MAYO CLINIC

Prediction of Progression in Barrett's Esophagus Using a Tissue Systems Pathology Test: A Pooled Analysis of International Multicenter Studies

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COURTESY OF CASTLE BIOSCIENCES

Background

- Progression in Barrett's esophagus (BE) without dysplasia (NDBE) is uncommon

- Progression in LGD is variable
  - Heterogeneous pathology interpretation
  - Ablation versus Surveillance

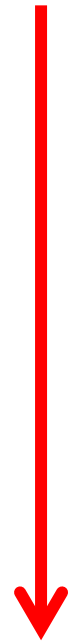
Grade of Dysplasia	Progression to EAC or HGD
No dysplasia (6847 patients)	3.3/1000 p-yr
LGD (2694 patients)	7.6-17.3 /1000 p-yr
HGD (236 patients)	65.8/1000 p-yr

COURTESY OF CASTLE BIOSCIENCES

Rastogi GI Endoscopy 2008, Wani Am J Gastroenterology 2009, Desai Gut 2011, Singh GI Endoscopy 2014

Background
DYSPLASIA grade : SOLE DETERMINANT of Management recommendations

Grade of Dysplasia	Progression rate EAC or HGD	Management
No dysplasia (6847 patients)	3.3/1000 p-yr	Surveillance 3-5 years
LGD (2694 patients)	7.6-17.3 /1000 p-yr	EET OR Q 6-12m surveillance
HGD (236 patients)	65.8/1000 p-yr	EET



COURTESY OF CASTLE BIOSCIENCES

Rastogi GI Endoscopy 2008, Wani Am J Gastroenterology 2009, Desai Gut 2011, Singh GI Endoscopy 2014 , Shaheen Am J Gastroenterology 2016

Background
Limitations of dysplasia and surveillance

- Risk of progression is variable within dysplasia strata
  - *Dysplasia is not sole risk factor for progression*
- Not cost effective (NDBE)
- Endoscopists are not compliant with recommendations
- Missed dysplasia/EAC : 25-33%

Factor	Hazard ratio (95% C.I.)
Demographics	
Age	1.03 (1.01-1.05)
Male gender	2.16 (1.84-2.53)
Lifestyle	
Smoking	1.47 (1.09–1.98)
Alcohol	1.11 (0.81–1.52)
BMI (per unit)	1.04 (0.93–1.17)
BE characteristics	
LGD (vs. NDBE)	4.25 (2.58–7.00)
Length of BE (per cm)	1.25 (1.16–1.36)
Medications	
NSAIDs	0.72 (0.50–1.04)
Statins	0.48 (0.31–0.73)
PPIs	0.55 (0.32–0.96)

COURTESY OF CASTLE BIOSCIENCES

Krishnamoorthi, Iyer CGH 2018. Abrams CGH 2008, Visrodia Gastroenterology 2016

Implications of predicting progression personalized management ?

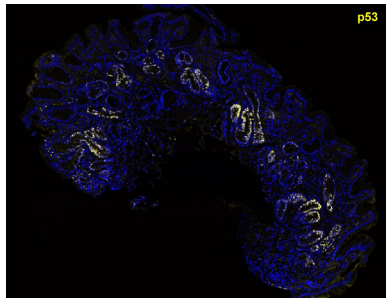
- Change in management :
  - Low risk : decrease or stop surveillance ?
  - High risk : intensive surveillance or proactive ablation ?

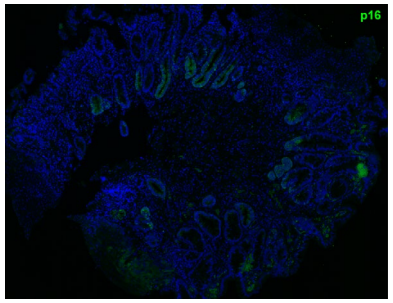
- May make BE management of more efficient and cost effective
  - Targeting surveillance to and preventing EAC in those at highest risk
  - Decreasing costs of ineffective/unnecessary surveillance

COURTESY OF CASTLE BIOSCIENCES

TissueCypher® ASSAY

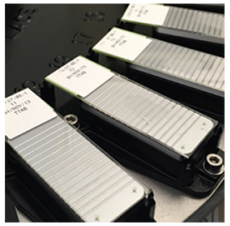
Laboratory-developed test done in a CLIA-certified laboratory performed on FFPE tissue





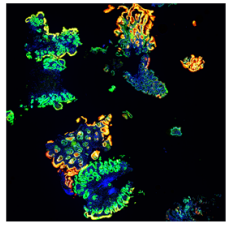
Multiplexed Immunofluorescence Slide Labeling

9 protein-based biomarkers plus Hoechst labeling of nuclei



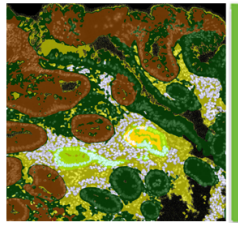
Whole Slide Fluorescence Scanning

4 registered channels of image data for each slide



TissueCypher® Image Analysis Platform [2]

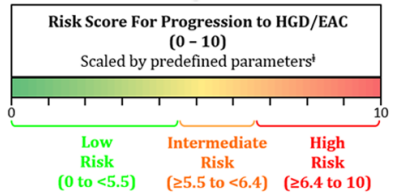
15 predefined quantitative measurements of biomarkers & morphology (features)

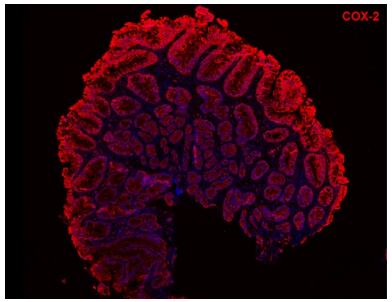


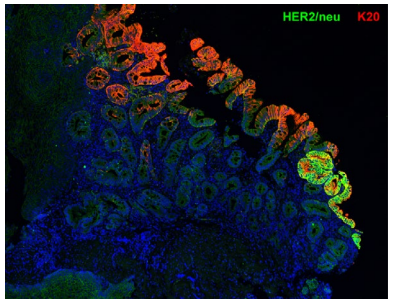
15 Features

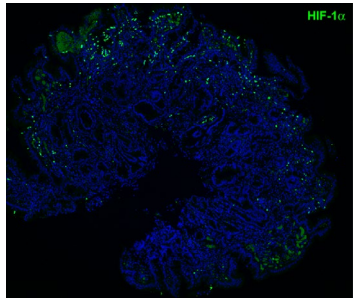
TissueCypher® Risk Classifier [3,4]

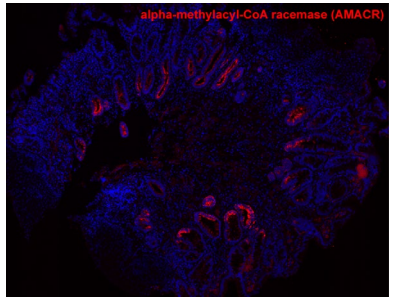
15 features integrated to produce a Risk Score (0-10) & Risk Class (Low, Int, High)

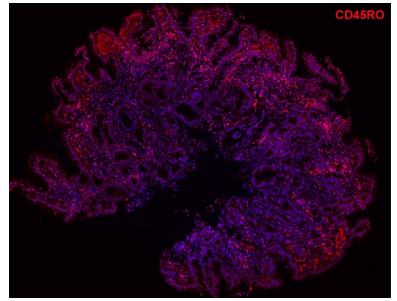


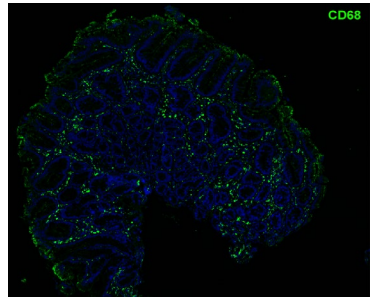












COURTESY OF CASTLE BIOSCIENCES

[1]Cernostics, Inc., Pittsburgh, PA, USA. CLIA ID: 39D2110302. [2]Prichard JW, et al. Journal of Pathology Informatics 2015;6:48. [3]Critchley-Thorne et al., Cancer Epidemiol Biomarkers Prev. 2016;25(6):958-68. [4]Critchley-Thorne et al., Cancer Epidemiol Biomarkers Prev. 2017;26(2):240-248.

AIMS

- We aimed to assess the **predictive ability** of a tissue systems pathology test performed on paraffin embedded (FFPE) tissue (TissueCypher®) to predict **incident progression** in BE patients without dysplasia (NDBE), indefinite dysplasia (IND) or low grade dysplasia (LGD) by combining **patient level** data from **four independent case-control studies** done in institutions in the USA and Europe.

COURTESY OF CASTLE BIOSCIENCES

4 Pooled Studies
4 US Institutions, AMC Netherlands, SURF Trial Europe (9 sites)

Study	Design	Study Size NDBE,IND,LGD	Comments
Critchley Thorne CEBP 2016	Retrospective Nested Case Control	79 P 287 NP	3 Tier Classifier developed
Davison et al AJG 2020	Retrospective Case Control	58 P 210 NP	3 Tier Classifier validated independent sample
Frei et al CTG 2020	Retrospective Nested Case Control REBUS prospective cohort	38 P 39 NP	3 Tier Classifier Spatial and temporal analysis
Frei et al AJG 2021	Retrospective Cohort study, SURF trial screening cohort	155 patients with community LGD diagnosis	Performance of assay compared to academic pathologists

Methods

- Pooled patient level data
  - Age, sex, BE length, HH, race, initial and confirmed path
- Non-progressors matched to progressors by age and sex
- Incident progression : HGD or EAC > 1 year after initial BE diagnosis
- Multivariable Conditional Logistic Regression
  - Association of TissueCypher^{R} assay with progression
  - Comparison of model prediction of progression (C statistic)
    - With and without TissueCypher assay results
- Subset analysis : NDBE patients alone

COURTESY OF CASTLE BIOSCIENCES

Cancer Epidemiol Biomarkers Prev. 2016;25(6):958-68; Am J Gastroenterol. 2020 Jun;115(6):843-852; Clinical & Translational Gastroenterology in Press ; American Journal of Gastroenterology 2021 in press

BASELINE CHARACTERISTICS

	Expert Path Diagnosis			
Variable	IND N=29	LGD N=43	NDBE N=403	Total **N=475**
Mean (SD) Age	60.4 (7.6)	62.8 (11.0)	61.0 (11.1)	61.1 (10.9)
Male Sex (%)	19 (65.5%)	41 (95.3%)	312 (77.4%)	372 (78.3%)
Long segment BE, N (%)	11 (39.3%)	30 (69.8%)	267 (68.1%)	**308 (66.5%)**
Hiatal Hernia present, N (%)	25 (86.2%)	32 (84.2%)	320 (85.1%)	377 (85.1%)
Progressors, N(%)	9 (31.0%)	31 (72.1%)	112 (27.8%)	**152 (32.0%)**
TissueCypher® risk score Mean (SD)	4.9 (1.6)	6.1 (1.6)	4.4 (1.8)	4.6 (1.8)
TissueCypher® risk class				
High	**5 (17.2%)**	**22 (51.2%)**	**51 (12.7%)**	**78 (16.4%)**
Intermediate	4 (13.8%)	8 (18.6%)	49 (12.2%)	61 (12.8%)
Low	20 (69.0%)	13 (30.2%)	303 (75.2%)	336 (70.7%)

Predictors of progression ALL PATIENTS (NDBE+LGD+IND) without Tissuecypher

	OR	95%CI		p value
Age	**1.047**	**0.996**	**1.102**	**0.0361**
Sex M vs F	3.078	0.773	12.258	0.0554
Expert diagnosis IND vs ND	2.131	0.870	5.223	0.0490
Expert diagnosis LGD vs ND	**7.99**	**3.838**	**16.654**	**0.0000**
Hiatal hernia Y vs N	0.772	0.419	1.422	0.7968
BE segment length in cm	**1.151**	**1.033**	**1.282**	**0.0053**

C Statistic = 0.68

(95% CI 0.61, 0.77)

COURTESY OF CASTLE BIOSCIENCES

PREDICTORS OF PROGRESSION ALL PATIENTS (NDBE+LGD+IND) WITH TissueCypher

	OR	95%CI		p value
Age	1.028	0.972	1.087	0.1699
Sex M vs F	2.357	0.543	10.227	0.1262
Expert diagnosis IND vs ND	1.870	0.693	5.046	0.1081
Expert diagnosis LGD vs ND	**3.496**	**1.594**	**7.670**	**0.0009**
Hiatal hernia Y vs N	0.771	0.398	1.493	0.7797
BE segment length in cm	**1.135**	**1.005**	**1.282**	**0.0208**
Tissuecypher High vs Low	**7.809**	**4.056**	**15.034**	**<0.00001**
Tissuecypher Intermediate vs Low	**1.805**	**1.006**	**3.235**	**0.0238**

C Statistic = 0.76

´95% CI 0.68, 0.83)

P < 0.00001

Likelihood ratio test comparison between C Statistic of model with vs without Tissuecypher

COURTESY OF CASTLE BIOSCIENCES

PREDICTION OF PROGRESSION
NDBE patients only without Tissuecypher

	OR	95%CI		p value
Age	**1.059**	**0.996**	**1.126**	**0.0341**
Sex M vs F	2.377	0.531	10.642	0.1288
Hiatal hernia Y vs N	0.664	0.311	1.420	0.8544
BE Segment length (cm)	**1.182**	**1.035**	**1.349**	**0.0067**

C Statistic = 0.62
(95% CI 0.52, 0.72)

COURTESY OF CASTLE BIOSCIENCES

PREDICTION OF PROGRESSION
NDBE patients only with Tissuecypher

	OR	95%CI		p value
Age	1.037	0.965	1.114	0.1620
Sex M vs F	1.023	0.198	5.289	0.4889
Hiatal hernia Y vs N	0.514	0.214	1.234	0.9318
Segment length in cm	1.152	0.992	1.339	0.0321
Tissuecypher High vs Low	**18.07**	**6.570**	**49.710**	**<0.00001**
Tissuecypher Intermediate vs Low	**1.936**	**0.929**	**4.034**	**0.0388**

C Statistic = 0.72
(95% CI 0.62, 0.82)

P < 0.00001
Likelihood ratio test comparison between C Statistic of model with vs without Tissuecypher

COURTESY OF CASTLE BIOSCIENCES

Advantages of Tissuecypher Assay

- Can be done on FFPE tissue
- Automated, Quantitative : lack of subjectivity
- Methods and cut off thresholds for risk levels are standardized and set
- Results validated in multiple independent sample sets
- Utility : LGD and NDBE
  - LGD : Ablation is recommended, ?used in borderline cases
  - NDBE

COURTESY OF CASTLE BIOSCIENCES

CONCLUSIONS

- Progression rates are low in NDBE, making current surveillance practice inefficient
- Prediction of progression in BE
  - Clinical parameters alone : modest accuracy
  - Addition of validated biomarker panel such as TissueCypher can augment the accuracy of prediction
- Implications
  - Personalize management of BE patients
    - High versus Low risk scores

COURTESY OF CASTLE BIOSCIENCES

