Primary screening test development for ESCC

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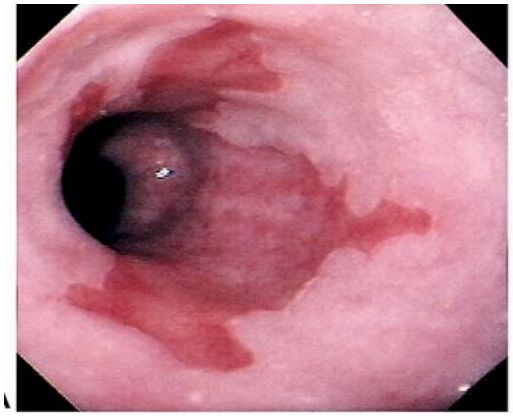
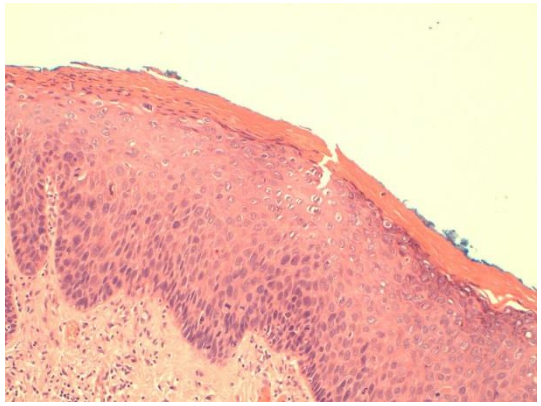
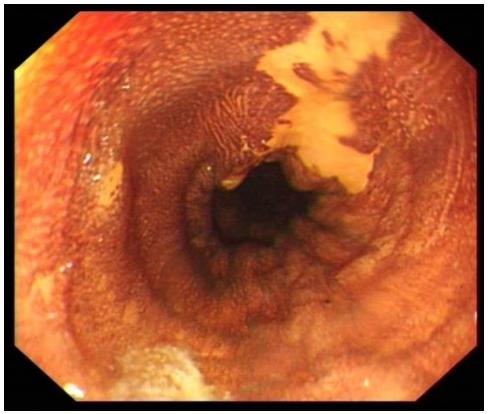
Endoscopic detection of early lesions

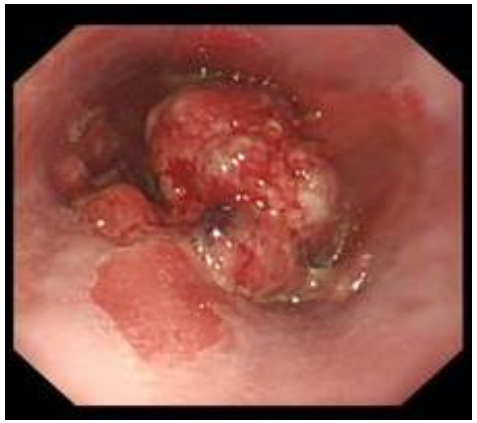


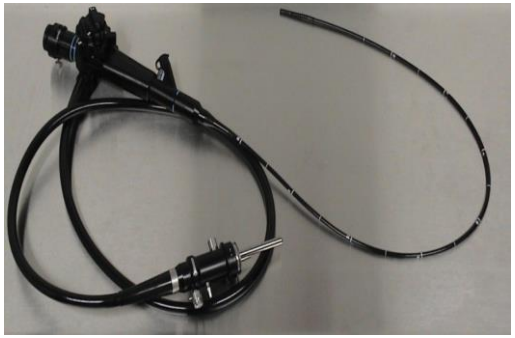




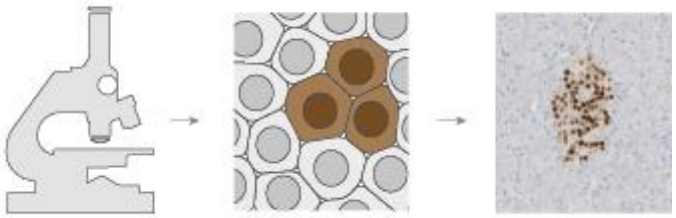
Barrett's to adenocarcinoma

Squamous dysplasia to
Squamous cell carcinoma





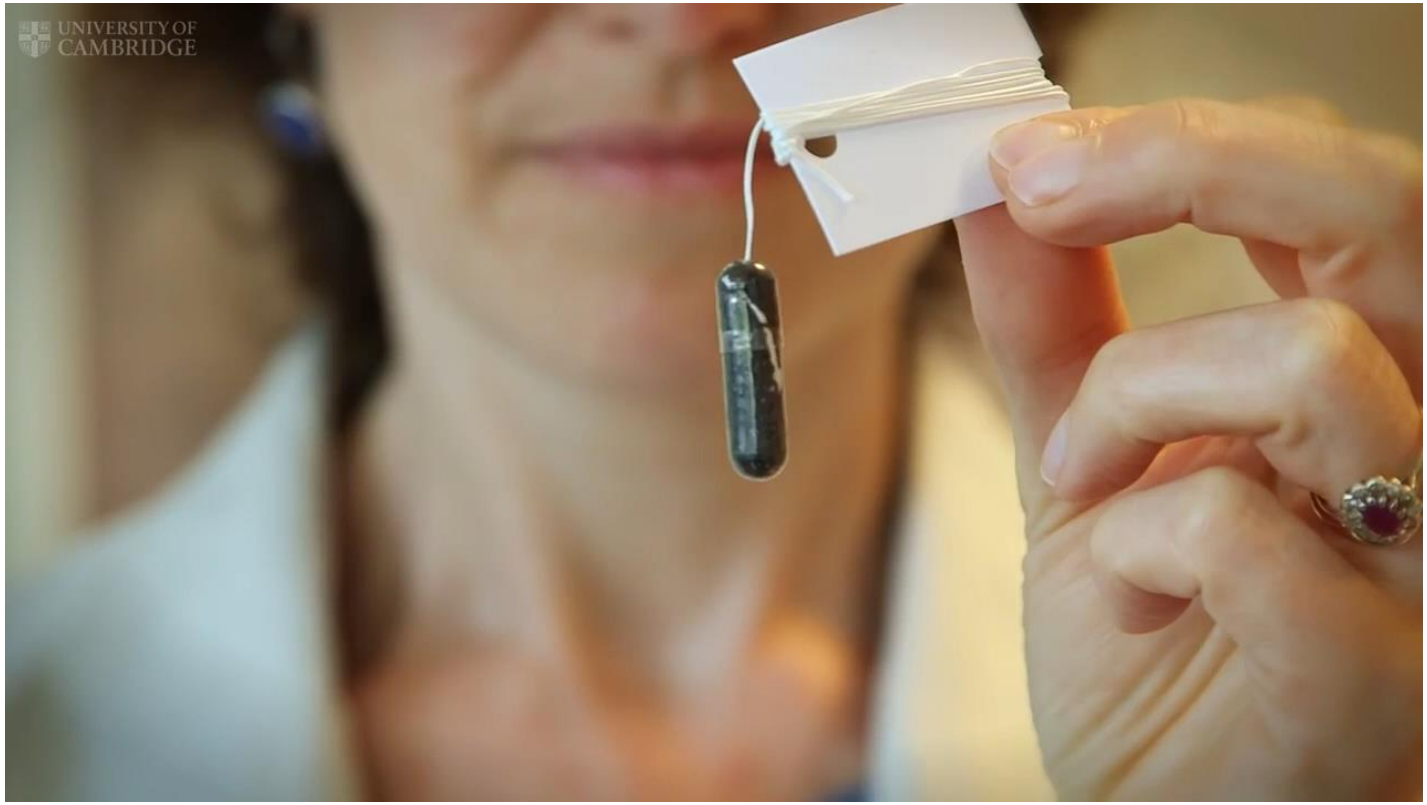
Concept of device + biomarkers



Objective biomarker assays for diagnosis and risk stratification

Non-endoscopic cell collection (prototype 2001)
Collect along entire oesophagus and minimise sampling bias

Pan-oesophageal sample collection in primary care



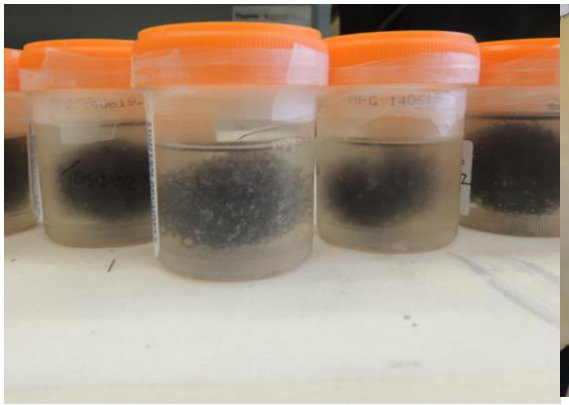
Barrett's trial data > 3,000 patients (pilot, BEST1 and BEST2 trials)

- Safe
- Acceptable
  - 80% preferred Cytosponge to endoscopy
  - Often tolerated better than endoscopy (p=0.0003)
- Transferable technology in rural settings
- Economics favourable

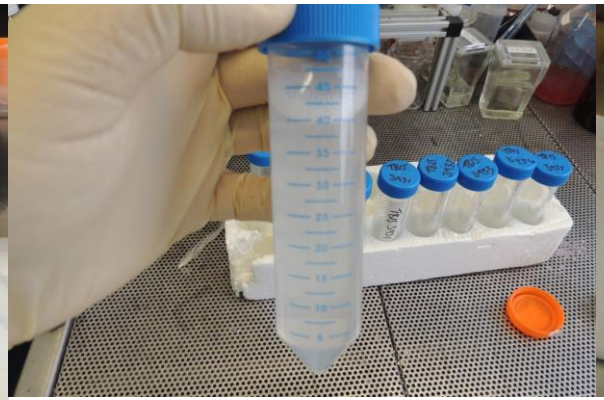
Kadri S….Fitzgerald RC BMJ 2010; 341: c4372 (BEST1)
Ross-Innes…Fitzgerald *PLOS Medicine 2015; doi: 10.1371 (BEST2)*
Benaglia T et al *Gastroenterology. 2013 Jan; 144:62-73*

Laboratory Processing

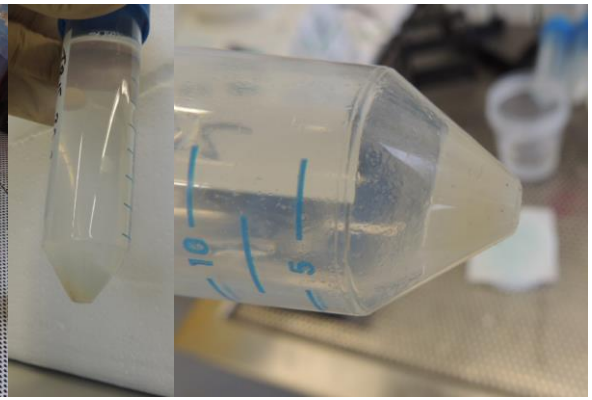
- High throughput capacity
- Preserving tissue architecture



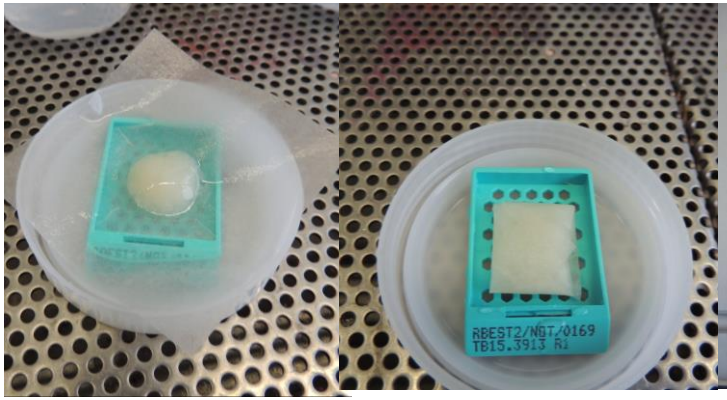
Shake and vortex



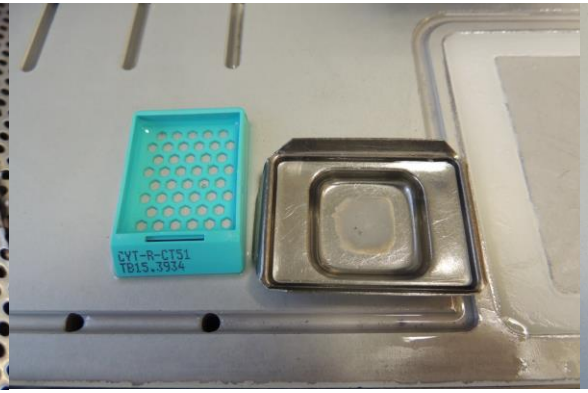
Shake and vortex, spin down to cell pellet

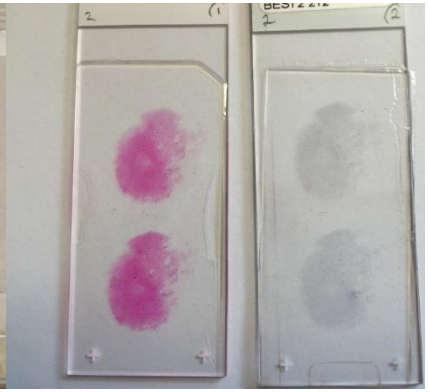


Make a thrombin clot

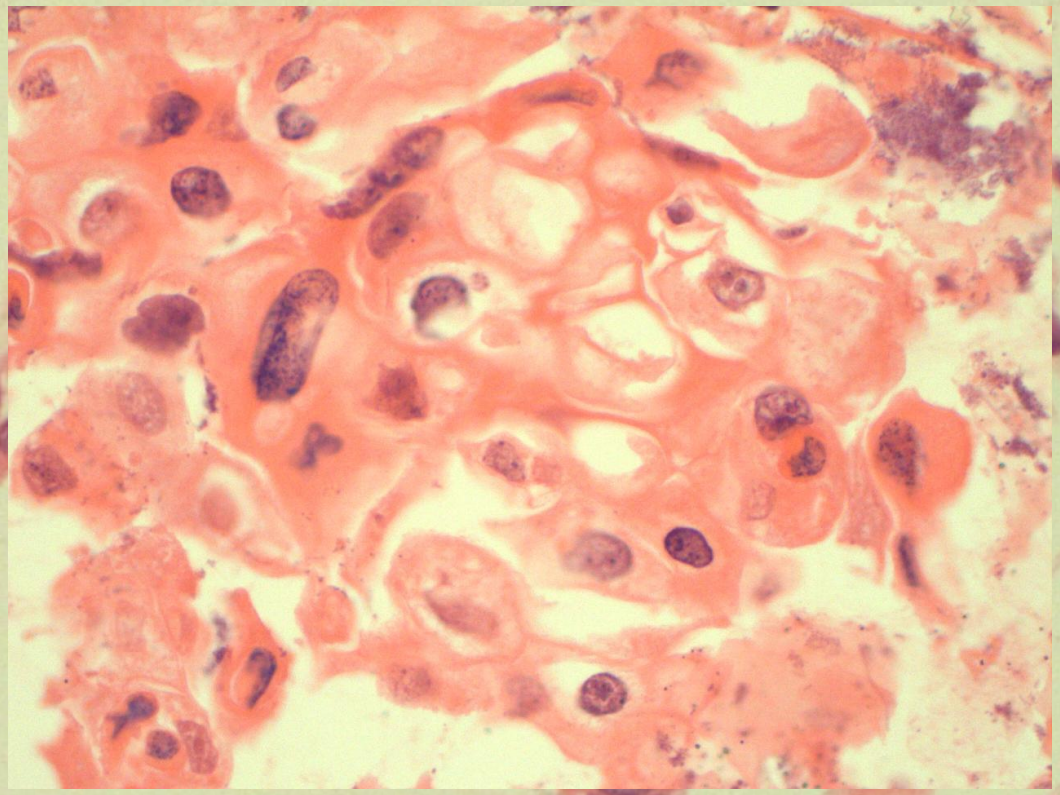


Process clot to a paraffin block

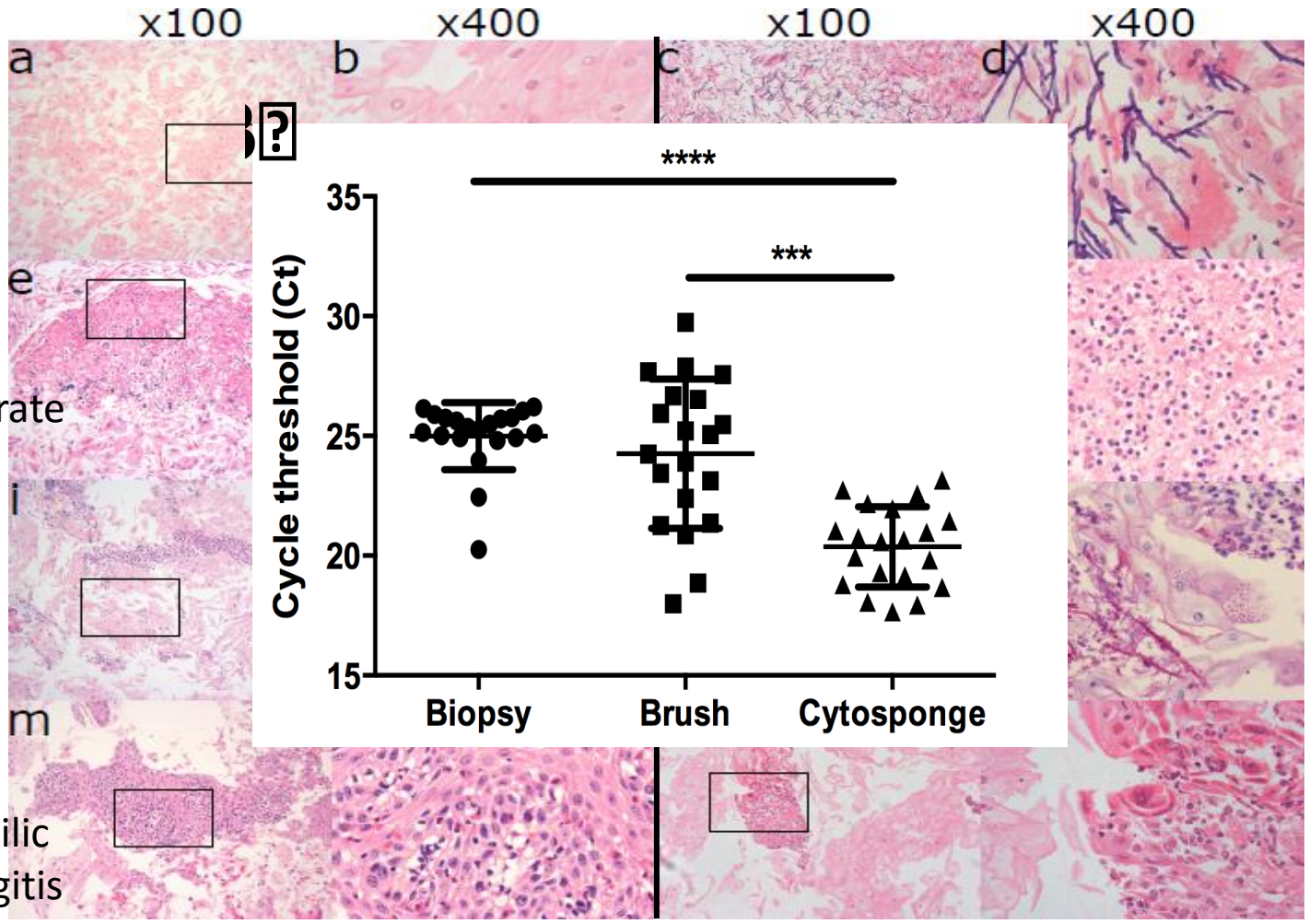




Stained slides
Sections for DNA extraction



Immune cells and pathogens on Cytosponge



Normal

Immune Cell infiltrate

Candida

Eosinophilic oesophagitis

Aspergillus

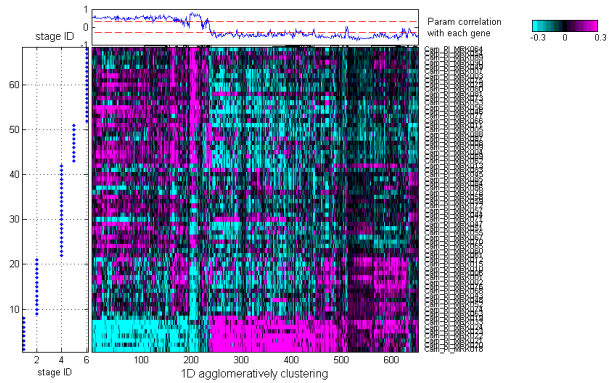
Ulcerated

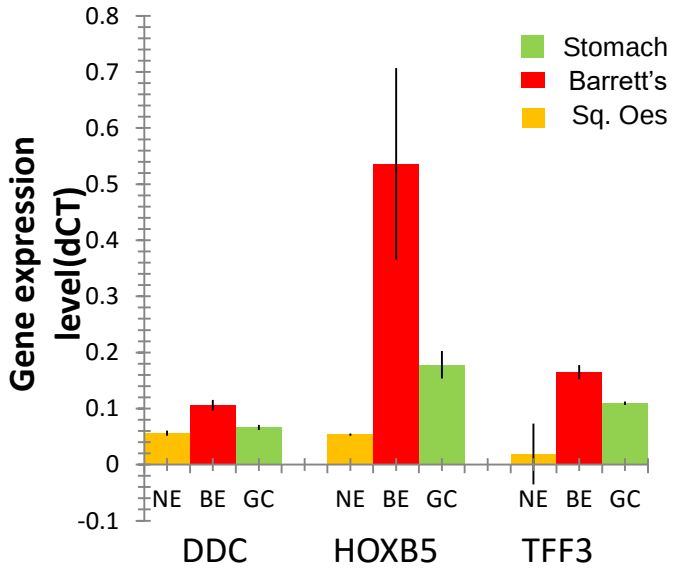
Candida (PAS stain)

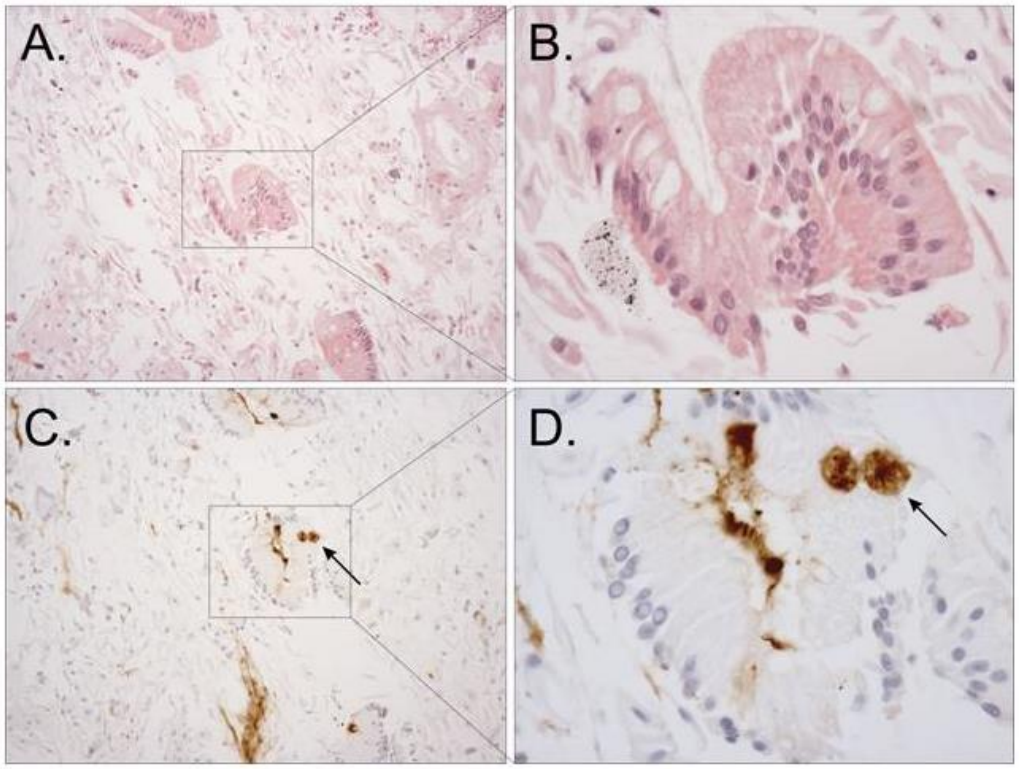
Herpes

Paterson et al J of Histopathology 2016 *in press;* Fels-Elliott et al*Lancet Gastro & Hepatol in press 2016*

Biomarker experience from Barrett's







Antibody to TFF3

Lao-Sirieix *et al.* GUT, 2009

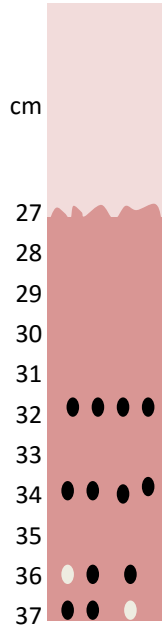
Accuracy data for TFF3 in detecting Barrett's (UK data BEST trials)

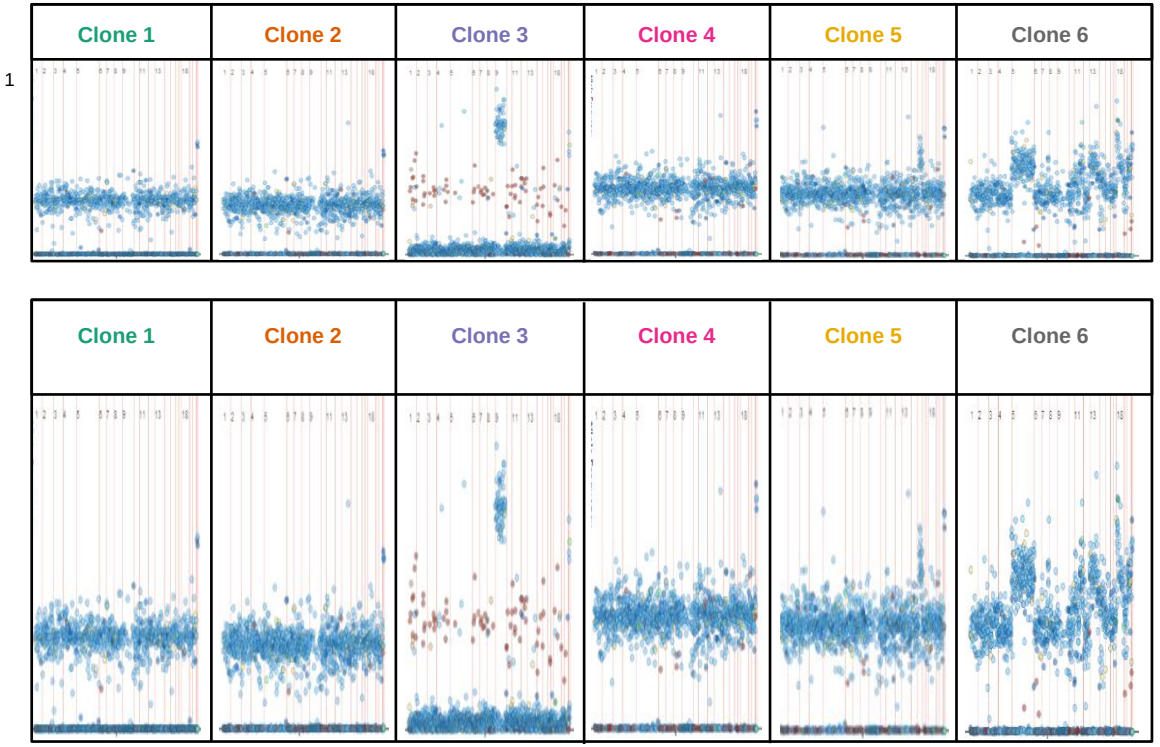
Study	Publication Year	Study type	Setting	Barrett's length (cm)	Sensitivity % (95% CI)	Specificity % (95% CI)
Pilot N= 40	2008	Cohort	2ndary care	≥C1	78.0 (64.0-89.0)	94.0 (87.0-98.0)
BEST1 N= 500	2010	Prospective	1ary care	≥C1	73.3 (44.9-92.2)	93.8 (91.3-95.8)
				≥C2	90.0 (55.5-99.7)	93.5 (90.9-95.5)
BEST2 N= 1,100	2014	Case:Control	2ndary care	≥C1	79.5 (75.9-82.9)	92.4 (89.5-94.7)
				≥C2	83.9 (80.0-87.3)	
				≥C3	87.2 (83.0-90.6)	

Kadri S….Fitzgerald RC BMJ 2010; 341: c4372 (BEST1)

Ross-Innes…Fitzgerald PLOS Medicine 2015; doi: 10.1371 (BEST2)

Cytosponge captures entire clonal architecture





X axis for each clone chr 1-23
Y axis VAF for each mutation

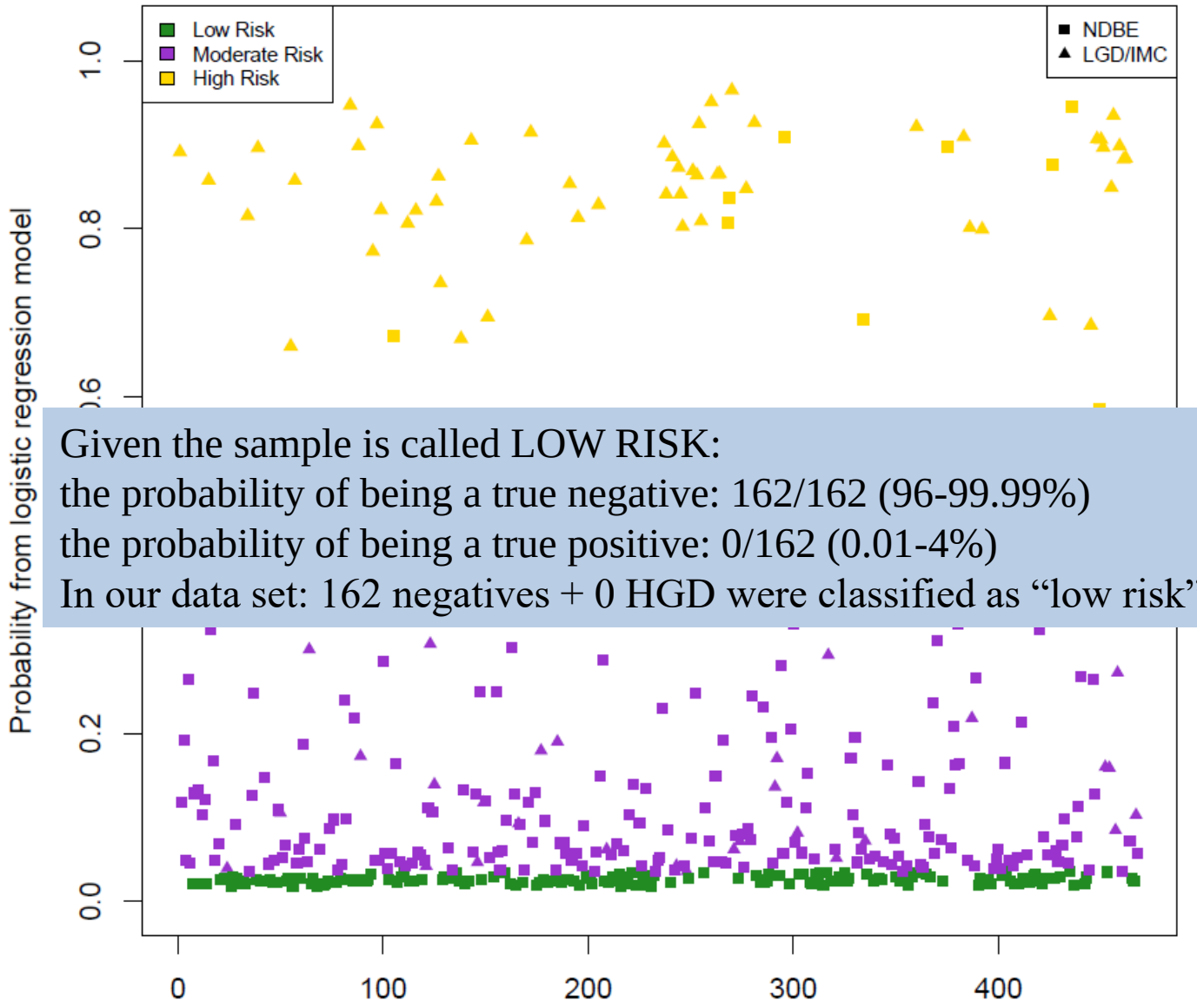
One of 1,437 SNVs

Ross-Innes et al *Nature Genetics* 2015

Barrett's Risk stratification panel

(Age, BMI, Barrett's length, atypia, p53 status)

(BEST2 n=468)

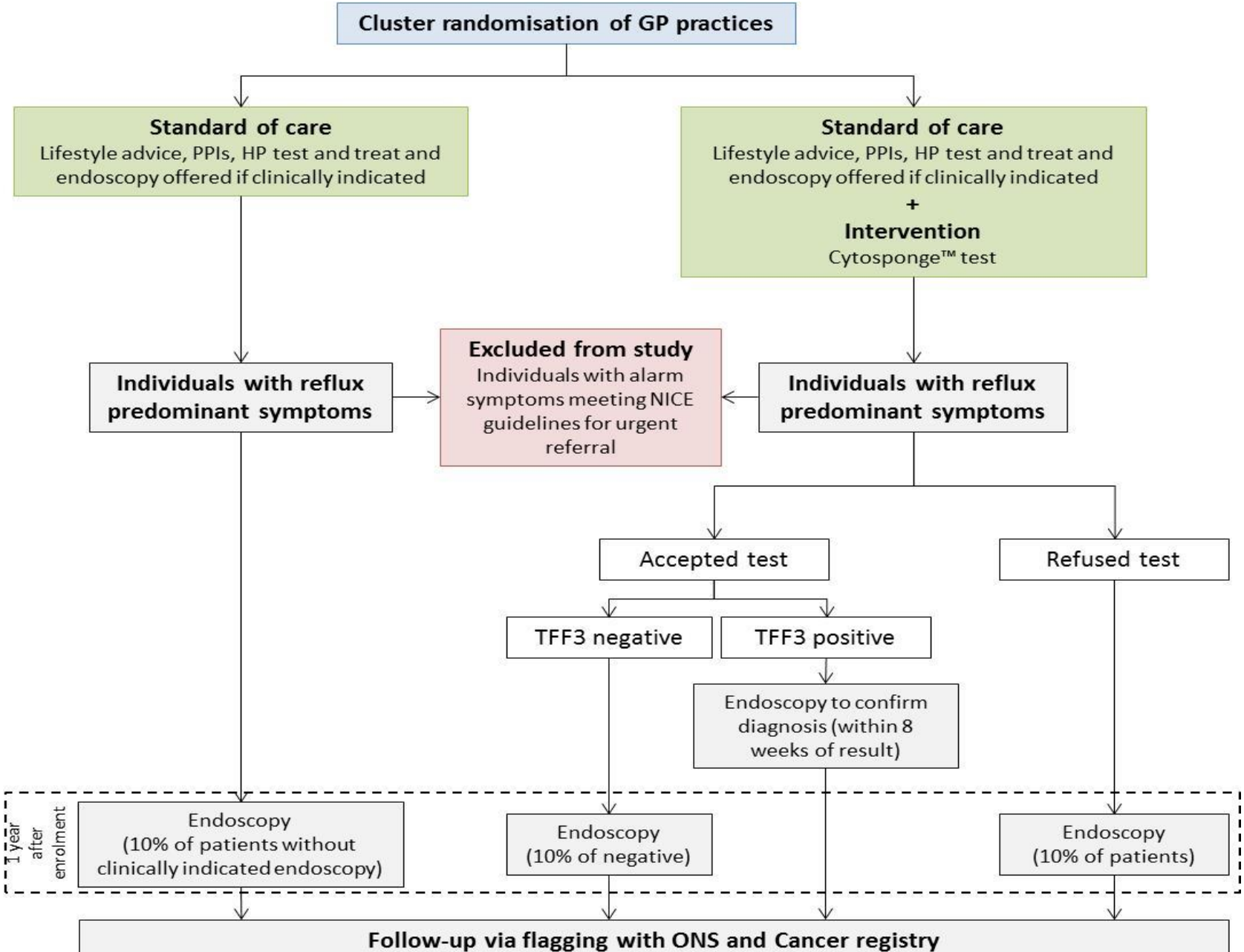


Given the sample is called LOW RISK:
the probability of being a true negative: 162/162 (96-99.99%)
the probability of being a true positive: 0/162 (0.01-4%)
In our data set: 162 negatives + 0 HGD were classified as "low risk"

Ross-Innes et al The Lancet Gastro & Hepatology 2016 *In press*

Weaver et al Nature Genetics 2014; 46: 837-43

BEST3 Trial Design (n=4,000 randomised)



Cytosponge for ESCC – China and Iran pilot studies using atypia and p53 IHC

Accuracy data– Iran pilot study

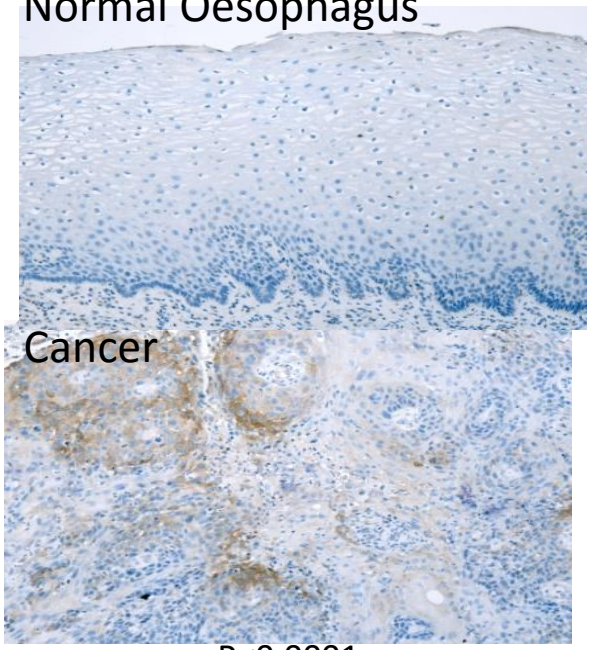
	Endoscopic examination	
Cytological examination	**ESD (all types)**	**High-grade ESD**
ASC		
Sensitivity (95% CI)	50% (29–71%)	100% (51–100%)
Specificity (95% CI)	99% (96–99%)	97% (94–98%)
PPV (95% CI)	69% (39–90%)	31% (10–61%)
NPV (95% CI)	97% (94–98%)	100% (98–100%)
Accuracy (95% CI)	96% (93–98%)	97% (94–99%)
P53 positivity		
Sensitivity (95% CI)	22% (9–45%)	100% (51–100%)
Specificity (95% CI)	89% (85–92%)	89% (85–92%)
PPV (95% CI)	11% (4–28%)	11% (4–28%)
NPV (95% CI)	95% (91–97%)	100% (98–100%)

N=344
N=131 unstained lesions
N=18 with dysplasia
of which 4 mod/severe

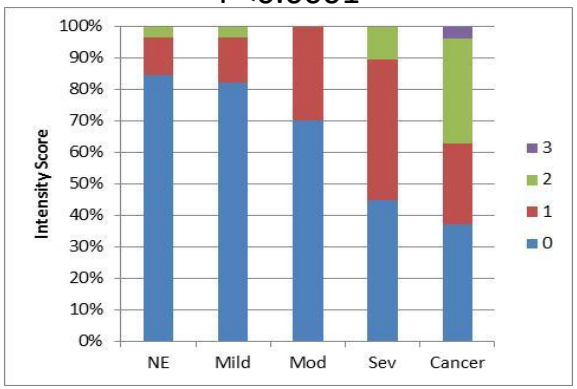
Roshandel et al Br J Cancer 2014

Immunohistochemical biomarkers for ESCC

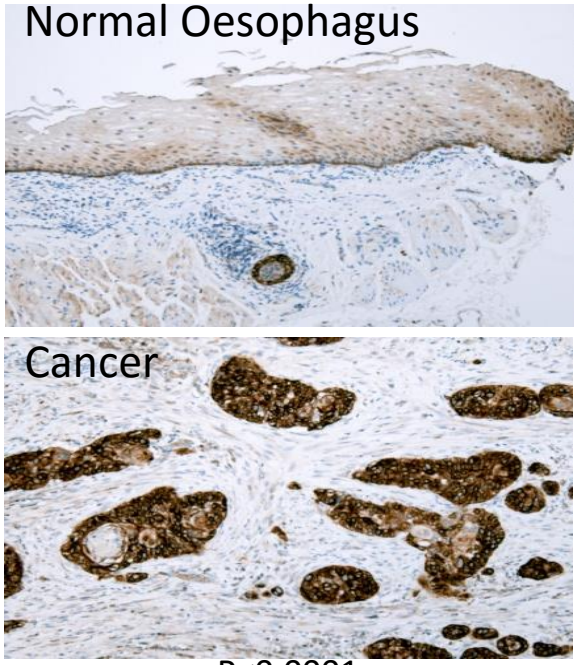
TNFAIP3



P<0.0001



CHN1



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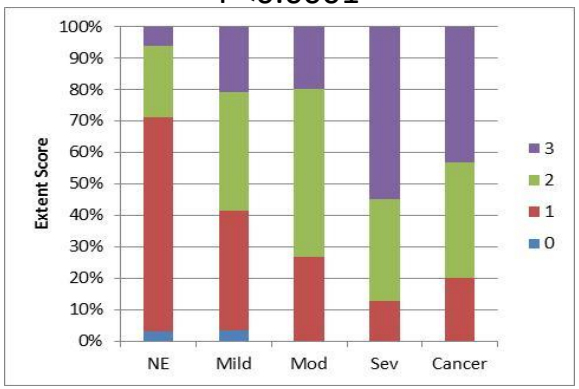
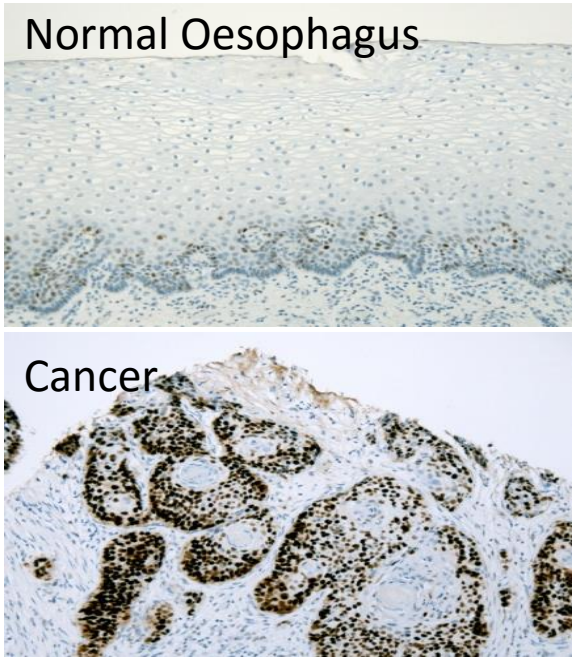


P53



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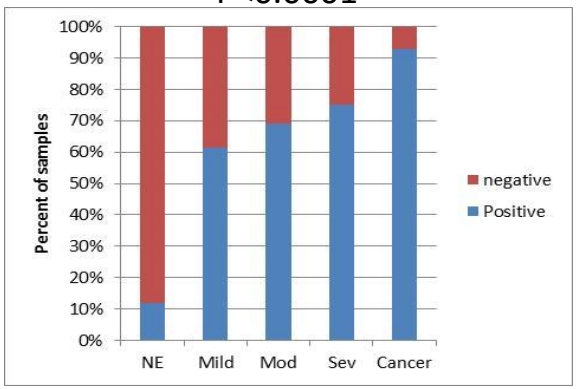
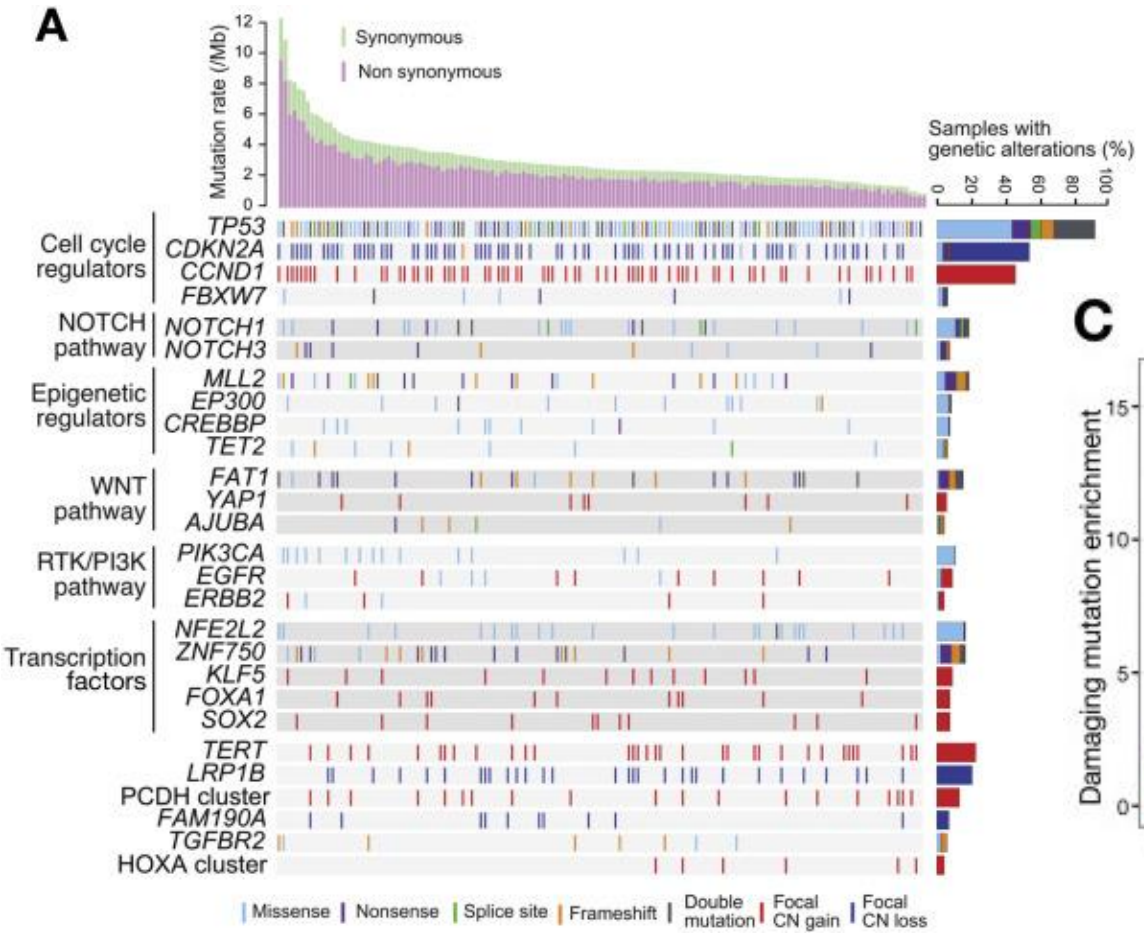
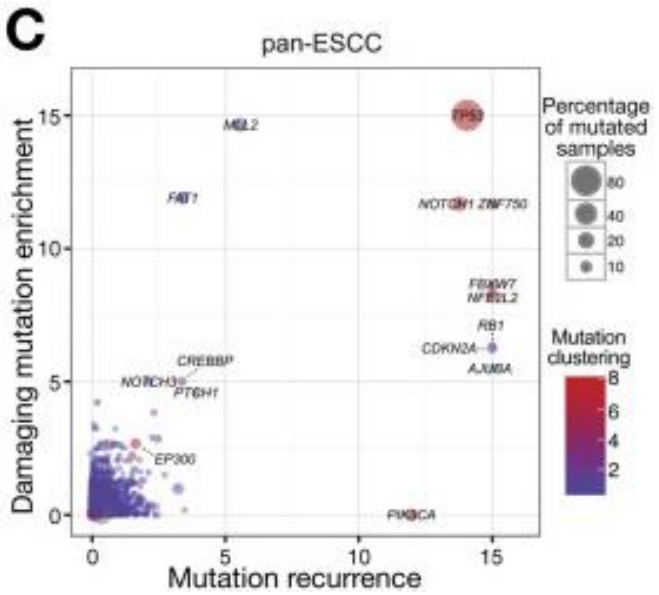


Cancer Prevention Research 2016

ESCC somatic mutation landscape: p53 most recurrent mutation





Sawada et al Gastroenterology 150: 1171-1182

Nan Hu et al. Cancer Res 2016;76:1714-1723

Conclusions

- Cytosponge + assays for diagnosing Barrett's with second tier to risk stratify is promising
- Non-endoscopic screening is attractive concept for high incidence areas of ESCC
  - primary care based, high throughput, economics favourable, acceptable
  - Iranian NESP (n=4,000) and China CICAMS Cytosponge trials (n=2,000) will evaluate further

Discussion points

- Biomarker assays need to be developed
  - Atypia too subjective
  - Immunoassays may not be objective, or accurate enough for ESCC/dysplasia
  - Genetic markers attractive and sequencing costs coming down
- Need large sample collections (dysplasia and early cancers) for biomarker testing
- Optimal trial designs and logistics

Acknowledgments