# Circulating Tumor DNA to Assess Characteristics of Colorectal Cancer and Completeness of Surgical Resection

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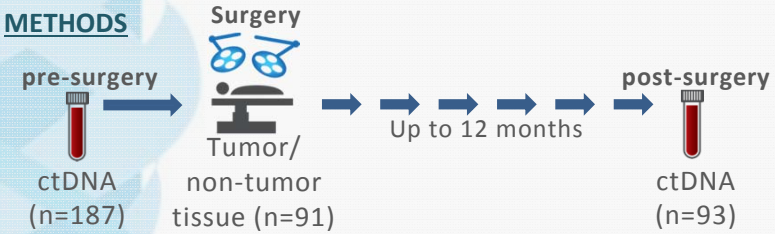
## BACKGROUND

- There is a need for a sensitive biomarker-based test that informs adequacy of surgery and therapy in patients diagnosed with colorectal cancer (CRC).
- Methylation in *BCAT1* and *IKZF1* are common events in CRC tissue, with these methylated genes detectable as circulating tumor DNA (ctDNA) in plasma [1-3].
- Detection of these biomarkers using the blood test Colvera™, has been high sensitivity and specificity for CRC [2, 3].

## AIM

To determine how the Colvera™ test results relate to methylation in tissue, cancer characteristics, and completeness of surgical resection.

## METHODS




- Blood and tissue samples were analyzed in triplicate for methylated *BCAT1/IKZF1* with real-time PCR. Samples were positive if ≥ 1 replicate was positive for methylated *BCAT1* or *IKZF1*. Tissue % methylation was calculated as total mass of *BCAT1* and *IKZF1* / total amount of DNA *(ACTB)*.
- Data were analysed to compare:

(1) % Methylation of non-cancer and cancer tissues (Mann-Whitney)
(2) Tissue and ctDNA results with cancer features (Chi-square)
(3) ctDNA test results with risk for residual disease (margins involved, metastases present or nature of node involvement; logistic regression analysis).

## RESULTS: Tissue methylation

- 98.9% (90/91) of tumor tissues had methylation of *BCAT1* or *IKZF1*.
- Methylation in tumor tissue was greater than that in non-tumor tissue (p<0.05, Figure 1).

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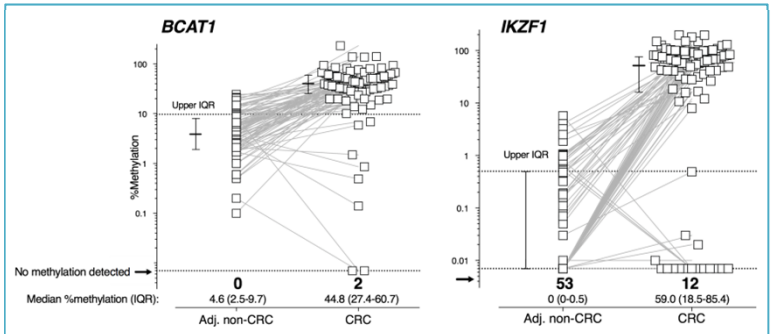


**FIGURE 1. Methylated *BCAT1* and *IKZF1* in matched tumor and adjacent non-tumor tissues.**

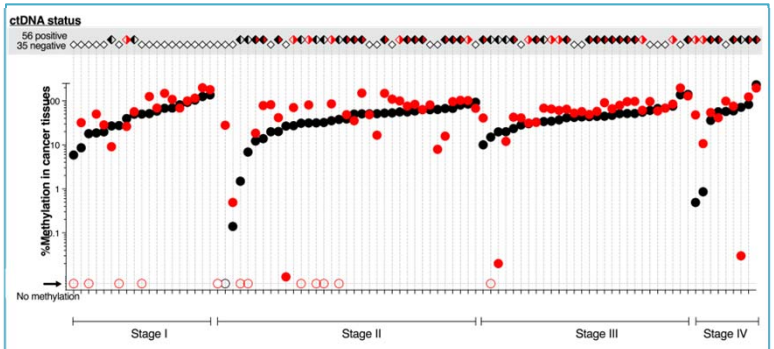


**FIGURE 2. Relationship between methylation in tissue and ctDNA positivity.** No color, negative; black, *BCAT1* positive; red, *IKZF1* positive.

## RESULTS: Methylation and tumor features

- Tissue methylation was not affected by stage (p>0.05, Fig. 2), but methylated *BCAT1* was higher in patients ≥65 years (p=0.039) and in proximal tumors (p=0.029).
- ctDNA was detected in 116/187 (62.0%) of cases at diagnosis. ctDNA sensitivity by AJCC stage was: I, 6/40 (15%); II, 35/54 (65%); III, 47/63 (75%); IV, 29/34 (85%), with ctDNA more likely to be detected with later stage of the cancer (p<0.001, Fig. 2).
- ctDNA had a higher positivity rate with increased T stage (p<0.001), N stage and M stage (both p=0.001), increased size (p<0.001), distal location (p=0.011), and lymphatic invasion (p=0.002).

## RESULTS: ctDNA and risk for residual disease

- Following surgery, 74.5% (35/47) of patients who were ctDNA-positive at diagnosis became negative, most within 3-4 months (Figure 3).
- Presence of ctDNA following surgery was independently associated with features suggestive of residual disease (close margins, apical node involved, or distant metastases), OR 33.3 (95%CI: 3.4-327.2).
- Of the 12 cases who remained ctDNA positive after surgery, incomplete resection was observed in 5/12 (41.7%) compared to 1/35 (2.9%) who became negative (p=0.003).

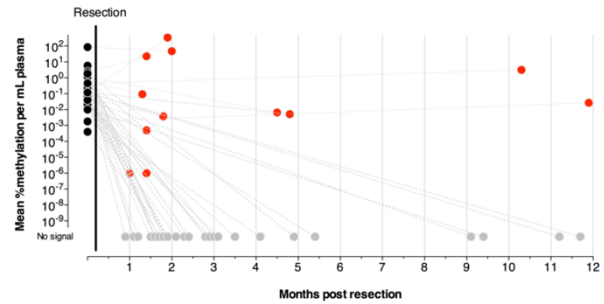


**FIGURE 3. ctDNA status before and after resection of the primary cancer in 47 cases who were positive prior to resection.**

## CONCLUSION

- Patients who are positive for ctDNA post-surgery have a higher likelihood of incomplete surgical resection.
- These results from Colvera™ have implications for guiding recommendations for adjuvant therapy and surveillance strategies.

## REFERENCES

1. Mitchell, S.M., et al. BMC Cancer 2014; 14: doi: 10.1186/1471-2407-14-54.
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3. Symonds, E.L., et al. Clin Transl Gastroenterol 2016; 7: 137.