A Novel 2-Gene Blood Test for Colorectal Cancer Recurrence

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BACKGROUND
Despite apparent clearance of colorectal cancer (CRC) following initial therapy, recurrence will develop in 30-40% of cases. U.S. guidelines for CRC monitoring recommend quarterly/four yearly blood testing for carcinoembryonic antigen (CEA), but its sensitivity and specificity is suboptimal. We have developed a novel blood test for detection of methylated BCAT1 and IKZF1 DNA for detection of CRC (1).

STUDY SYNOPSIS
Objectives
To estimate the sensitivity and specificity of the 2-gene blood test (methylated BCAT1 and IKZF1) for detection of clinically confirmed recurrent CRC and to compare the 2-gene test performance with that of CEA.

Study Design
An observational study collecting blood for BCAT1/IKZF1 and CEA testing. Radiological imaging, usually CT scan, but also MRI, PET, surgery, etc, was used for verification of clinical status of recurrence. Figure 1.

Study Cohort
Patients who had undergone curative treatment for primary CRC and scheduled for diagnostic follow-up as part of surveillance for recurrent CRC.

Methods
KEDTA-blood was collected from CRC surveillance patients (excluding those undergoing active follow-up as part of surveillance for recurrent CRC.

RESULTS
Study Cohort: 332 enrolments, 64% men, average age 64 years at diagnosis (range: 31-85 years), CRC stages: 74 Stage I, 49 Stage II, 90 Stage III, 37 Stage IV, 35 unstaged. 120 subjects with confirmed recurrence (median 2.3 mo), 94.1% (114/120) of patients who were confirmed recurrent CRC and to compare the 2-gene test performance with that of CEA.

Sensitivity estimates (Table 1): Local recurrence: 2-gene 55% (95%CI: 23-83), CEA 18% (95%CI: 23-52). Specificity estimates: Local recurrence: 2-gene 88% (95%CI: 77-93), CEA 28% (95%CI: 6-41).

Test concordance: In 11 cases with local recurrence, 55% were 2-gene positive, with only 2 (18%) positive by both tests (p=0.06). In 19 cases with distant recurrence, 68% and 26% were BCAT1/IKZF1 methylation and CEA positive, respectively (p=0.008). No cases with confirmed recurrence were CEA positive only. In patients with no evident disease, 19% were positive for one test but not the other (2-gene blood test, 14%; CEA, 4%; p=0.05).

Apparent false-positives: The 2-gene blood test may detect recurrence not apparent on imaging until a later time point. Figure 1.

Following resection (median 2.3 mo), 94.1% (30/32) of patients who were BCAT1/IKZF1 methylation positive prior to treatment showed either no detectable BCAT1/IKZF1 (27%) or significantly reduced levels in blood (27%).

CONCLUSION
Two-gene test positivity correlated with local (55%) and distant (18%) recurrence with 27-34% more recurrence cases detected than with CEA. The 2-gene test appears to be better than CEA for recurrence monitoring. Studies evaluating effect on survival are now warranted.


Figure 1. Study Design

Figure 2. Study enrolment and outcomes

Figure 3. Monitoring profiles of selected apparent false-positives

Table 1. Positivity rates for testing for methylation markers versus CEA

<table>
<thead>
<tr>
<th>Recurrence</th>
<th>Local CEA</th>
<th>Distant CEA</th>
<th>Local 2-gene</th>
<th>Distant 2-gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Recurrence</td>
<td>4% (1/25)</td>
<td>4% (1/25)</td>
<td>14% (3/21)</td>
<td>4% (1/25)</td>
</tr>
<tr>
<td>CEA (cut-off, 5ng/mL)</td>
<td>18% (2/11)</td>
<td>26% (5/19)</td>
<td>86% (95%CI: 77-92)</td>
<td>13% (3/24)</td>
</tr>
<tr>
<td>BCAT1/IKZF1</td>
<td>5% (1/21)</td>
<td>13% (2/15)</td>
<td>80% (95%CI: 67-93)</td>
<td>25% (5/20)</td>
</tr>
</tbody>
</table>

Table 2. False-positives

<table>
<thead>
<tr>
<th>Status of Recurrence</th>
<th>Status of Recurrence</th>
<th>Status of Recurrence</th>
<th>Status of Recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt 804 (IIIC)</td>
<td>Pt 1279 (IIA)</td>
<td>30 cases with recurrence</td>
<td>90 cases with no recurrence</td>
</tr>
<tr>
<td>CEA</td>
<td>BCAT1/IKZF1</td>
<td>McNemar’s p-value 0.005</td>
<td>McNemar’s p-value 0.005</td>
</tr>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>12</td>
<td>11</td>
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