**Background**

The methylation-circulating tumor DNA (ctDNA) biomarkers BCAT1 and IKZF1 are common events in colorectal cancer (CRC). Play a role in its development and drugs targeting BCAT1 are available. As these biomarkers disappear from blood after surgery in most patients, a prospective study was conducted to assess the relationship between their post-surgery presence and risk for residual disease as well as survival.

**Study Synopsis**

Methods
DNA was extracted from at least 3.9 mL of K3 EDTA plasma collected within 12 months of initial surgical resection, bisulfite converted and assayed for methylated BCAT1 and IKZF1 as previously described. Detection of either marker was related by logistic regression to pathologically-determined presence or risk of residual disease (“RD”, margins involved, metastases present or apical node involvement). A Cox Proportional Hazards (PH) model was used to determine an association with CRC recurrence. Time to recurrence was measured from date of surgery to first positive radiological evidence of recurrence and censored at last radiological follow-up.

Results

**Table 2. Characteristics of cases at surgery categorized according to post-surgery ctDNA status (n=172)**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. (%)</th>
<th>Post-surgical ctDNA status, No. (%)</th>
<th>OR (95% CI)</th>
<th>p2</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Resection margins involved</td>
<td>4 (2)</td>
<td>3 (2)</td>
<td>1 (4)</td>
<td>1.7 (0.2-17.4)</td>
</tr>
<tr>
<td>B. Number of nodes involved by tumor</td>
<td>0</td>
<td>112 (65)</td>
<td>100 (69)</td>
<td>12 (43)</td>
</tr>
<tr>
<td>C. Apical node involved</td>
<td>7 (4)</td>
<td>2 (1)</td>
<td>5 (18)</td>
<td>15.4 (2.8-84.3)</td>
</tr>
<tr>
<td>D. Distant metastasis remaining after surgery</td>
<td>4 (2)</td>
<td>1 (1)</td>
<td>3 (10)</td>
<td>17.2 (1.7-171.6)</td>
</tr>
<tr>
<td>E. T4 Stage/peritoneal involvement</td>
<td>32 (19)</td>
<td>21 (15)</td>
<td>11 (39)</td>
<td>3.8 (1.6-9.2)</td>
</tr>
<tr>
<td>Less than 12 nodes sampled</td>
<td>32 (19)</td>
<td>26 (18)</td>
<td>6 (21)</td>
<td>1.2 (0.5-3.4)</td>
</tr>
</tbody>
</table>

Incomplete non-surgical treatment at time of venesection

Any of A, C, D or E above

Any of A or D above

Any of A, C or D above

Any of A, C, D or E above

Odds Ratio determined by univariate logistic regression analysis; *Wald test, p-value; †Included in multivariate logistic regression.

**Funding**

Funding from NRHM Australia (ID#1006242). Part funding from Clinical Genomics Pty. Ltd. (Australia).

**Figure 1. Disposition and outcomes of study cohort**

**Figure 2. Characteristics of cases at diagnosis, study design and results**

**Figure 3. Kaplan-Meier estimate for recurrence-free survival in patients according to their post-surgery ctDNA status**

**Conclusion**

- CRC cases positive for these ctDNA biomarkers within 12 months of surgery are at increased risk of residual disease and subsequently for recurrence.
- This has implications for adjuvant therapy and monitoring of cases; randomised studies are now indicated to determine if such can provide survival benefit.

**References**