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. US guidelines for CRC monitoring recommend quarterly/biannual 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 K3EDTA-blood was collected from CRC surveillance patients (excluding those undergoing active treatment). DNA was isolated from 4mL plasma and analyzed for methylated *BCAT1* and *IKZF1* DNA (1). The presence of either marker was considered positive. The CEA concentration was measured using the DiaSorin LIAISON CEA test. CEA levels ≥5ng/mL were deemed positive. True- and falsepositive rates for the 2-gene test and CEA were calculated and compared in all clinically confirmed cases as determined by the temporally closest radiological imaging results. Sensitivity and specificity estimates were derived from calculated true- and false positive rates.

Figure 1. Study Design

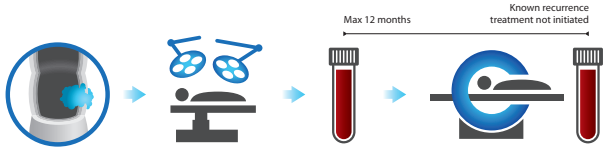

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Figure 2. Study enrolment and outcomes

332 enrolled

212 excluded (not under surveillance, 68; recurrence status unknown, 107; other cancers, 6; new CRC, 2; residual disease, 15; blood testing not within defined timeframe, 14).

30 cases with recurrence

		CEA +	CEA –
BCAT1/IKZF1	+	7	12
	–	0	11

McNemar's p value: 0.0005

90 cases with no recurrence

		CEA +	CEA –
BCAT1/IKZF1	+	0	13
	–	4	73

McNemar's p value: 0.05

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

	Recurrence		No Recurrence
	Local	Distant	
2-gene blood test	**55%** (6/11)	**68%** (13/19)	**14%** (13/90)
CEA (cut-off, 5ng/mL)	**18%** (2/11)	**26%** (5/19)	**4%** (4/90)

RESULTS

Study Cohort: 332 enrolees, 64% men, average age 64 years at diagnosis (range: 31-85 years), CRC stages: 74 Stage I, 96 Stage II, 90 Stage III, 37 Stage IV, 35 unstaged. 120 subjects with confirmation of recurrence status, including 30 cases with recurrence (25%).

Sensitivity estimates (Table 1):

Local recurrence:	2-gene: 55% (95%CI: 23-83)	CEA: 18% (95%CI: 23-52)
Distant recurrence:	2-gene: 68% (95%CI: 43-87)	CEA: 26% (95%CI: 9-51)
Specificity estimates:	2-gene: 86% (95%CI: 77-92)	CEA: 96% (95%CI: 89-99)

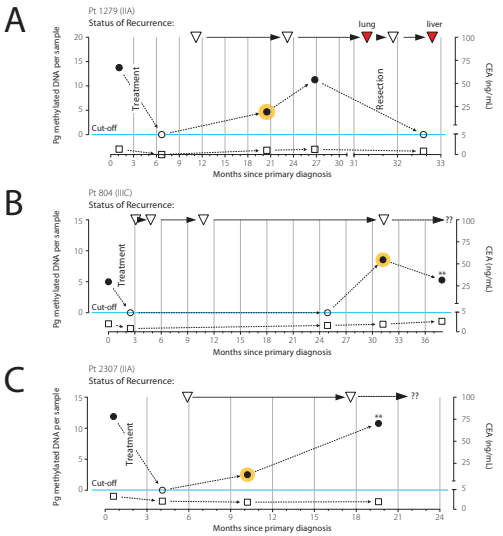
Test concordance: In 11 cases with local recurrence, 55% were 2-gene positive, with only 2 (18%) positive by both tests (p=0.06). In 19 cases with distant recurrence, 68% and 26% were *BCAT1/IKZF1* methylation and CEA positive, respectively (p=0.008). No cases with confirmed recurrence were CEA positive 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 3.

Following resection (median 2.3mo), 94.1% (32/34) of patients who were *BCAT1/IKZF1* methylation positive prior to treatment showed either no detectable *BCAT1/IKZF1* (74%) or significantly reduced levels in blood (21%) .

Figure 3. Monitoring profiles of selected apparent false-positives

Examples of apparent false-positive 2-gene blood results (marked in yellow) based on temporally closest clinical confirmation of recurrence status. Triangles: Radiological imaging. Circles: 2-gene blood testing, squares; CEA testing. Open symbols: negative or not present; filled symbols: positive or present (triangles filled in red when recurrence was verified). Asterisks: results not included in primary data analysis due to no follow-up imaging results. (A) The 2-gene blood test indicated recurrence ~10 months prior to clinical confirmation of pulmonary recurrence even though an intervening CT scan was negative. (B-C): Examples of apparent false-positives where further followup imaging will clarify status (if true or false positive). Horizontal (blue) line indicates positivity thresholds for the 2-gene blood test (any methylation signal) and for CEA (cut-off: 5ng/mL).



CONCLUSION

Two-gene test positivity correlated with local (55%) and distant (68%) recurrence with 2.7-fold 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.

References: **1.** Pedersen *et al. BMC Cancer* 2015;15(1):654