Methylated circulating tumor-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 tumors shed DNA into blood and circulating tumor 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*. 2014;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. Levels of methylated *BCAT1/IKZF1* in tissue

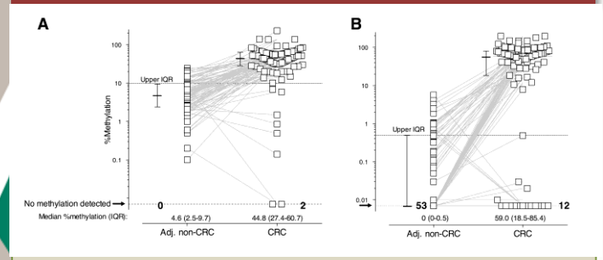

Figure 1: *BCAT1* (A) and *IKZF1* (B) methylation in cancer and adjacent non-neoplastic tissues (91 pairs)

- Mean methylation in cancer tissues were 48.0% and 60.5% (respectively), compared to 6.8% and 0.4% in non-neoplastic tissues (each p<0.0001).

1 & 2. Association between tissue methylation and detection in blood

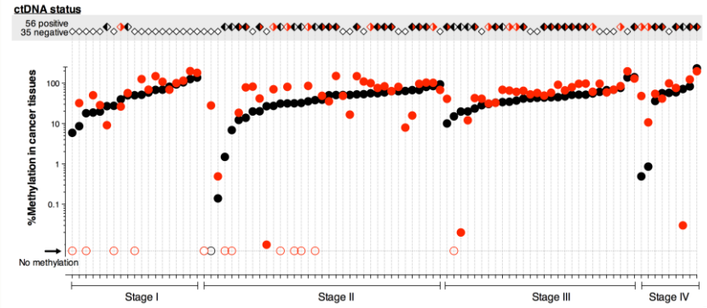

Figure 3: Relationship between methylation in tissue and ctDNA positivity according to cancer stage (manuscript submitted). Top grey panel shows ctDNA positivity; open diamonds, ctDNA negative; black/white, *BCAT1* positive only; white/red, *IKZF1* positive only; black/red: ctDNA methylated in both genes. 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.

1. Tissue methylation (cont.)

- Significantly higher methylation of either *BCAT1* or *IKZF1* was seen in 84/91 (92%) **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).

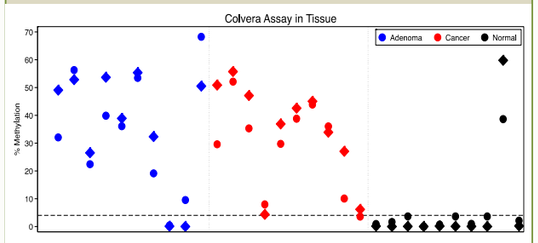

Figure 2: Marker methylation in cancer, adenoma and normal tissues. Circles, *BCAT1;* diamonds, *IKZF1*

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

3. Comparison with FIT

TABLE 1. Colonoscopy finding	ctDNA positive (%)	FIT positive (%)
Colorectal Cancer (N = 66)	41 (62.1%)	52 (78.8%)
Stage I (N = 17)	7 (41.2%)	13 (76.5%)
Stage II (N = 25)	19 (76.0%)	20 (80.0%)
Stage III (N = 17)	10 (58.8%)	13 (76.0%)
Stage IV (N = 7)	5 (71.4%)	6 (86.0%)
Adenoma (N = 448)	41 (9.2%)	138 (30.8%)
Advanced (N = 189)	16 (8.5%)	80 (42.3%)
Non-advanced (N = 259)	25 (9.7%)	58 (22.4%)
Non-neoplastic lesions (N=621)	48 (7.7%)	94 (15.1%)
No evidence of disease (N=246)	19 (7.7%)	25 (10.2%)

- A FIT (OC-Sensor, cut-off 10 µg Hb/g) and *BCAT1/IKZF1* 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 .
- Symonds, *Clin Trans Gastroenterol.* 2016; 14;7:e137.

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).

4. Effect of resection on ctDNA

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

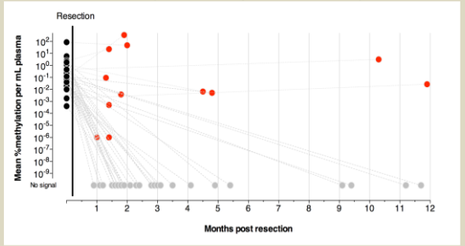

Figure 4: ctDNA status before and after resection of the primary cancer. Black circles: ctDNA positive cases before resection (n=47); red circles: cases who tested positive after resection (n=12); grey circles: cases who tested negative after resection.

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.