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

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

Objective biomarker assays for diagnosis and risk stratification
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

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)

<table>
<thead>
<tr>
<th>Study</th>
<th>Publication Year</th>
<th>Study type</th>
<th>Setting</th>
<th>Barrett’s length (cm)</th>
<th>Sensitivity % (95% CI)</th>
<th>Specificity % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pilot</td>
<td>2008</td>
<td>Cohort</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt;ary care</td>
<td>≥C1</td>
<td>78.0 (64.0-89.0)</td>
<td>94.0 (87.0-98.0)</td>
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<tr>
<td>BEST1</td>
<td>2010</td>
<td>Prospective</td>
<td>1&lt;sup&gt;st&lt;/sup&gt;ary care</td>
<td>≥C1</td>
<td>73.3 (44.9-92.2)</td>
<td>93.8 (91.3-95.8)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>≥C2</td>
<td>90.0 (55.5-99.7)</td>
<td>93.5 (90.9-95.5)</td>
</tr>
<tr>
<td>BEST2</td>
<td>2014</td>
<td>Case:Control</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt;ary care</td>
<td>≥C1</td>
<td>79.5 (75.9-82.9)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>≥C2</td>
<td>83.9 (80.0-87.3)</td>
<td>92.4 (89.5-94.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>≥C3</td>
<td>87.2 (83.0-90.6)</td>
<td></td>
</tr>
</tbody>
</table>

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)

Cluster randomisation of GP practices

- Standard of care
  - Lifestyle advice, PPIs, HP test and treat
  - Endoscopy offered if clinically indicated

  Individuals with reflux predominant symptoms

  Excluded from study
  - Individuals with alarm symptoms meeting NICE guidelines for urgent referral

  Accepted test
  - TFF3 negative
  - TFF3 positive
    - Endoscopy to confirm diagnosis (within 8 weeks of result)

  Refused test

Endoscopy (10% of patients without clinically indicated endoscopy)

Follow-up via flagging with ONS and Cancer registry
Cytosponge for ESCC – China and Iran pilot studies using atypia and p53 IHC
# Accuracy data – Iran pilot study

## Cytological examination

<table>
<thead>
<tr>
<th></th>
<th>ESD (all types)</th>
<th>High-grade ESD</th>
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<tbody>
<tr>
<td><strong>ASC</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity (95% CI)</td>
<td>50% (29–71%)</td>
<td>100% (51–100%)</td>
</tr>
<tr>
<td>Specificity (95% CI)</td>
<td>99% (96–99%)</td>
<td>97% (94–98%)</td>
</tr>
<tr>
<td>PPV (95% CI)</td>
<td>69% (39–90%)</td>
<td>31% (10–61%)</td>
</tr>
<tr>
<td>NPV (95% CI)</td>
<td>97% (94–98%)</td>
<td>100% (98–100%)</td>
</tr>
<tr>
<td>Accuracy (95% CI)</td>
<td>96% (93–98%)</td>
<td>97% (94–99%)</td>
</tr>
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</table>

## P53 positivity

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<table>
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</thead>
<tbody>
<tr>
<td>Sensitivity (95% CI)</td>
<td>22% (9–45%)</td>
<td>100% (51–100%)</td>
</tr>
<tr>
<td>Specificity (95% CI)</td>
<td>89% (85–92%)</td>
<td>89% (85–92%)</td>
</tr>
<tr>
<td>PPV (95% CI)</td>
<td>11% (4–28%)</td>
<td>11% (4–28%)</td>
</tr>
<tr>
<td>NPV (95% CI)</td>
<td>95% (91–97%)</td>
<td>100% (98–100%)</td>
</tr>
</tbody>
</table>

**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

Normal Oesophagus

Cancer

P<0.0001

CHN1

Normal Oesophagus

Cancer

P<0.0001

P53

Normal Oesophagus

Cancer

P<0.0001

Cancer Prevention Research 2016
ESCC somatic mutation landscape: p53 most recurrent mutation

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Sawada et al. Gastroenterology 150: 1171-1182

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