Methylated circulating tumour-derived DNA for detection of colorectal cancer—relationship of methylated BCAT1 or IKZF1 to tissue expression and comparison with FIT.

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BACKGROUND

• Solid tumours shed DNA into blood and circulating tumour DNA (ctDNA) can be detected by tests for mutation and hypermethylation.
• The development of colorectal cancer (CRC) is accompanied by extensive epigenetic changes, including hypermethylation of specific genes.
• We have previously described the discovery and validation of a range of novel hypermethylated genes characteristic of colorectal neoplastic tissue and described 2 methylation markers, BCAT1 and IKZF1 that exhibit low abundance in plasma of cases without neoplasia.
• Mitchell BMC Cancer. 2016;14:54

AIM

To explore their value as screening biomarkers for CRC, these two biomarkers were evaluated in a series of four studies, described here.

1. Tissue methylation (cont.)

• Significantly higher methylation of either BCAT1 or IKZF1 was seen in 84/91 (92.3%) cancer tissues, compared with non-neoplastic specimens (p < 0.001).
• Comparing tissue methylation in adenomas, CRC and normal colon (each n=10), levels in either marker were similar to those in cancers (Fig. 2).

2. Detection in Blood (cont.)

• ctDNA was detected in 116 (62.0%) cases (Fig. 3) and was significantly more likely to be detected with later stage (p < 0.001) and distal tumor location (p = 0.004).
• ctDNA sensitivity by AJCC stage was: I, 6/40 (15%); II, 35/54 (65%); 47/63 (75%); 29/34 (85%) (Fig. 3).
• There was no relationship between degree of methylation in tissue and detection in blood. (Fig. 3).

3. Comparison with FIT

<table>
<thead>
<tr>
<th>TABLE 1. Colonoscopy finding</th>
<th>ctDNA positive (%)</th>
<th>FIT positive (%)</th>
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<tr>
<td>Colorectal Cancer (N = 66)</td>
<td>47 (62.1%)</td>
<td>52 (78.8%)</td>
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<tr>
<td>Stage I (N = 17)</td>
<td>7 (41.2%)</td>
<td>13 (76.5%)</td>
</tr>
<tr>
<td>Stage II (N = 25)</td>
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<td>Stage IV (N = 7)</td>
<td>5 (71.4%)</td>
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<td>Adenoma (N = 448)</td>
<td>41 (9.2%)</td>
<td>138 (30.8%)</td>
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<td>Advanced (N = 189)</td>
<td>16 (8.5%)</td>
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<td>Non-advanced (N = 259)</td>
<td>25 (9.7%)</td>
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• A fit (OC-Sensor, cut-off 10 µg Hb/g) and BCAT1/KZF1 ctDNA test were completed in 1381 scheduled for colonoscopy.
• Sensitivity for CRC was 62% for the ctDNA test and 79% for FIT (p<0.05, Table 1).
• Specificity (from cases without CRC) was 92% for the ctDNA test and 81% for FIT (p=0.001).
• Fit was more sensitive for advanced adenoma (42.3% vs 8.5%, p=0.01) and for stage I cancer than the ctDNA test.

4. Effect of resection on ctDNA

• Of 47 CRC patients ctDNA-positive at diagnosis, 35 (74.5%) became negative after resection (manuscript submitted).

CONCLUSIONS

• These studies have shown that BCAT1 and IKZF1 methylation are common in CRC and adenomas.
• Despite methylation in adenoma tissues, ctDNA is not detected in these cases.
• Detection of ctDNA in blood is CRC stage dependent and is unrelated to degree of tissue methylation.
• Sensitivity is only equivalent to FIT in stages II-IV cancer; FIT is more sensitive for adenomas and stage I cancer.
• There is rapid reversion from being positive with ctDNA to negative following resection in most patients.

Figure 1: 1. Tissue methylation (cont.)

• Significantly higher methylation of either BCAT1 or IKZF1 was seen in 84/91 (92.3%) cancer tissues, compared with non-neoplastic specimens (p < 0.001).
• Comparing tissue methylation in adenomas, CRC and normal colon (each n=10), levels in either marker were similar to those in cancers (Fig. 2).

Figure 2: Marker methylation in cancer, adenoma and normal tissues. Circles, BCAT1; diamonds, IKZF1

• Tissue methylation levels were independent of stage, (Figs. 2 & 3) and all pathological variables.

Figure 3: Relationship between methylation in tissue and ctDNA positivity according to cancer stage (manuscript submitted).

Top grey panel shows ctDNA positivity; open diamonds, ctDNA negative; black/white, BCAT1 positive only; white/red, IKZF1 positive only; black/red: ctDNA methylated in both genes. Bottom panel shows graphical representation of methylation levels in cancer tissues (closed circles: black, BCAT1; red, IKZF1). Tissues with no detectable BCAT1 and/or IKZF1 are indicated with open circles.

Figure 4: ctDNA status before and after resection of the primary cancer. Black circles: ctDNA-positive cases before resection (n=47); red circles: cases who tested positive after resection (n=12); grey circles: cases who tested negative after resection.

Figure 5: Levels of methylated BCAT1 and IKZF1 in tissue and plasma of cases without neoplasia.

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