heterogeneity, including inflammatory drivers of EAC development. By analyzing biomarkers that identify molecular and cellular changes during progression to HGD/EAC, TissueCypher can objectively detect indicators of progression that may precede morphologic changes that can be observed by traditional pathology review and reduce the impact of variable pathology review on management decisions for patients with BE.

Development and Validation of the TissueCypher Barrett's Esophagus Test

The 15-feature risk prediction algorithm that underlies the TissueCypher® Barrett's Esophagus test was developed in a training cohort (n=183) and then tested in a separate validation cohort (n=183) of patients with BE with known outcome data. The training and validation cohorts included patients with outcome data demonstrating progression to HGD/EAC at least one year later (incident progressors) and patients with outcome data showing no disease progression (non-progressors).[26] In this initial clinical validation study by Critchley-Thorne et al. (2016), TissueCypher predicted future progression from NDBE, IND, and LGD to HGD/EAC within 5 years, independent of clinicopathologic variables.[26]

The test provided more accurate risk stratification than age, sex, segment length, expert pathology diagnosis, and use of p53 as a single biomarker. TissueCypher identified a high-risk group of patients who were at a significantly increased risk (>9-times greater risk; $p<0.0001$) of developing HGD/EAC when compared with patients identified as low risk for progression. The study demonstrated that TissueCypher can objectively guide management of patients with BE, identifying low-risk patients who can safely avoid unnecessary treatment and extend surveillance intervals, as well as high-risk patients who would benefit from therapeutic intervention or more intensive surveillance. Because effective therapies are widely available to prevent and treat early EAC,[6–9] TissueCypher's ability to more accurately predict risk of progression in BE relative to current standard-of-care practices can facilitate more personalized, risk-aligned management of BE patients for better health outcomes.

A

	OR	95% CI		*P* value
Age	1.03	0.97	1.09	.34
Sex (male vs female)	2.36	0.54	10.23	.25
Expert diagnosis (IND vs ND)	1.87	0.69	5.05	.22
Expert diagnosis (LGD vs ND)	3.50	1.59	7.67	.002
Hiatal hernia (yes vs no)	0.77	0.40	1.40	.44
Segment length in cm	1.14	1.01	1.28	.04
TissueCypher risk class (high vs low)	7.81	4.06	15.03	< .001
TissueCypher risk class (intermediate vs low)	1.81	1.01	3.24	.05

	C-statistic	95% CI		*P* value*
	0.76	0.68	0.83	< .001

C, Concordance; *CI*, confidence interval; *EAC*, esophageal adenocarcinoma; *HGD*, high-grade dysplasia; *IND*, indefinite for dysplasia; *LGD*, low-grade dysplasia; *ND*, no dysplasia; *OR*, odds ratio.
*For the likelihood ratio test comparison between the c-stat of this model vs the model without TissueCypher for the ND subgroup.

B

	OR	95% CI		*P* value
Age	1.04	0.97	1.11	.32
Sex (male vs female)	1.02	0.20	5.29	.98
Hiatal hernia (yes vs no)	0.51	0.21	1.23	.14
Segment length in cm	1.15	0.99	1.34	.06
TissueCypher risk class (high vs low)	18.07	6.57	49.71	< .001
TissueCypher risk class (intermediate vs low)	1.94	0.93	4.03	.0776

	C-statistic	95% CI		*P* value*
	0.72	0.62	0.82	< .001

C-statistic, Concordance statistic; *CI*, confidence interval; *EAC*, esophageal adenocarcinoma; *NDBE*, nondysplastic Barrett's esophagus; *ND*, no dysplasia; *OR*, odds ratio.
*For the likelihood ratio test comparison between the c-statistic of this model vs the model without TissueCypher for the ND subgroup.

Figure 4: Multivariable conditional logistic regression model and C-statistic values using TissueCypher classes for prediction of incident progression and prevalent HGD/EAC. **A)** Prediction of incident progression and prevalent HGD/EAC in patients with NDBE, IND, and LGD (n=590). **B)** Prediction of incident progression and prevalent HGD/EAC in patients with NDBE only (n=489). Adapted from Iyer et al., 2022 (Supplemental Tables 10 and 11).[41]

"TissueCypher provides objective risk stratification in community-diagnosed LGD."

TissueCypher Consistency in Demonstrating Improved Risk Stratification in BE: Independent, Blinded Studies

In subsequent validation studies, results from TissueCypher have consistently risk-stratified patients for BE progression better than clinical variables and pathology review.[11,28,29,38] Furthermore, future incident progression from NDBE, IND, and LGD to HGD/EAC was predicted more accurately by TissueCypher relative to clinicopathologic variables in an independent, blinded study (n=268 patients) conducted by two U.S. institutions (Davison et al., 2020).[28] This study provided further support for adherence to long-interval surveillance intervals (3-5 years) as recommended by guidelines for patients at low risk for progression, but also revealed that patients in the cohort with NDBE and a high-risk TissueCypher score progressed at a higher rate than patients having a diagnosis of LGD that was confirmed by an expert GI pathologist (26% versus 22% in 5 years). Current standard-of-care practice based on histopathology fails to identify any high-risk patients with NDBE; on the other hand, not only does TissueCypher identify these high-

risk NDBE patients, but does so at an early stage, enabling escalation of management, such as EET or short-interval surveillance, which have both been correlated with improved health outcomes.

Another independent, blinded study of 76 patients, who were prospectively followed as part of a registry in the Netherlands (Frei et al., 2020), validated TissueCypher's ability to risk-stratify patients with NDBE.[11] TissueCypher identified high-risk patients with NDBE who were more than five-times as likely to progress to HGD/EAC compared to patients with NDBE identified as low risk by TissueCypher. The TissueCypher high-risk patients with NDBE progressed at 6.9%/year, a rate that exceeds those reported for expert-confirmed LGD.[4,19,20] The study further demonstrated that TissueCypher can identify high-risk patients with NDBE who are universally unable to be identified by standard-of-care clinicopathologic assessment and may benefit from therapeutic intervention or short-interval surveillance, as well as low-risk patients who could be managed by extending surveillance to 3-5 years in adherence with guideline recommendations.

Across these studies, TissueCypher was found to have a high negative predictive value (NPV) for risk stratification, ranging from 96-98% and supporting management decisions that avoid unnecessary treatment and potentially extend surveillance intervals for patients with a low-risk TissueCypher result.

TissueCypher in Community-Based Diagnoses of LGD

In patients with a community-based diagnosis of LGD, current standard-of-care practice includes referral to an expert GI pathologist for confirmation of diagnosis.[4,6,12,25,31,39] The majority (73-85%) of community-diagnosed LGD cases are down-staged to IND or NDBE during expert pathology review.[31,33–36] This down-staging is largely due to significant observer variability that exists among both generalist and expert pathologists. Notably, even expert GI pathologists over- and under-diagnose LGD.[32,35,36]

Frei et al. (2021) reported in another blinded cohort (n=155) study that TissueCypher provides objective risk stratification in community-diagnosed LGD.[29] Patients with community-diagnosed LGD and a TissueCypher high-risk result had more than six-times the risk of progressing to HGD/EAC relative to those with a TissueCypher low-risk result. Of the patients who were down-staged to NDBE by expert pathology review, TissueCypher identified >50% of patients who progressed to HGD/EAC. Additionally, Khoshiwal et al. (2022) recently reported that TissueCypher outperformed the sensitivity and specificity of pathology review (n=30 generalist and expert pathologists) for predicting progression to HGD/EAC in patients with community-diagnosed LGD.[38] These studies further support TissueCypher's ability to identify high-risk patients for early intervention or increased surveillance.

In addition to predicting incident progression to HGD/EAC, TissueCypher can identify abnormalities associated with prevalent HGD/EAC. Patients with prevalent HGD/EAC are often missed (~27% of HGD/EAC cases) at initial endoscopy, which delays accurate diagnosis and appropriate treatment.[40] Critchley-Thorne et al. (2017) demonstrated that TissueCypher can identify presence of missed, prevalent HGD and EAC.[27] In a cohort (n=175) of patients with expert-diagnosed NDBE, IND, or LGD from four institutions in the U.S. and Europe, 30 patients were diagnosed with HGD/EAC during repeat endoscopy <1 year later. These patients were, therefore, assumed to have been missed for prevalent HGD/EAC at the baseline endoscopy. The cohort also included 145 patients

Milestones
The History of TissueCypher

As shown by the timeline in **Figure 3**, there have been many milestones during the development and validation of TissueCypher.

1) In 2015, feasibility of the TissueCypher technology for analysis of BE tissues was reported by Prichard et al.[10]

2) That same year, the National Institutes of Health awarded a grant for $1.2 million for independent clinical validation of TissueCypher.

3) The development and clinical validation of TissueCypher to predict future progression from NDBE, IND, and LGD to HGD/EAC within five years of diagnosis was first reported from a multi-site, international study by Critchley-Thorne et al. in 2016.[26]

4) Later that same year, TissueCypher was launched as a clinical laboratory-developed test (LDT) via a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory.

5) In 2017, the clinical validation of TissueCypher for detection of prevalent HGD/EAC that is missed at diagnosis was published by Critchley-Thorne and colleagues from another multi-site study.[27]

6) In 2018, the TissueCypher tissue systems pathology/spatialomics approach for objective quantification of biomarkers in the context of tissue architecture for development of predictive cancer pathology models was described.[42]

7) In May 2019, the American Medical Association (AMA) established a Current Procedural Terminology (CPT) code, CPT Code 0108U, as a unique identifier for TissueCypher for all payers.

8) A cost analysis study by Hao et al. was published in 2019, demonstrating that TissueCypher is cost-effective within 5 years, and showed that TissueCypher-guided care resulted in fewer patients progressing to HGD/EAC and reduced EAC-related deaths.[43]

9) A multi-site, independent, blinded study by Davison et al. was published in 2020, demonstrating further clinical validation of TissueCypher for predicting future progression from NDBE, IND, and LGD to HGD/EAC in patients with BE.[28]

10) Also in 2020, Frei et al. reported an independent clinical validation of TissueCypher for predicting future progression from NDBE to HGD/EAC in a prospectively-followed BE patient registry.[11]

11) As of January 1, 2021, TissueCypher's CPT Code 0108U was listed in Medicare's Clinical Laboratory Fee Schedule (CLFS).

12) The clinical utility of TissueCypher was demonstrated in a study by Diehl et al., published in 2021, in which TissueCypher results significantly impacted management decisions for patients with NDBE, IND, or LGD; resulting in risk-aligned increases or decreases in management intensity for 55% of the cohort.[30]

13) Frei et al. also reported in 2021, the clinical validation of TissueCypher for detecting prevalent HGD/EAC in patients with LGD via an independent, blinded study of a cohort derived from screening for the surveillance versus radiofrequency ablation (SURF) randomized controlled trial.[29]

14) TissueCypher was acquired by Castle Biosciences, Inc. in 2021.

15) In 2022, Iyer et al. reported results from an independent, pooled analysis of multi-center, international studies, demonstrating further clinical validation of TissueCypher.[41]

16) The Centers for Medicare and Medicaid Services (CMS) granted Advanced Diagnostic Laboratory Test (ADLT) status to TissueCypher in 2022, providing further support for the TissueCypher systems pathology approach to management of patients with BE. The ADLT status is a unique designation, indicating TissueCypher provides unique clinical diagnostic information that cannot be gained by other tests, biomarkers, or other clinical variables.

with surveillance data showing no progression of disease. A TissueCypher high-risk result was associated with 46-times the likelihood of having prevalent HGD/EAC relative to a TissueCypher low-risk result.

TissueCypher: Added Value to Current Risk Assessment for Management of Patients with BE

Several studies have demonstrated that TissueCypher can add value to current standard-of-care practices by refining risk stratification in BE. An independent, pooled analysis by Iyer et al. (2022) using patient-level data (n=590) from international, multi-center studies assessed the value of TissueCypher when used adjunctly with clinicopathologic variables to predict risk of progression to HGD/EAC in patients with NDBE, IND, or LGD.[41] This patient population included incident progressors (n=152), patients with missed prevalent HGD/EAC (n=38), and non-progressors (n=400). A TissueCypher high-risk result outperformed currently used clinicopathologic factors (i.e., age, segment length, sex, presence of hiatal hernia, and expert pathologist-confirmed LGD) in multivariable models as an independent predictor of increased risk of incident progression to HGD/EAC (p<0.001, **Figure 4A**).

In a subset analysis of patients with NDBE, TissueCypher also demonstrated improved risk stratification relative to clinicopathologic factors (p<0.001, **Figure 4B**), identifying patients who had at least 18-times increased risk of incident progression and would benefit from more intensive management. Compared with risk assessment via clinicopathologic factors alone, the addition of TissueCypher results revealed significantly improved prediction of progression within five years (p<0.0001).[41]

Clinical Utility of TissueCypher to Guide Management Decisions for BE Patients

A recent study of clinical utility by Diehl et al. (2021) showed that TissueCypher results changed 55% of clinical management decisions in a cohort of patients with NDBE, IND, or LGD.[30] A high-risk TissueCypher result was associated with 22% of the change in clinical decisions, which resulted in escalation of management, including endoscopic eradication therapy or shorter surveillance intervals. A TissueCypher low-risk result was associated with 33% of the change in clinical decisions, which led to de-escalation of management, i.e., surveillance only or extended surveillance intervals within clinical guidelines (p<0.0001). These changes in management decisions were aligned with the individual patient's risk stratification by TissueCypher for a more personalized approach to improve health outcomes. These studies corroborate the added value of TissueCypher for guiding clinical management of patients with BE.

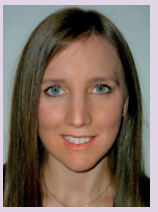
As a result of the growing body of clinical validity and utility evidence, a recent clinical practice update from the American Gastroenterological Association on latest innovations for BE screening and surveillance suggests clinical use of TissueCypher as part of best practice advice in the management of patients with NDBE.[25] Current American College of Gastroenterology guidelines also note that the body of published literature on TissueCypher raises the possibility that the test "may provide some benefit in a subset of patients with BE, particularly in those without dysplasia."[4] TissueCypher can be used adjunctly with clinicopathologic assessment to refine risk stratification of patients with BE. Taken together, a substantial body of evidence supports the use of TissueCypher's spatialomics approach to guide objective management of BE, identifying high-risk patients who may benefit from therapeutic intervention or intensive surveillance as well as low-risk patients who may be effectively managed by surveillance in 3-5 years without therapy.

"As a result of the growing body of clinical validity and utility evidence, a recent clinical practice update from the American Gastroenterological Association on latest innovations for BE screening and surveillance suggests clinical use of TissueCypher as part of best practice advice in the management of patients with NDBE."

The Value of the Spatialomics Approach to Precision Pathology: Castle's 'Patients-First' Approach to Innovative Tests

The objective, spatialomics-based prediction test, TissueCypher, has demonstrated added value to current clinicopathologic factors (including expert pathology review) for improving risk stratification of individual patients with BE to improve health outcomes,[27,30,41] exemplifying Castle Biosciences' 'Patients First' approach to precision medicine test innovation.

Traditional clinicopathologic approaches for risk assessment of patients with BE are limited by several variables (e.g., adherence to biopsy protocols, patient follow-up, observer variability and subjectivity during pathology review).[4,31,32]



Rebecca Critchley-Thorne, PhD, VP R&D, Spatialomics & GI

Rebecca Critchley-Thorne serves as Vice President, Research & Development at Castle Biosciences' Spatialomics and Gastroenterology division. She previously was the Co-Founder and Chief Scientific Officer at Cernostics, Inc., that was acquired by Castle Biosciences in December 2021. Dr. Critchley-Thorne led the development of the TissueCypher® computational pathology platform as well as the TissueCypher® Barrett's Esophagus test and the clinical studies supporting its use. She completed training as a postdoctoral fellow at Stanford University where she focused on highly multiplexed analysis of biomarkers to understand mechanisms of immune dysfunction in various cancer types. She completed doctoral work in cancer immunotherapy at Imperial College and Cancer Research UK in London, UK, and earned a B.S. (Hons) degree in Pharmacology from the University of Sheffield, UK. Dr. Critchley-Thorne is the author of many medical and scientific publications, principal investigator on NIH-funded research studies, and an inventor on several of Castle Biosciences' patents.

Craig Munroe, MD, Medical Director, GI

Dr. Munroe is the Medical Director for GI at Castle Biosciences, Inc. He was previously the Associate Chief for Clinical Innovation, and an interventional endoscopist at the University of California, San Francisco. Dr. Munroe has been active in clinical care, research, and new technology in medicine throughout his career. Prior to UCSF, Dr. Munroe served as Chief of Gastroenterology from 2017-2020 at Kaiser Permanente, San Francisco. Dr. Munroe earned his Doctor of Medicine from Georgetown University, completed his Internal Medicine residency at Massachusetts General Hospital, GI fellowship at Stanford, and Interventional Endoscopy fellowship at UCSD.

Mary A. Hall, MBA, PhD, Medical Writer II, R&D

Mary Hall is a Medical Writer, Research & Development, serving multiple product lines at Castle Biosciences, and has co-authored many scientific publications throughout her career. Previously, Dr. Hall served as an Assistant Professor and Research Scientist in the Center for Molecular Imaging – Institute of Molecular Medicine, UTHealth, Houston, TX where she focused on prostate cancer, wound healing, and molecular imaging. Her postdoctoral training was conducted at Research, Inc., VAMC, Memphis, TN and the Department of Immunology, MD Anderson Cancer Center, Houston. She completed her doctoral work at Southern Illinois University-Carbondale (SIUC) in molecular biology, microbiology, and biochemistry with a focus in immunology. Prior to her PhD, she served as a Senior Research Assistant in the Department of Pediatrics – Infectious Diseases at Baylor College of Medicine, Houston and earned her MBA from the University of Houston and her bachelor's in microbiology from SIUC.

TissueCypher can be used adjunctly with current standard-of-care practices and guidelines to improve prediction of risk of progression to HGD/EAC in patients with NDBE, IND, or LGD as well as to detect prevalent HGD/EAC that may be missed on initial pathology review. This approach places patients first, providing clinically actionable information to help guide management decisions for individual patients who are at low risk and can safely avoid unneeded and costly treatment or frequent surveillance, and those who are at intermediate or high risk and could benefit from more intensified surveillance intervals or treatment. As effective therapies are readily available to prevent progression in BE and treat early EAC,[6–9] the 'Patients First' approach using TissueCypher aligns with more personalized, risk-appropriate management of patients with BE for better health outcomes.

The design, development, and validation of TissueCypher has been focused on achieving a more precise and objective tool for assessing individual risk of progression in BE. Multiple validation studies have shown that TissueCypher consistently outperforms current standard-of-care practices for risk-stratifying patients with BE.[11,26–30,41] Thus, the spatialomics approach of TissueCypher brings precision pathology to the clinic for more personalized care of patients with BE to improve health outcomes. JoPM

Summary Points

The TissueCypher Barrett's Esophagus Test

- Objective, fully automated, spatialomics-based, tissue systems pathology (TSP-9) test predicts an individual patient's risk of progression from NDBE, IND, or LGD to HGD/EAC within five years of endoscopy.
- Predicts both future risk of developing HGD/EAC and presence of prevalent HGD/EAC often missed by current standard-of-care practices.
- Commercially available as a send-out test and performed by Castle Biosciences' CLIA-certified laboratory.
- Performed on existing sections of FFPE tissue from biopsies acquired during BE screening and surveillance endoscopies.
- Individual patient risk score, risk class (low, intermediate, or high risk), and a 5-year probability of progression to HGD/EAC provided in clinical report.
- Test results can be incorporated into current standard-of-care guidelines to improve individual patient risk stratification and refine patient management to improve health outcomes.
- Validated in multiple, multi-site, international studies with consistent, robust risk-stratification performance.
- Outperforms current clinicopathologic factors and pathology review in predicting risk of progression to HGD/EAC.
- ADLT status awarded under the Medicare CLFS (CPT Code: 0108U).

References

1. Lewis SM, Asselin-Labat ML, Nguyen Q, et al. Spatial omics and multiplexed imaging to explore cancer biology. Nat Methods. 2021;18(9):997-1012. doi:10.1038/s41592-021-01203-6
2. Wu Y, Cheng Y, Wang X, Fan J, Gao Q. Spatial omics: Navigating to the golden era of cancer research. Clin Transl Med. 2022;12(1):e696. doi:10.1002/ctm2.696
3. Bingham GC, Lee F, Naba A, Barker TH. Spatial-omics: Novel approaches to probe cell heterogeneity and extracellular matrix biology. Matrix Biol. 2020;91-92:152-166. doi:10.1016/j.matbio.2020.04.004
4. Shaheen NJ, Falk GW, Iyer PG, et al. Diagnosis and Management of Barrett's Esophagus: An Updated ACG Guideline. Am J Gastroenterol. 2022;117(4):559-587. doi:10.14309/ajg.0000000000001680
5. Naini BV, Souza RF, Odze RD. Barrett's Esophagus: A Comprehensive and Contemporary Review for Pathologists. Am J Surg Pathol. 2016;40(5):e45-66. doi:10.1097/PAS.0000000000000598
6. American Gastroenterological Association, Spechler SJ, Sharma P, Souza RF, Inadomi JM, Shaheen NJ. American Gastroenterological Association medical position statement on the management of Barrett's esophagus. Gastroenterology. 2011;140(3):1084-1091. doi:10.1053/j.gastro.2011.01.030
7. Schlottmann F, Patti MG, Shaheen NJ. Endoscopic Treatment of High-Grade Dysplasia and Early Esophageal Cancer. World J Surg. 2017;41(7):1705-1711. doi:10.1007/s00268-017-3977-8
8. Konda VJA, Ellison A, Codipilly DC, Han J, Samarasena J, Iyer PG. Quality in Barrett's Esophagus: Diagnosis and Management. Techniques and Innovations in Gastrointestinal Endoscopy. Published online February 19, 2022. doi:10.1016/j.tige.2022.01.009
9. Desai M, Rösch T, Sundaram S, et al. Systematic review with meta-analysis: the long-term efficacy of Barrett's endoscopic therapy-stringent selection criteria and a proposal for definitions. Aliment Pharmacol Ther. 2021;54(3):222-233. doi:10.1111/apt.16473
10. Prichard JW, Davison JM, Campbell BB, et al. TissueCypher(TM): A systems biology approach to anatomic pathology. J Pathol Inform. 2015;6:48. doi:10.4103/2153-3539.163987
11. Frei NF, Konte K, Bossart EA, et al. Independent Validation of a Tissue Systems Pathology Assay to Predict Future Progression in Nondysplastic Barrett's Esophagus: A Spatial-Temporal Analysis. Clin Transl Gastroenterol. 2020;11(10):e00244. doi:10.14309/ctg.0000000000000244
12. ASGE STANDARDS OF PRACTICE COMMITTEE, Qumseya B, Sultan S, et al. ASGE guideline on screening and surveillance of Barrett's esophagus. Gastrointest Endosc. 2019;90(3):335-359.e2. doi:10.1016/j.gie.2019.05.012
13. Schlottmann F, Molena D, Patti MG. Gastroesophageal reflux and Barrett's esophagus: a pathway to esophageal adenocarcinoma. Updates Surg. 2018;70(3):339-342. doi:10.1007/s13304-018-0564-y
14. Gregson EM, Bornschein J, Fitzgerald RC. Genetic progression of Barrett's oesophagus to oesophageal adenocarcinoma. Br J Cancer. 2016;115(4):403-410. doi:10.1038/bjc.2016.219
15. Sampliner RE. Management of nondysplastic barrett esophagus with ablation therapy. Gastroenterol Hepatol (N Y). 2011;7(7):461-464.
16. Krishnamoorthi R, Mohan BP, Jayaraj M, et al. Risk of progression in Barrett's esophagus indefinite for dysplasia: a systematic review and meta-analysis. Gastrointest Endosc. 2020;91(1):3-10.e3. doi:10.1016/j.gie.2019.07.037
17. Thota PN, Lee HJ, Goldblum JR, et al. Risk stratification of patients with barrett's esophagus and low-grade dysplasia or indefinite for dysplasia. Clin Gastroenterol Hepatol. 2015;13(3):459-465.e1. doi:10.1016/j.cgh.2014.07.049
18. 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(3):220-227; quiz e26. doi:10.1016/j.cgh.2010.11.008
19. Wani S, Falk GW, Post J, et al. Risk factors for progression of low-grade dysplasia in patients with Barrett's esophagus. Gastroenterology. 2011;141(4):1179-1186, 1186.e1. doi:10.1053/j.gastro.2011.06.055
20. Singh S, Manickam P, Amin AV, et al. Incidence of esophageal adenocarcinoma in Barrett's esophagus with low-grade dysplasia: a systematic review and meta-analysis. Gastrointest Endosc. 2014;79(6):897-909.e4; quiz 983.e1, 983.e3. doi:10.1016/j.gie.2014.01.009
21. Thrift AP. Global burden and epidemiology of Barrett oesophagus and oesophageal cancer. Nat Rev Gastroenterol Hepatol. 2021;18(6):432-443. doi:10.1038/s41575-021-00419-3
22. Cook MB, Coburn SB, Lam JR, Taylor PR, Schneider JL, Corley DA. Cancer incidence and mortality risks in a large US Barrett's oesophagus cohort. Gut. 2018;67(3):418-529. doi:10.1136/gutjnl-2016-312223
23. Rastogi A, Puli S, El-Serag HB, Bansal A, Wani S, Sharma P. Incidence of esophageal adenocarcinoma in patients with Barrett's esophagus and high-grade dysplasia: a meta-analysis. Gastrointest Endosc. 2008;67(3):394-398. doi:10.1016/j.gie.2007.07.019
24. Overholt BF, Lightdale CJ, Wang KK, et al. Photodynamic therapy with porfimer sodium for ablation of high-grade dysplasia in Barrett's esophagus: international, partially blinded, randomized phase III trial. Gastrointest Endosc. 2005;62(4):488-498. doi:10.1016/j.gie.2005.06.047
25. Muthusamy VR, Wani S, Gyawali CP, Komanduri S. AGA Clinical Practice Update on New Technology and Innovation for Surveillance and Screening in Barrett's Esophagus: Expert Review. Clinical Gastroenterology and Hepatology. 2022;0(0). doi:10.1016/j.cgh.2022.06.003
26. Critchley-Thorne RJ, Duits LC, Prichard JW, et al. A Tissue Systems Pathology Assay for High-Risk Barrett's Esophagus. Cancer Epidemiol Biomarkers Prev. 2016;25(6):958-968. doi:10.1158/1055-9965.EPI-15-1164
27. Critchley-Thorne RJ, Davison JM, Prichard JW, et al. A Tissue Systems Pathology Test Detects Abnormalities Associated with Prevalent High-Grade Dysplasia and Esophageal Cancer in Barrett's Esophagus. Cancer Epidemiol Biomarkers Prev. 2017;26(2):240-248. doi:10.1158/1055-9965.EPI-16-0640
28. Davison JM, Goldblum J, Grewal US, et al. Independent Blinded Validation of a Tissue Systems Pathology Test to Predict Progression in Patients With Barrett's Esophagus. Am J Gastroenterol. 2020;115(6):843-852. doi:10.14309/ajg.0000000000000556
29. Frei NF, Khoshiwal AM, Konte K, et al. Tissue Systems Pathology Test Objectively Risk Stratifies Barrett's Esophagus Patients With Low-Grade Dysplasia. Am J Gastroenterol. 2021;116(4):675-682. doi:10.14309/ajg.0000000000001037
30. Diehl DL, Khara HS, Akhtar N, Critchley-Thorne RJ. TissueCypher Barrett's esophagus assay impacts clinical decisions in the management of patients with Barrett's esophagus. Endosc Int Open. 2021;9(3):E348-E355. doi:10.1055/a-1326-1533
31. Wani S, Rubenstein JH, Vieth M, Bergman J. Diagnosis and Management of Low-Grade Dysplasia in Barrett's Esophagus: Expert Review From the Clinical Practice Updates Committee of the American Gastroenterological Association. Gastroenterology. 2016;151(5):822-835. doi:10.1053/j.gastro.2016.09.040
32. Vennalaganti P, Kanakadandi V, Goldblum JR, et al. Discordance Among Pathologists in the United States and Europe in Diagnosis of Low-Grade Dysplasia for Patients With Barrett's Esophagus. Gastroenterology. 2017;152(3):564-570.e4. doi:10.1053/j.gastro.2016.10.041
33. Wani S, Mathur S, Sharma P. How to manage a Barrett's esophagus patient with low-grade dysplasia. Clin Gastroenterol Hepatol. 2009;7(1):27-32. doi:10.1016/j.cgh.2008.08.014
34. Odze RD. Diagnosis and grading of dysplasia in Barrett's oesophagus. J Clin Pathol. 2006;59(10):1029-1038. doi:10.1136/jcp.2005.035337
35. Curvers WL, ten Kate FJ, Krishnadath KK, et al. Low-grade dysplasia in Barrett's esophagus: overdiagnosed and underestimated. Am J Gastroenterol. 2010;105(7):1523-1530. doi:10.1038/ajg.2010.171
36. Duits LC, Phoa KN, Curvers WL, et al. Barrett's oesophagus patients with low-grade dysplasia can be accurately risk-stratified after histological review by an expert pathology panel. Gut. 2015;64(5):700-706. doi:10.1136/gutjnl-2014-307278
37. Wani S, Williams JL, Komanduri S, Muthusamy VR, Shaheen NJ. Endoscopists systematically undersample patients with long-segment Barrett's esophagus: an analysis of biopsy sampling practices from a quality improvement registry. Gastrointest Endosc. 2019;90(5):732-741.e3. doi:10.1016/j.gie.2019.04.250
38. Khoshiwal AM, Frei NF, Duits LC, et al. 695: AN OBJECTIVE, FULLY AUTOMATED BARRETT'S RISK PREDICTION ASSAY OUTPERFORMS MOST PATHOLOGISTS IN RISK STRATIFYING BARRETT'S ESOPHAGUS WITH LOW GRADE DYSPLASIA. Gastroenterology. 2022;162(7, Supplement):S-174. doi:10.1016/S0016-5085(22)60414-1
39. Standards of Practice Committee – ASGE, Wani S, Qumseya B, et al. Endoscopic eradication therapy for patients with Barrett's esophagus-associated dysplasia and intramucosal cancer. Gastrointest Endosc. 2018;87(4):907-931.e9. doi:10.1016/j.gie.2017.10.011
40. Visrodia K, Singh S, Krishnamoorthi R, et al. Magnitude of Missed Esophageal Adenocarcinoma After Barrett's Esophagus Diagnosis: A Systematic Review and Meta-analysis. Gastroenterology. 2016;150(3):599-607.e7; quiz e14-15. doi:10.1053/j.gastro.2015.11.040
41. Iyer PG, Codipilly DC, Chandar AK, et al. Prediction of Progression in Barrett's Esophagus Using a Tissue Systems Pathology Test: A Pooled Analysis of International Multicenter Studies. Clin Gastroenterol Hepatol. Published online February 22, 2022:S1542-3565(22)00190-2. doi:10.1016/j.cgh.2022.02.033
42. DeWard A, Critchley-Thorne RJ. Systems Biology Approaches in Cancer Pathology. In: von Stechow L, ed. Cancer Systems Biology: Methods and Protocols. Methods in Molecular Biology. Springer; 2018:261-273. doi:10.1007/978-1-4939-7493-1_13
43. Hao J, Critchley-Thorne R, Diehl DL, Snyder SR. A Cost-Effectiveness Analysis Of An Adenocarcinoma Risk Prediction Multi-Biomarker Assay For Patients With Barrett's Esophagus. Clinicoecon Outcomes Res. 2019;11:623-635. doi:10.2147/CEOR.S221741