What is the Current Role of Biomarkers and Risk Stratification of Barrett's Esophagus?

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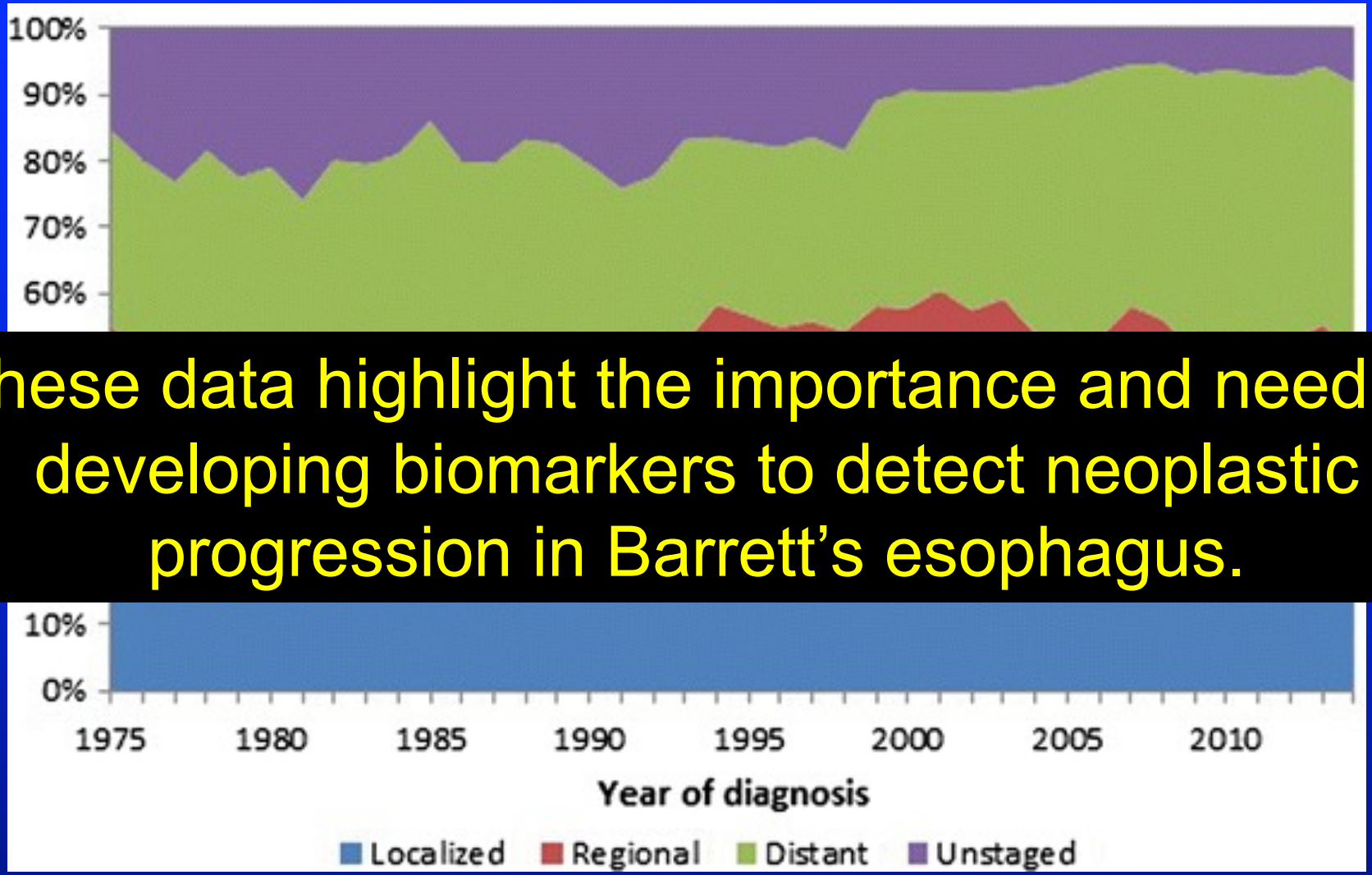
Endoscopic Surveillance Strategies Are Ineffective



These data highlight the importance and need of developing biomarkers to detect neoplastic progression in Barrett's esophagus.

Thrift AP. Dig Dis Sci 2018;63:1988-1996.

Biomarker-Guided Surveillance Strategy: 50 Year-Old White Men with GERD Symptoms Followed 30 Years

Strategy	Cancers	Cancer Deaths
No surveillance	366	356
Dysplasia-guided surveillance	315 (↓14%)	157 (↓56%)
"Perfect" biomarker-guided surveillance	172 (↓53%)	46 (↓87%)

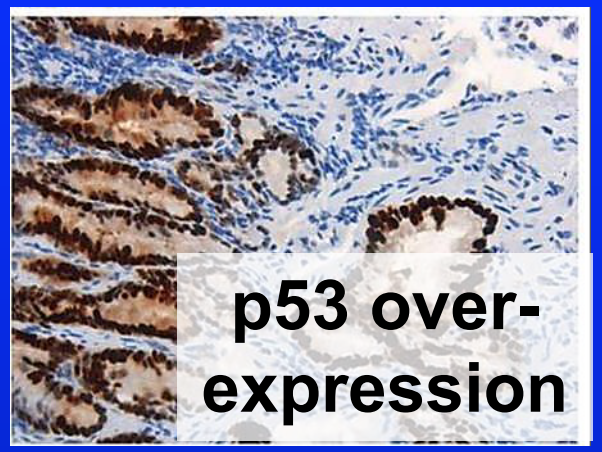
Rubenstein et al. AP&T, 22; 135-146, 2005

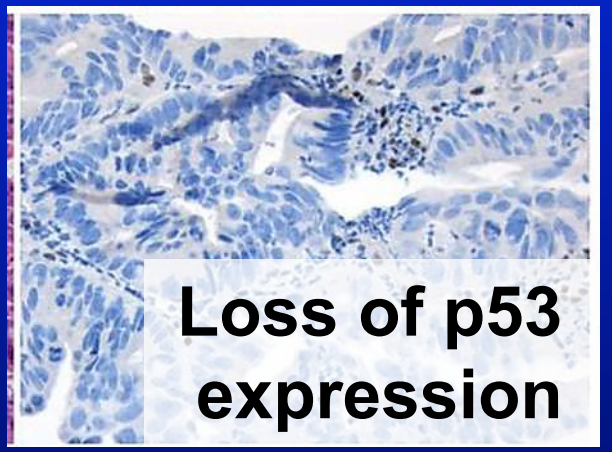
p53 Immunostaining

Largest body of evidence as a biomarker
Easy and commonly used by pathologists

- Mutations that inactivate but stabilize p53 show overexpression
- Mutations that prevent translation show loss of expression

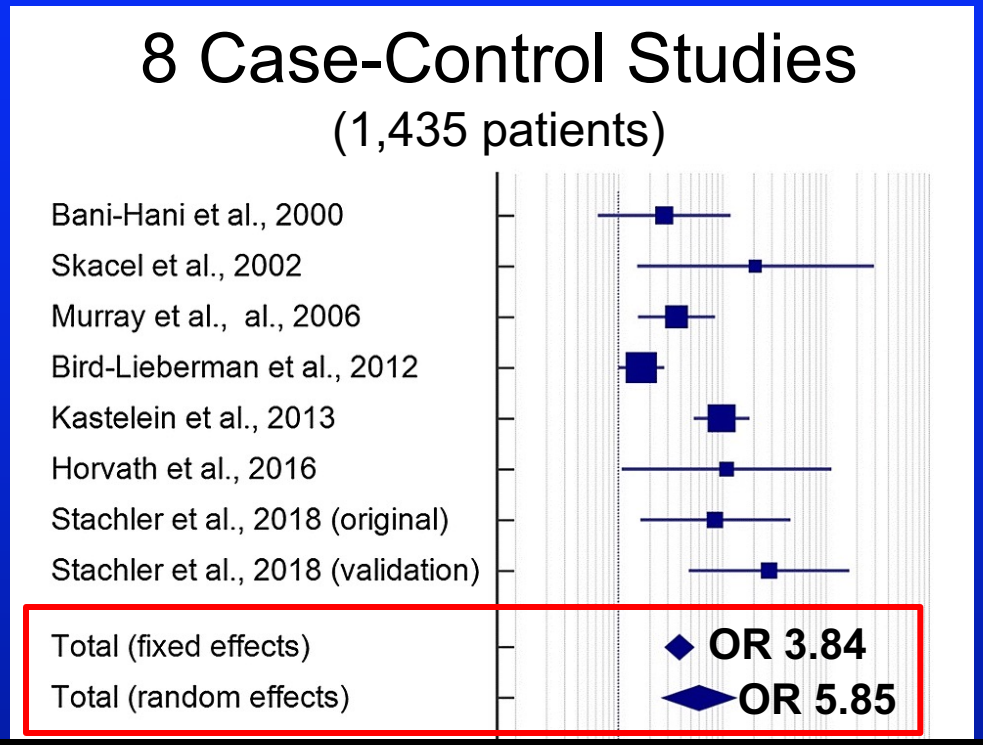
Aberrant p53 Expression

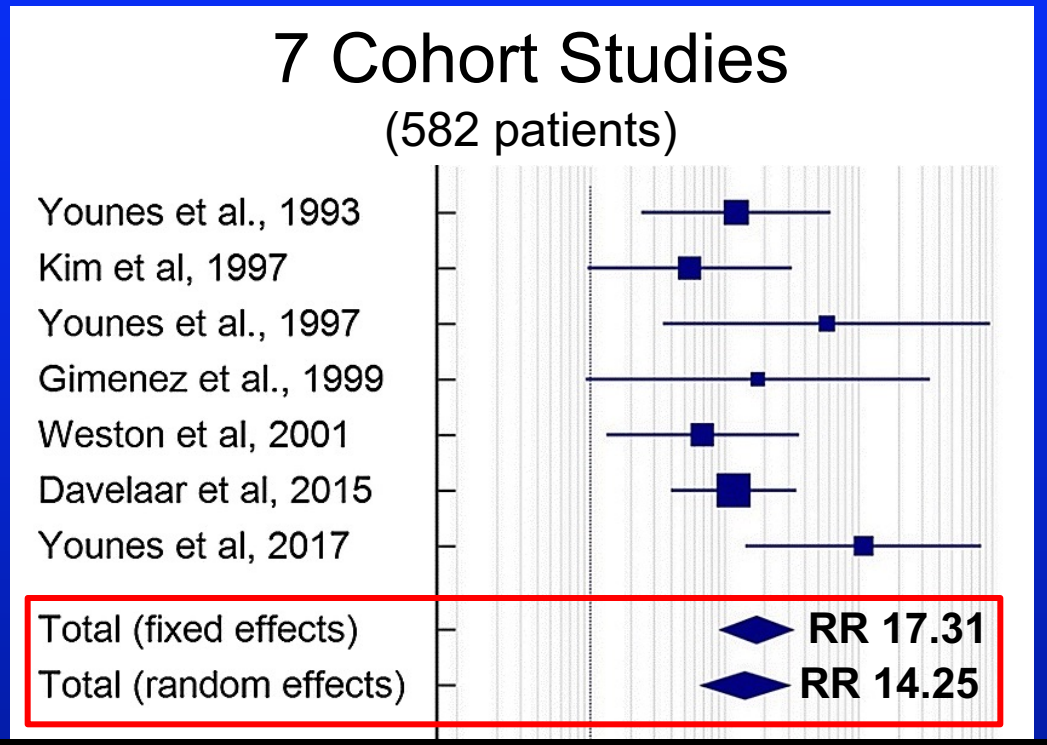




Meta-Analyses of Risk for Neoplastic Progression in BE Based on p53 Immunostaining

- Studies that used p53 IHC in baseline Barrett's biopsies, with follow-up evaluating neoplastic progression





Case-control and cohort studies show consistent, strong, and significant associations between aberrant p53 IHC and progression to high grade dysplasia or cancer.

Meta-Analyses of Risk for Neoplastic Progression in BE with LGD Based on p53 Immunostaining

- 2 studies exclusively enrolled LGD patients
  - Odds ratio was 21 in the case-control study
  - Relative risk was 5.7 in the cohort study

Scoring of p53 is subject to interpretation by the pathologist and there are no widely accepted standard criteria for “abnormal p53 staining”.

Specificity for p53 Immunostaining to Predict Risk

- p53 overexpression: Sensitivity 62%; Specificity 80%
- Loss of p53 expression: Sensitivity 31%; Specificity 98%

Altaf K et al. Br J Surg 2017;104:493-502.

Srivastava et al. Am J Surg Pathol 2017;41:e8-e21.

Abnormal TP53 Predicts Risk of Progression in Patients with Barrett's Esophagus Regardless of a Diagnosis of Dysplasia

- Standardized criteria for abnormal p53 IHC were developed and validated in 233 Barrett's patients (183 NDBE, 50 HGD)
- [illegible]
- [illegible]
- [illegible] follow-up ≥1 year later matched for age and sex
- Abnormal p53 IHC: sensitivity 49.7%%, specificity 93.8%, OR 58, and HR 5 for progression
- Abnormal p53 staining was found > 5 years before the detection of dysplasia

Prospective validation of these criteria for abnormal p53 IHC by a large, community-based pathology practice confirmed their ability to predict neoplastic progression in NDBE.

Redston M et al. medRxiv 2020.10.18.20213561.

Systems Biology

- Views tissue as a system with multiple compartments to be analyzed for genetic, immunologic, vascular, and morphologic features of neoplastic progression
- TissueCypher™ (Cernostics)
  - Uses formalin-fixed, paraffin-embedded Barrett's biopsies
  - Analyses for a panel of biomarkers associated with Barrett's neoplasia in epithelial, stromal, and immune cells

Biomarker	Process Involved	Abnormality Indicated
p53	Tumor suppressor, apoptosis	Nuclear overexpression or loss in epithelial cells
p16	Cell cycle control	Cellular loss in epithelial cells
HER2/neu	Cell growth, proliferation	Plasma membrane overexpression in epithelial cells
AMACR	Lipid metabolism	Overexpression in peroxisomes and mitochondria of epithelial cells
COX-2	Inflammation	Overexpression in epithelial and stromal cells
CD68	Macrophages	Stromal density and phenotype of macrophages
CD45RO	Memory lymphocytes	Stromal density
HIF-1α	Angiogenesis	Expression and subcellular localization in stromal cells
Cytokeratin-20	Metaplasia	Plasma membrane expression in epithelial cells

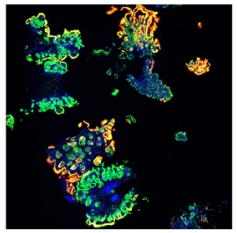
TissueCypher® For Predicting Risk of Neoplasia

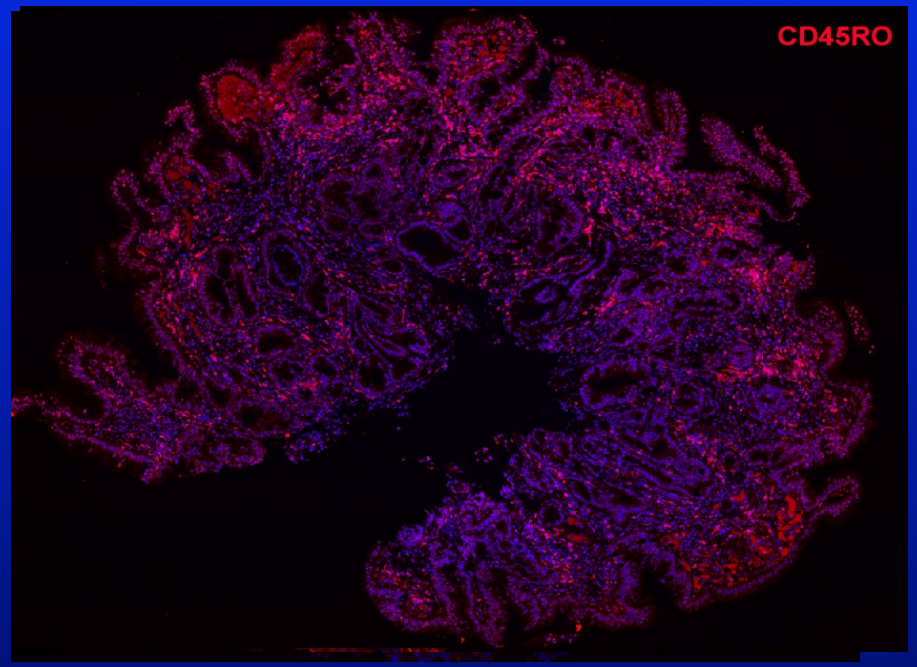
Multiplexed Immunofluorescence Slide Labeling

9 protein-based biomarkers plus Hoechst labeling of nuclei

Whole Slide Fluorescence Scanning

4 registered channels of image data for each slide





Studies on TissueCypher® for Predicting Neoplastic Progression of Barrett's Esophagus

5 independent studies of 239 progressors and 656 non-progressors at medical centers in US and Europe

Case-control studies show consistent, strong, and significant associations between a TissueCypher high-risk score and progression to high grade dysplasia or cancer and

between a TissueCypher low-risk score and *lack* of progression to high grade dysplasia or cancer.

- NIH funded study (Cleveland Clinic & UPMC) that independently validated ability of TissueCypher to predict

Non-dysplastic Barrett's patients who scored high-risk progress to high grade dysplasia or cancer at a rate higher than that with an expert pathologist-confirmed diagnosis of low grade dysplasia.

Dysplasia. Am J Gastroenterol. 2021 Apr;16(4):675-682.

- Further independent validation of TissueCypher's ability to predict progression patients with LGD (n=155)

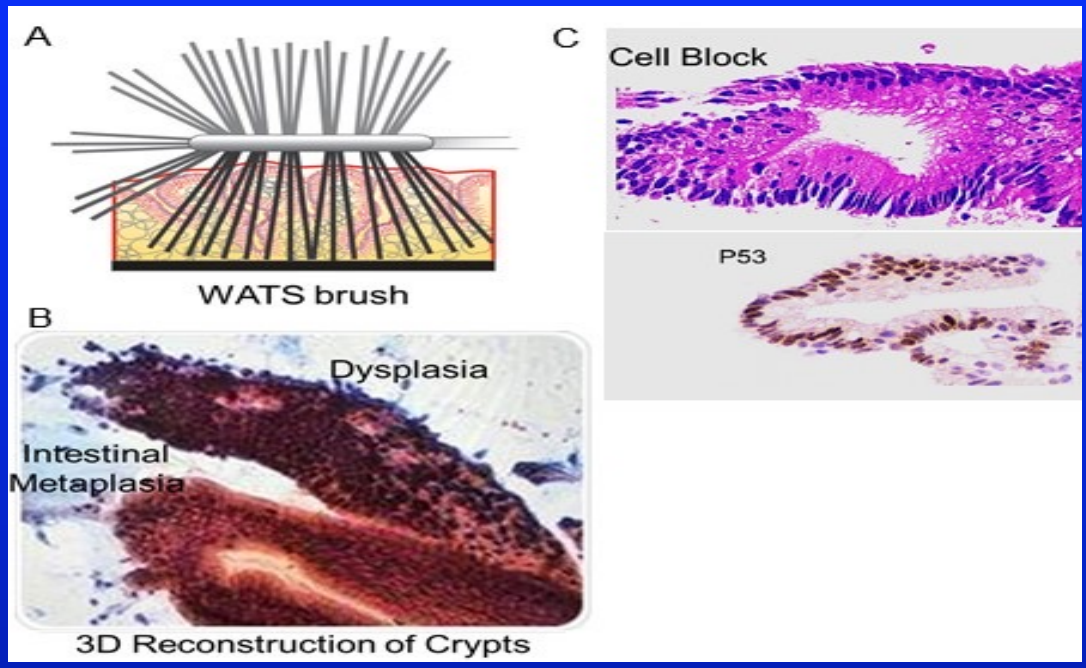
Prediction of Progression in Barrett's Esophagus Using a Tissue Systems Pathology Test: A Pooled Analysis of International Multicenter Studies

Iyer. DDW 2021

- 475 Barrett's patients (403 NDBE, 43 LGD, 29 IND) from 4 independent case-control studies in the US and Europe
- Cases: 152 patients developed HGD/EAC on follow-up ≥1 year later
- Controls: 323 patients **did not** develop HGD/EAC on follow-up ≥1 year later matched for age and sex
- Age, sex, histologic diagnosis, BE segment length, and hiatal hernia used in a clinical prediction model
- High risk score: sensitivity 38.6%, specificity 93.8% and independently predicted a ~8-fold ↑ in progression
- The TissueCypher risk score significantly improved the predictive value of the clinical model

Imaging Biomarker: Wide-Area Transepithelial Sampling (WATS) with Computer-Assisted 3D Analysis

- Abrasive brush scrapes an extensive area of the Barrett's segment
- Provides a thick tissue specimen
- Combination of AI neural network and 3D computer imaging of the tissue to identify abnormal cells for review by a pathologist.



Courtesy of Dr. Rob Odze and CDx

- Obtain a cytology specimen for morphological analysis as well as for immunostaining for proteins such as p53

The Addition of WATS to Forceps Biopsies Detects More Indefinite and LGD

- Community endoscopists at 21 sites enrolled 12,899 patients having *screening or surveillance* for Barrett's esophagus
  - FB found dysplasia in 88 pts (0.68%), WATS added 213 pts (1.65%)
  - WATS increased dysplasia detection rate from 0.68% to 2.33%
- *ASGE Conditional Recommendation*: In patients with known or suspected Barrett's esophagus, we suggest using WATS-3D **in addition** to Seattle protocol biopsy sampling.
  - WATS increased dysplasia detection rate from 2% to 5%
  - 74 additional patients: 70 indefinite/LGD, 4 HGD

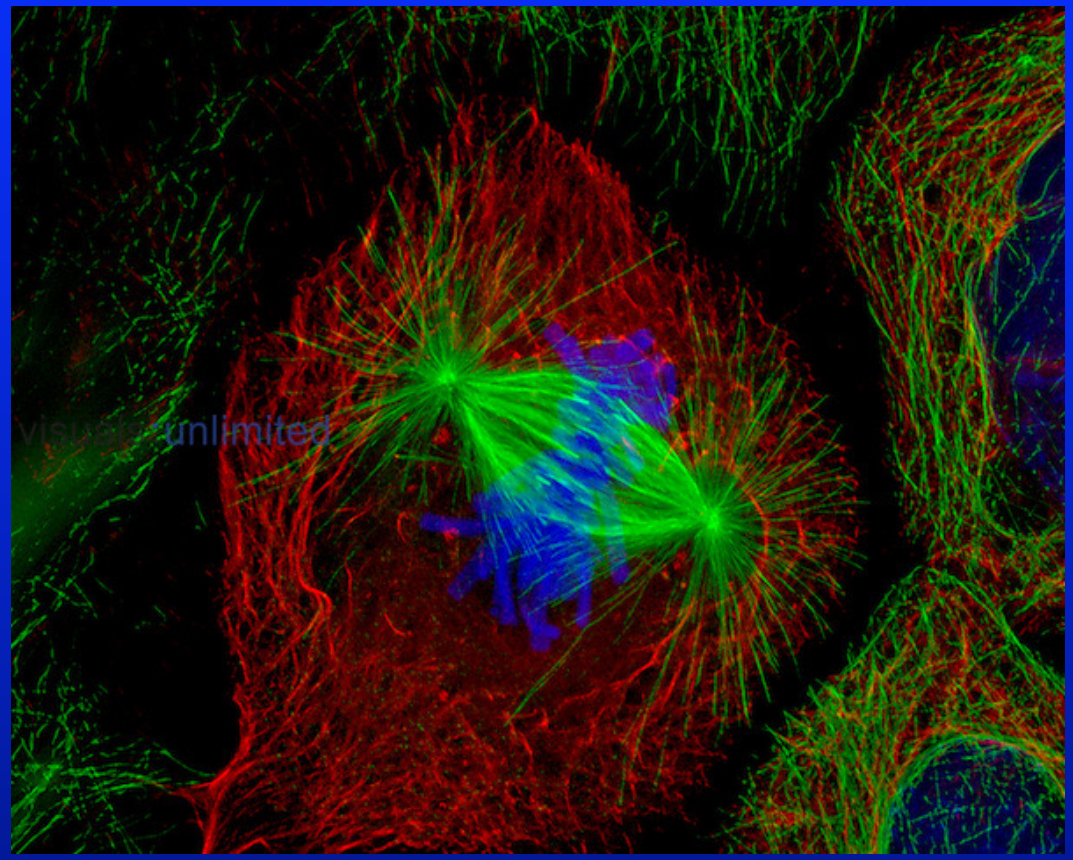
Smith MS et al. Dis Esoph 2019;32(3).

Tests of Genomic Instability For Predicting Neoplastic Progression

- **Genomic Instability**

(↑ genome alterations during cell division)

  - Small structural alterations (base pair mutation, MSI)
  - Large structural alterations [chromosome number (aneuploidy), structure-LOH]



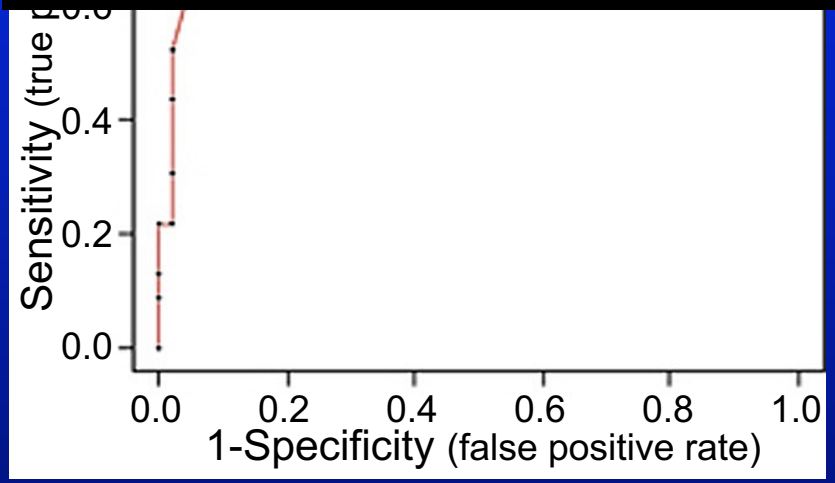
Mutational Load

- **Mutational Load** (Index of genomic instability)
  - BarreGEN®
    - Pathologist microdissects targets on H&E slides
    - LOH mutations and MSI at 10 loci for tumor suppressor genes assessed using PCR and quantitative capillary electrophoresis of DNA
    - Mutational load (ML) quantified on a scale of 0-10

Mutational Load Predicts Neoplastic Progression

- Case-Control Study
  - 23 Cases: Patients with NDBE or LGD at baseline who developed HGD/EAC on follow-up ≥1 year later
  - 46 Controls: Patients with NDBE or LGD at baseline who **did not** develop HGD/EAC on follow-up ≥1 year later

Contradictory study results limit the utility of mutational load for prediction of neoplastic progression in Barrett's esophagus.

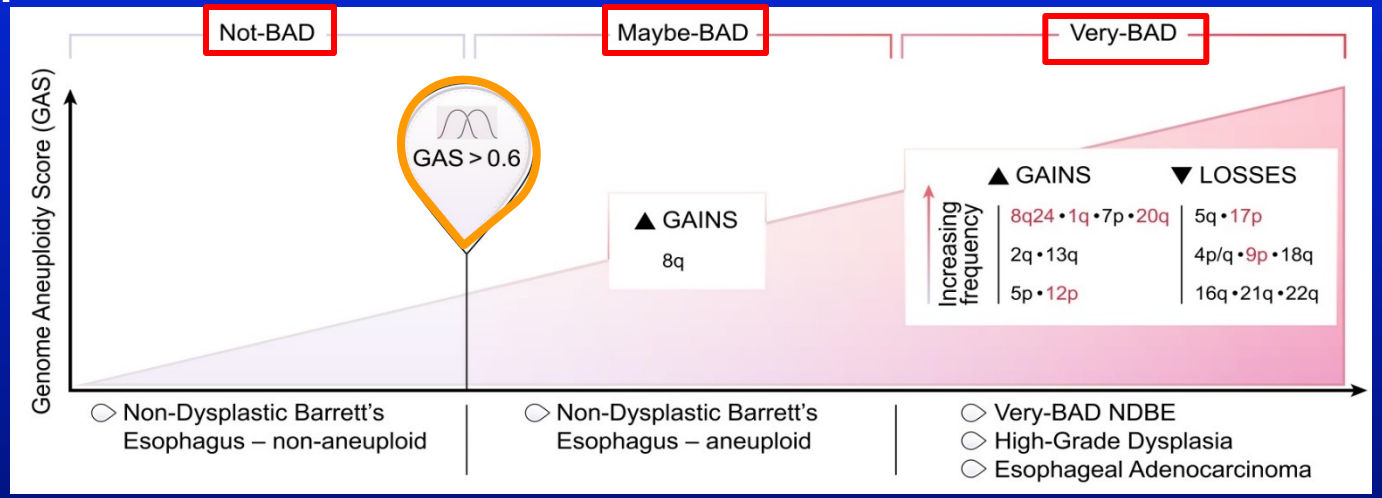


  - discrimination of ML in predicting progression (AUC 0.50 at ML≥1)
  - Used crude lysates rather than purified DNA

Eluri S et al. Dis Esoph 2018;31.

Barrett's Aneuploidy Decision: BAD

- Novel PCR-based method (RealSeq) to amplify genome-wide loci for sequencing allowing for the identification of single chromosomal arm gains or losses
- Performed on esophageal brushings
- Chromosomal arm level scores are integrated into a Global Aneuploidy Score (GAS)
- 6 specific chromosomal alterations- gain 1q, 12p, 20q and 8q24; loss 9p and 17p
- BAD Score: Global Aneuploidy Score + 6 chromosomal alterations



Douville…Chak Gastroenterology 2021, 160:2043-2054.

BAD Scores May Predict Neoplastic Progression

- Medical records were reviewed in a blinded fashion for 60 NDBE cases over a 36 month follow up period
  - 40 cases were classified as Not-BAD and none of these cases progressed to HGD/EAC
  - 16 cases were classified as Maybe-BAD and none of these cases progressed to HGD/EAC
  - 4 cases were Very-BAD and 2 of the 4 (50%) progressed

New, promising biomarker test that is easy to use (PCR-based) and can be performed on endoscopic brushings…

And the name is not BAD either!

Douville…Chak Gastro