Detection of variable methylation patterns improves colorectal cancer blood test sensitivity

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**Simplified Sample Collection**

- Collect K3EDTA blood
  - Blood can be kept for up to 7hrs at RT

- Isolate plasma (4.5mL)
  - Single centrifugation step, 10min RT,
  - Plasma can be kept for up to 24hrs at RT

**Simplified Sample Processing**

- Extract & bisulphite convert DNA
  - 48 samples per batch

- Assay - Qualitative (Yes/No) or Quantitative Result
  - Real-time PCR; triplex assay;
  - 2 methylated DNA cancer biomarkers plus QC marker
BCAT1 & IKZF1: Hypermethylated in neoplastic tissue

**BCAT1: branched-chain amino acid transaminase 1**
- Promotes apoptosis indirectly
- A direct c-myc target
- Mouse/yeast homologues suppress G1-to-S transition
- Disrupted expression increases growth in yeast

**IKZF1: Ikaros family zinc finger 1, DNA binding protein**
- Tumour suppressor in colorectal cancer HCT116 cells
- Negatively regulates Notch signalling.
- Hypermethylation-based loss of expression dictates:
  - down-regulation of DNA repair genes (MSH2),
  - up-regulation of cell-cycle progression genes,
  - inhibition of apoptosis and stem cell renewal

Mitchell et al; *BMC Cancer* 2014, 14:54
# CVP positivity rate by clinical status

<table>
<thead>
<tr>
<th>Clinical status</th>
<th>n</th>
<th>CVP 2-gene panel</th>
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<tbody>
<tr>
<td></td>
<td>2108</td>
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<tr>
<td>Non-neoplastic</td>
<td>1283</td>
<td>74</td>
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<tr>
<td>Non-advanced Adenomas</td>
<td>460</td>
<td>30</td>
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<tr>
<td>Advanced Adenomas</td>
<td>232</td>
<td>17</td>
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<tr>
<td>TIs (stage 0)</td>
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<td>0</td>
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<tr>
<td>Cancers</td>
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<td>85</td>
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<tr>
<td>Stage I</td>
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<td>7</td>
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<tr>
<td>Stage II</td>
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<td>36</td>
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<tr>
<td>Stage III</td>
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<td>30</td>
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<tr>
<td>Stage IV</td>
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<td>8</td>
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<tr>
<td>Unstaged</td>
<td>5</td>
<td>4</td>
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</tbody>
</table>

65% sensitivity (any cancer) / 94% specificity

Young et al., DDW 2014
Can we improve detection of early-stage cancers?

Tumour tissue heterogeneity:
• Varying methylation patterns
• May change during disease progression
• Will be reflected in cfDNA
• Could affect $BCAT1$ and/or $IKZF1$
Variable methylation at *IKZF1*


Fwd primer → Hydrolysis probe ← Rev primer

**IKZF1** assay redesign:
mixture of 8 probes to detect all possible methylation combinations
# IKZF1 positivity rate with modified assay

<table>
<thead>
<tr>
<th>Clinical status</th>
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<td></td>
<td>743</td>
<td>Full-meth</td>
<td>Vari-meth</td>
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<tr>
<td>Non-neoplastic</td>
<td>514</td>
<td>8</td>
<td>1.6</td>
<td>17</td>
<td>3.3</td>
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<td>Adenomas</td>
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<tr>
<td>TIs (stage 0)</td>
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<td>0</td>
<td>-</td>
<td>1</td>
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<tr>
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<td>11</td>
<td>33.3</td>
<td>17</td>
<td>51.5</td>
<td>0.04</td>
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<td>Stage I+II</td>
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<td>25</td>
<td>11</td>
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<td>0.07</td>
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<td>Stage III+IV</td>
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<td>5</td>
<td>55.6</td>
<td>6</td>
<td>66.7</td>
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</tbody>
</table>

**IKZF1 assay redesign improved positivity in early-stage cancers**
Assessment of modified CVP (IKZF1 partial methylation) in a clinical cohort

2013: Full-meth assay
2014: Partial IKZF1 meth assay

n = 677 (467N, 175A, 2 Stage 0, 33 CRC)

64% sensitivity/94% specificity

70% / 92%
Conclusion

• Improved blood-test sensitivity in early stage cancers
• IKZF1 methylation not complete in early stage cancers?
• Seeking opportunities to further investigate clinical utility

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Thank you!