TissueCypher Objectively Risk Stratifies Barrett's Esophagus Patients with Low-Grade Dysplasia

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Disclosures

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EA Bossart: Ownership interest in Cernostics (stock options)

RJ Critchley-Thorne: Ownership in Cernostics (stock, stock options and patents)

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Risk stratification in Barrett's surveillance

- Is based on histological review of surveillance biopsies by pathologists
- Low-grade dysplasia (LGD) is the best predictor of malignant progression

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Diagnosing LGD in Barrett's is challenging

- High inter-observer variability for the histological diagnosis of LGD

- **Guidelines**: LGD biopsies should be reviewed by an expert pathologist
  - LGD is overdiagnosed in 50-75% of the community based diagnoses
  - Such overdiagnosed cases do not have an increased risk for progression
  - Confirmed LGD carries 5-10% annual risk for progression

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Problems with pathology review of LGD cases

- It is unclear what defines an “expert pathologist”
- Access to an expert pathologist is not widely available
- Logistical challenges in transferring slides for such review

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We need an objective and easily accessible tool

- TissueCypher Barrett's Esophagus Assay is a commercially available, objective precision medicine tool for patients with Barrett's Esophagus (BE)
  - An automated assay of 4 standard histology slides
  - Automated labeling and imaging for multiple (9) immunofluorescence markers and nuclei
  - Fully automated computational pathology approach to quantification of the 9 protein-based biomarkers and nuclear morphology.

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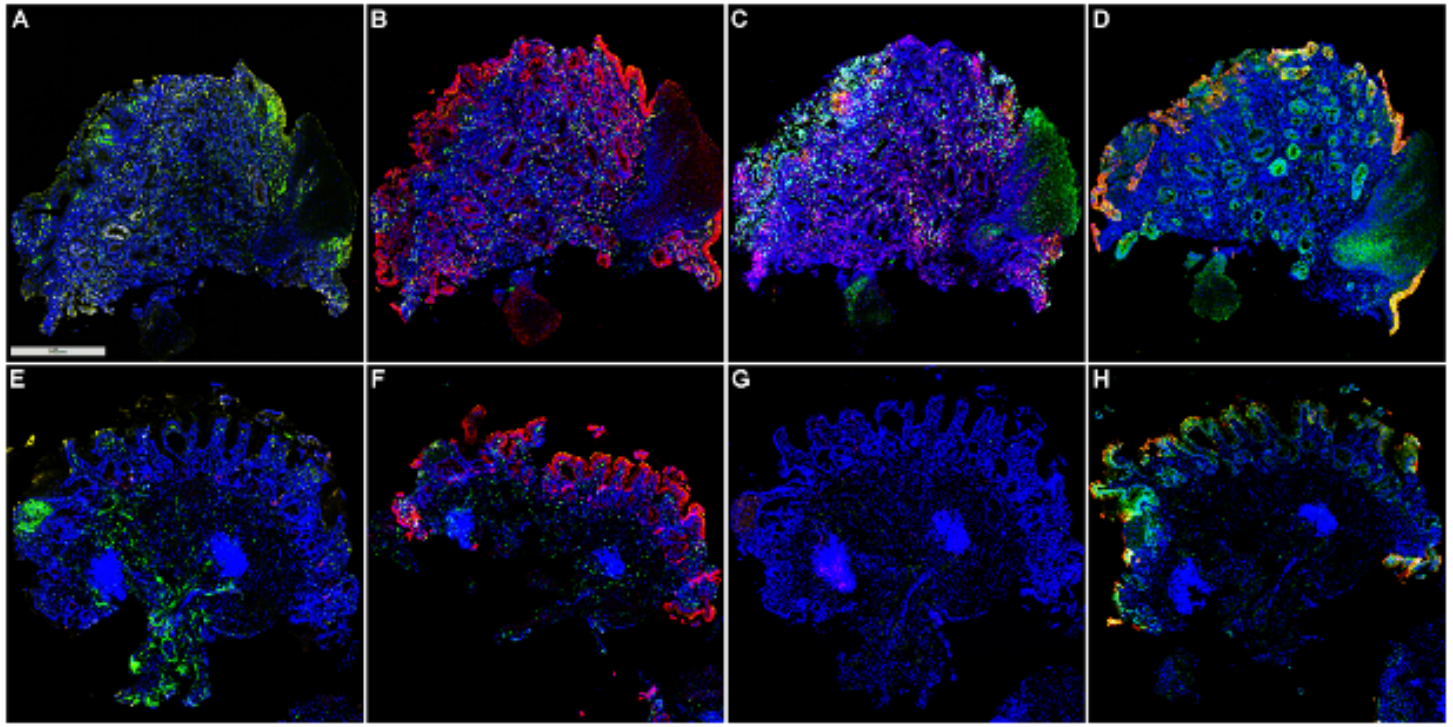
How does TissueCypher work?



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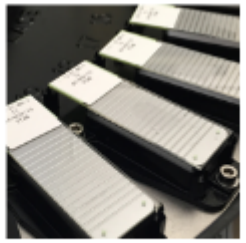
Four 5-micron sections from standard BE biopsies

Automated Multiplexed Immunofluorescence Labeling of 9 Biomarkers



4 slides for immunofluorescence staining (4 biomarkers per slide, incl. controls)

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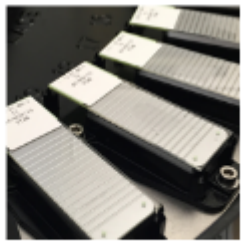
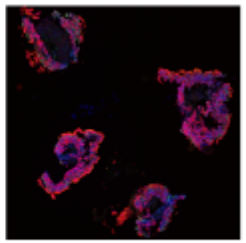


Whole Slide Fluorescence Scanning



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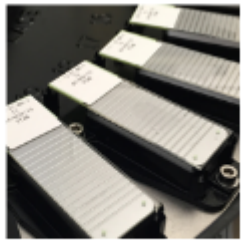

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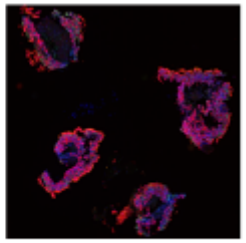
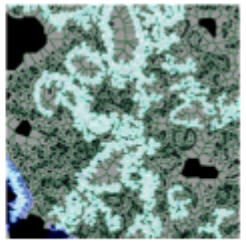


TissueCypher Image Analysis



15 Features automatically extracted by image analysis software

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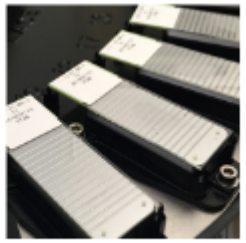

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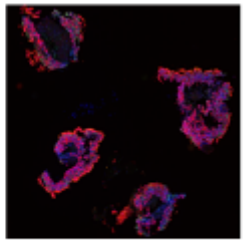

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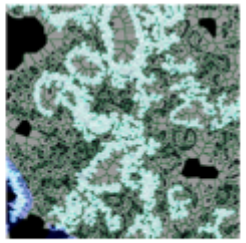

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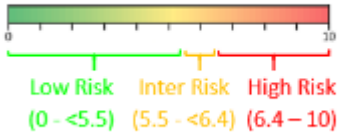
TissueCypher Risk Classifier

15 Features
Scaled & Weighted*
↓
Risk Score*



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TissueCypher has been extensively studied

- 5 peer-reviewed publications in high impact journals over the last 5 years
- All showing that TissueCypher can predict malignant progression in BE biopsies
- Case-control studies, not yet studied in a ‘true’ cohort study
- Not yet specifically studied for its value in LGD cases.

Critchley-Thorne et al, Cancer Epidemiol Biomakers Prev 2016
Critchley-Thorne et al, Cancer Epidemiol Biomarkers Prev 2017
Davison et al, AmJ Gastro 2020
Frei et al, AmJ Gastro 2020
Frei et al, Clinical and Translational Gastroenterology, 2020

COURTESY OF CASTLE BIOSCIENCES

Aim

- To evaluate the predictive value of TissueCypher in a cohort of 155 BE patients with a community-based diagnosis of LGD
- To benchmark its performance against a panel of 12 pathologists from the Netherlands and the US
- Including pathologists with a track record as “expert BE pathologist”

A cohort of 155 BE patients with LGD

- Derived from the screening cohort of the SURF trial: a RCT comparing Surveillance versus RFA for confirmed LGD (Phoa *et al*. JAMA 2014).

- All biopsies of the baseline LGD-endoscopy
  - 5-micron slides cut and assessed by TissueCypher
  - “Sandwich slides” (2 H&E slides and 1 IHC p53) digitized for pathology revision.
  - Worst biopsy score per endoscopy used as outcome for TC and pathologists.

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All biopsies reviewed by 12 pathologists

- Six **EXPERT** pathologists *(3 from the Netherlands, 3 from the US)*
  - Special interest in the field of Barrett's esophagus for over 10 years
  - Minimum case load of 5-10 mainly dysplastic cases per week
  - Co-authored >10 peer-reviewed publications in the field of BE
  - Actively involved in pathology training in BE

- Six **COMMUNITY-BASED** pathologists *(3 from the Netherlands, 3 from the US)*
  - Referring dysplastic BE cases to an expert pathologist

155 patients with a community-based diagnosis of LGD

- 79% males, median age 62 ± 10 years, median Barrett's length C3M4
- Median follow-up of 7.0 years (IQR 4.4 - 9.7)
- Mean number of 3 ± 2 endoscopies

- 25 developed HGD/EAC within 5 years (progressors)
- 130 did not progress to HGD/EAC within 5 years (non-progressors)

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How did our panel review the baseline biopsies?

	Dutch expert pathologists	US expert pathologists	Dutch community-based pathologists	US community-based pathologists
Downstaged to NDBE, (%)	60.0 (52.3 - 71.6)	76.8 (72.9 - 82.6)	59.4 (34.2 - 72.9)	44.3 (12.9 – 72.9)
IND, (%)				
Confirmed LGD, (%)	23.0 (16.8 - 29.7)	17.4 (14.2 - 20.0)	23.5 (10.5 - 40.6)	20.6 (12.9 – 35.5)
Progression to HGD or cancer during follow-up				
Progression of NDBE, (%)	8.2 (8.0 – 8.6)	8.9 (7.1 – 10.3)	9.2 (8.0 – 11.3)	12.1 (8.0 – 20.0)
Progression of IND, (%)				
Progression of LGD, (%)	41.0 (37.0 – 46.2)	51.3 (46.4 – 59.1)	44.9 (22.2 – 62.5)	45.8 (25.5 – 61.9)

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Illustrates the relevance of pathology review of LGD

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IND, (%)	17.0 (11.6 -21.3)	5.8 (3.2 - 7.1)	16.6 (7.9 - 25.2)	35.0 (13.5 - 74.2)
Confirmed LGD, (%)	23.0 (16.8 - 29.7)	17.4 (14.2 - 20.0)	23.5 (10.5 - 40.6)	20.6 (12.9 - 35.5)
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Progression of IND, (%)	12.6 (3.6 – 22.2)	6.1 (0 – 18.2)	12.2 (8.3 – 15.4)	14.1 (9.6 – 18.5)
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Significant subset scored indefinite for dysplasia

COURTESY OF CASTLE BIOSCIENCES

Do expert pathologists do a better job?

	Expert pathologists (n=6)	Community-based pathologists (n=6)
Downstaged to NDBE, (%)	68.4 (52.3 – 82.6)	47.6 (12.9 – 72.9)
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COURTESY OF CASTLE BIOSCIENCES

How did TissueCypher perform here?

TissueCypher	155 patients community-based LGD	25 patients progressed to HGD/EAC within 5-yr follow-up
Low-risk score (<5.5)	110 (71.0%)	8 (7.3%)
Intermediate risk (5.5-6.4)	24 (15.5%)	7 (29.2%)
High-risk (>6.4)	21 (13.5%)	10 (47.6%)

- TissueCypher downstaged the majority of community-based LGD cases
- Patients with a low-risk TC score have a low rate of progression to HGD/EAC

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- TissueCypher downstaged the majority of community-based LGD cases.
- Patients with a low-risk TC score have a low rate of progression to HGD/EAC
- Intermediate/high-risk TC scores have a similar high rate of progression to HGD/EAC

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Intermediate/high-risk (>5.5)	45 (29.0%)	17 (37.8%)

- TissueCypher identified 17/25 progressors: sensitivity 68%
- TissueCypher correctly downstaged 102/130 non-progressors: specificity 78.5%.
- How does this compare to the pathologists'performance?

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How does TC compare to the 12 pathologists?

Progression within 5 years	**TissueCypher**	**Pathologists**	
	Intermediate/high-risk *vs.* low risk score	LGD+IND *vs.* NDBE	LGD *vs.* IND+NDBE
Sensitivity	68.0%	67.1% (52.0-84.0)	52.8% (40.0-68.0)
Specificity	78.5%	65.4% (12.3-89.2)	85.1% (62.3-95.3)

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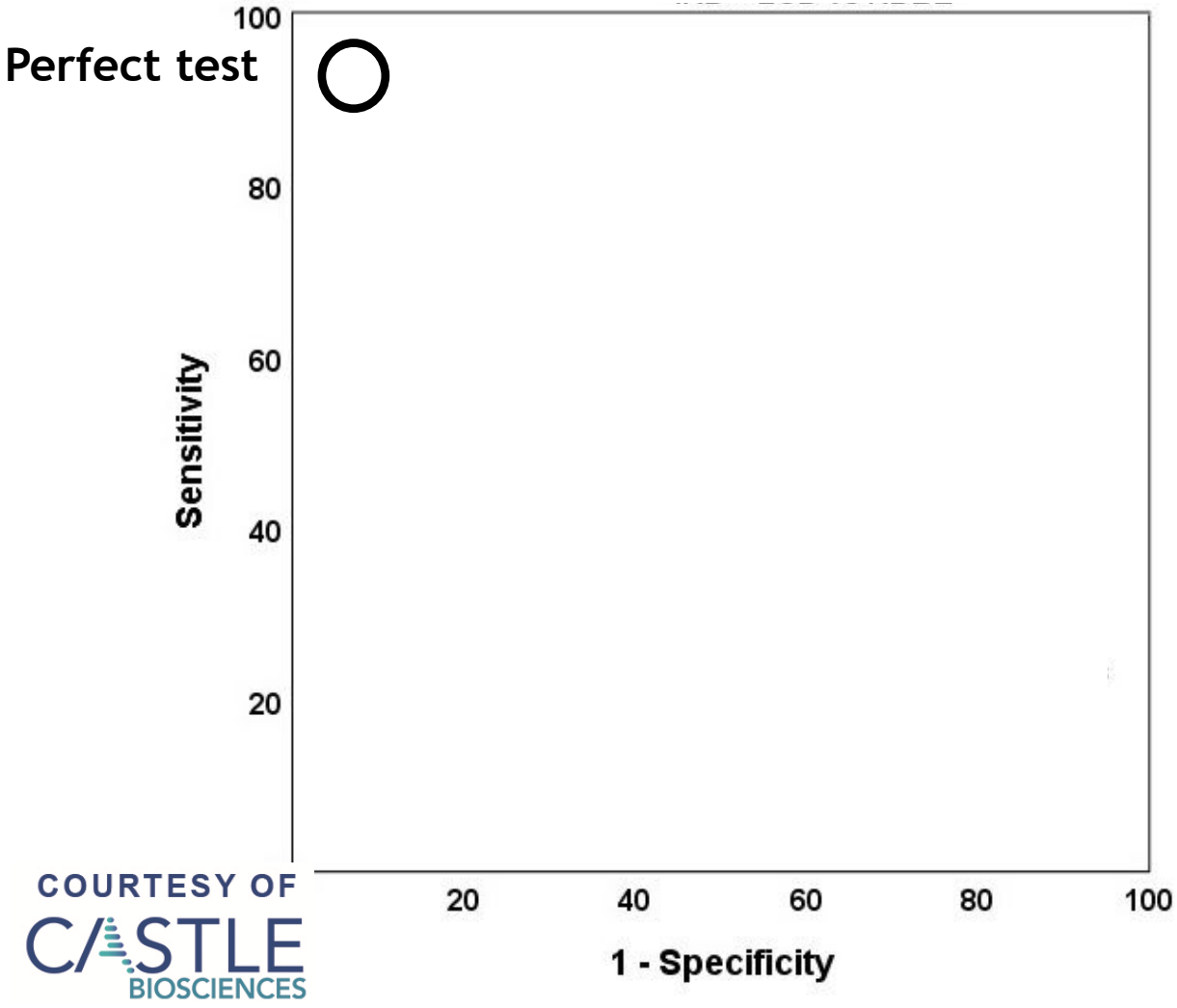
TissueCypher vs. Expert vs. Community-based pathologist



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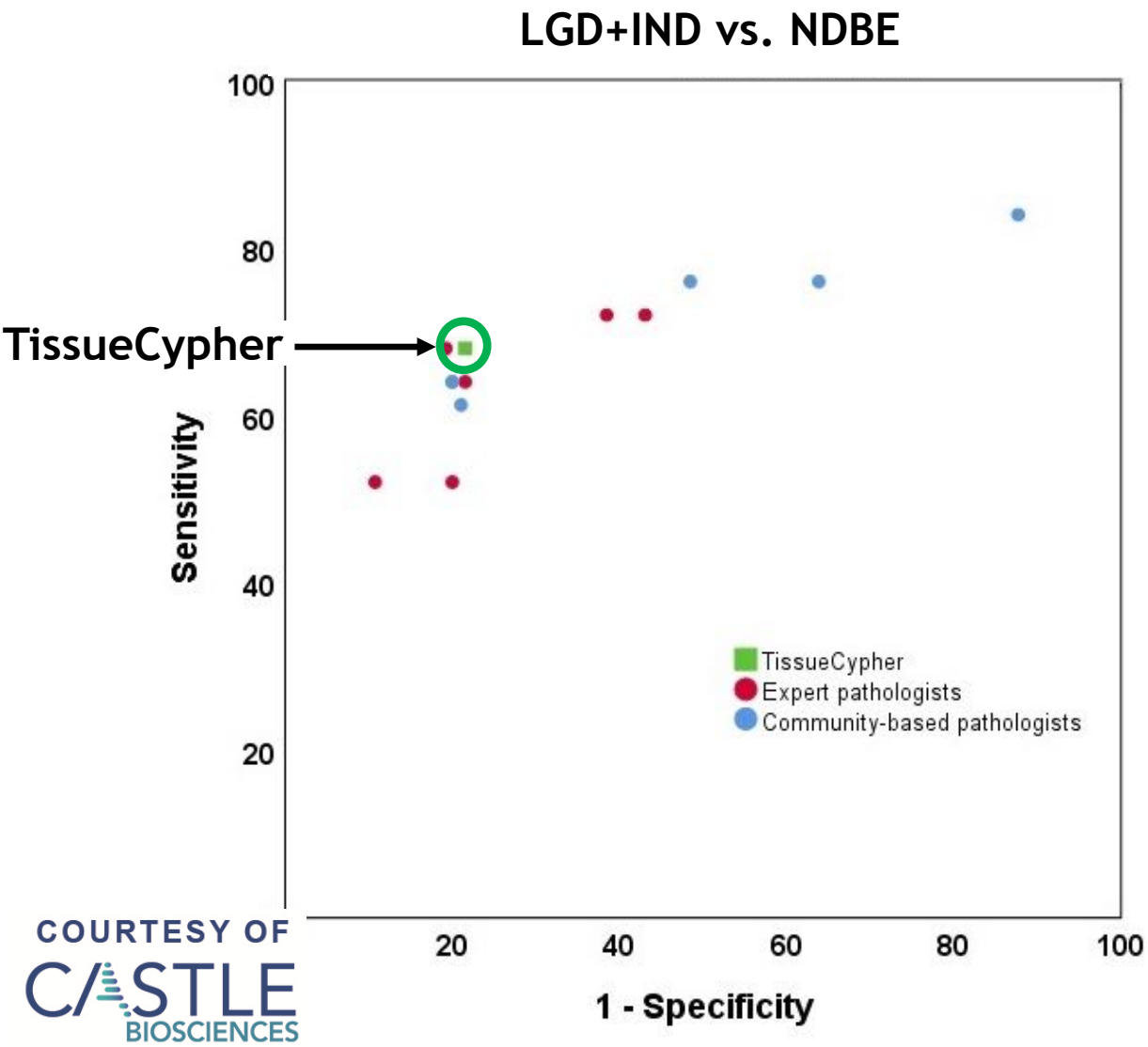

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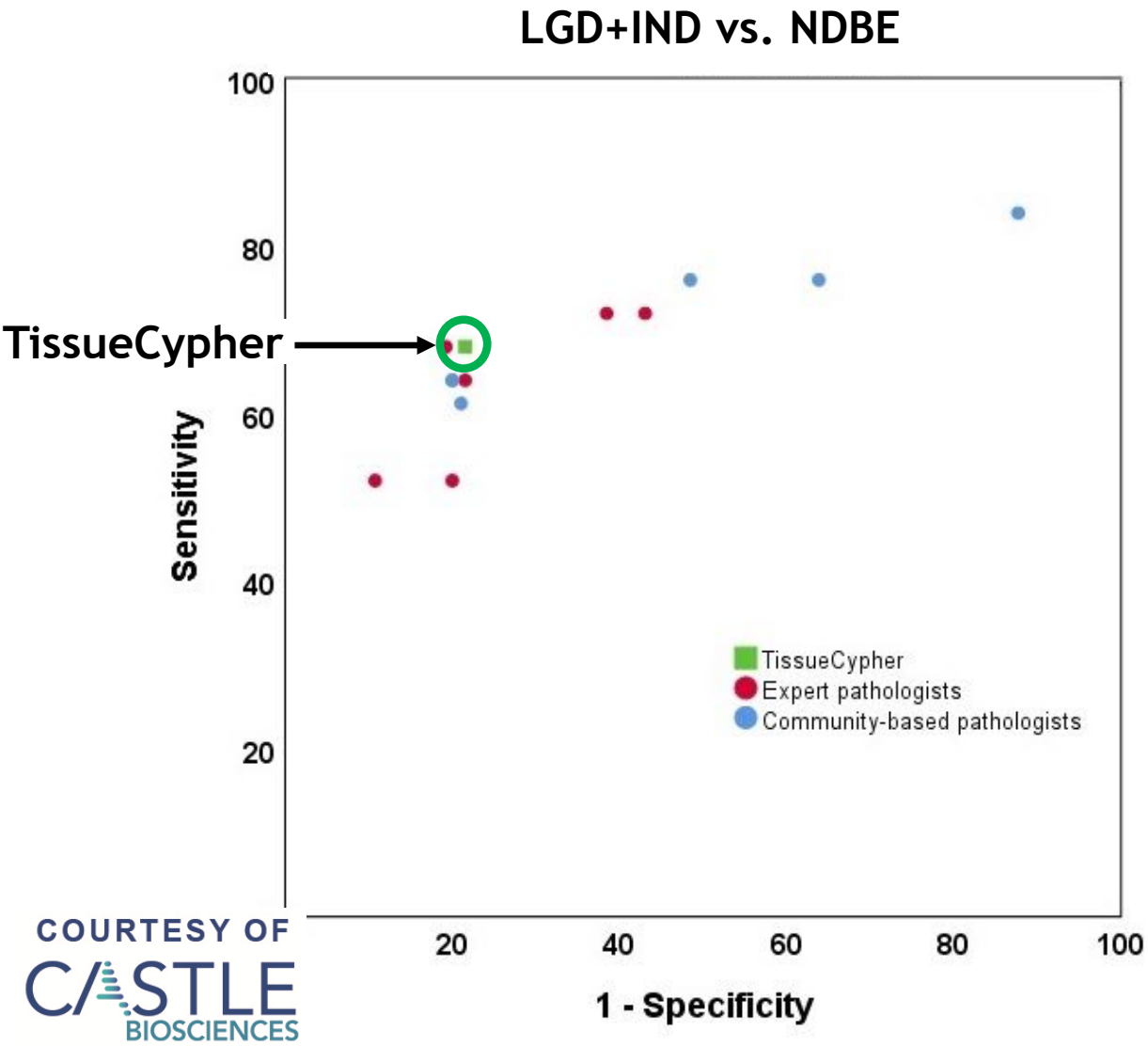

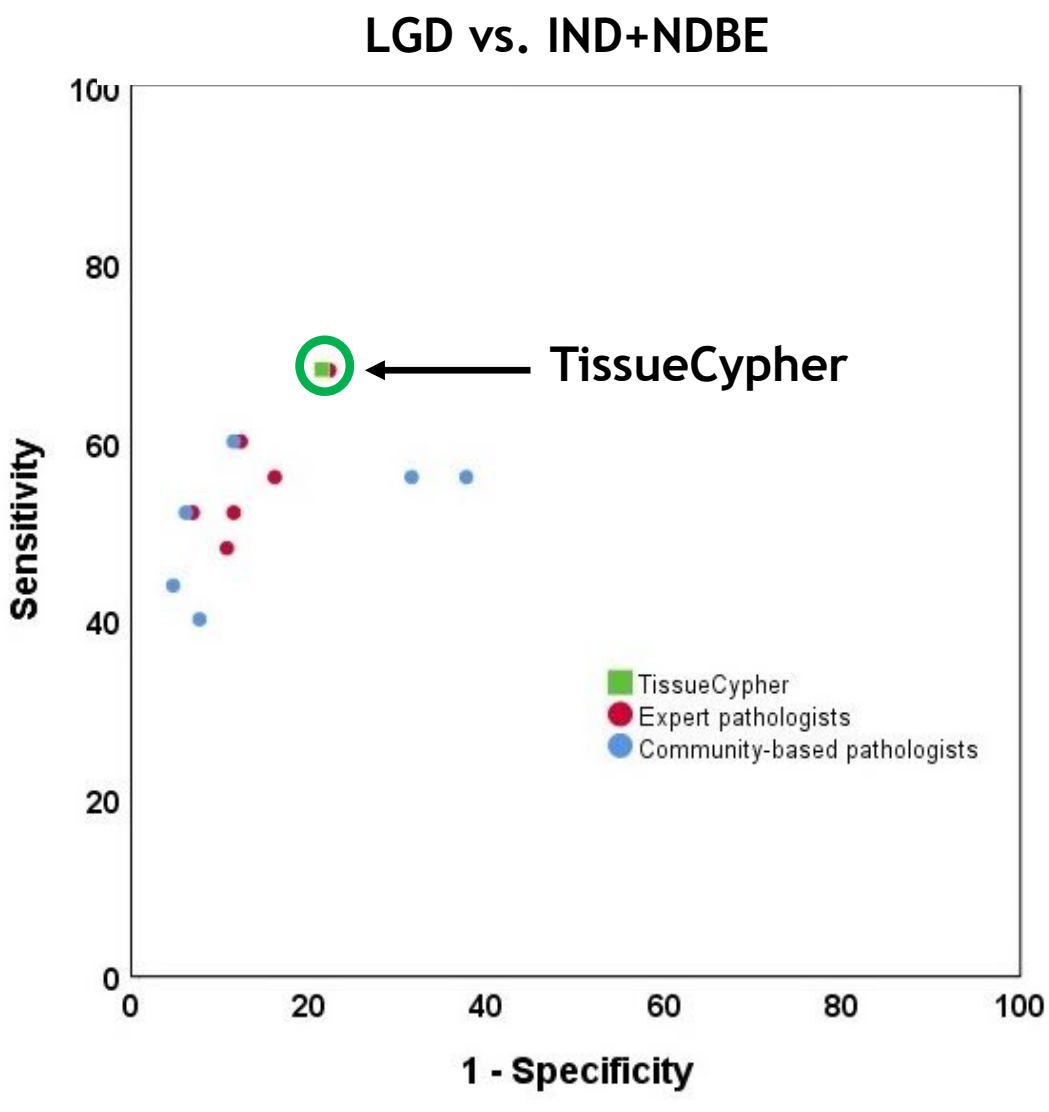

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Risk prediction of LGD in Barrett's

- **Is mandatory for all cases with a community-based LGD diagnosis**
  - Majority of cases will be down-staged to NDBE with low risk of progression
  - It identifies a subgroup with a high-risk of malignant progression

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- **Conventional pathology review has significant limitations**
  - Expert pathology is poorly defined and not widely available
  - Review is subjective and variable – even among expert pathologists
  - A significant subgroup is classified as indefinite for dysplasia

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  - A significant subgroup is classified as indefinite for dysplasia

- **TissueCypher is a more logical tool for risk stratifying LGD**
  - It is fully automated, objective and highly reproducible
  - Outperforms most pathologists
  - Is as good as the best performing expert pathologist

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Amsterdam UMC
Universitair Medische Centra

Thank you for your attention

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