

Circulating tumor DNA before and after resection for colorectal cancer

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Background and Aims

- *BCAT1* and *IKZF1* are methylated with high frequency in colorectal cancer (CRC) and detectable in blood (methylated circulating tumor-derived DNA, ctDNA).
- By assaying pre-and post-surgery blood samples for methylation in these two genes, in a prospective correlative study, we aimed to:
 1. Demonstrate the impact of tumor resection on ctDNA levels, and
 2. Identify factors associated with a positive test after resection.

Methods

- 93 people (age 31-86 years, median age 68yr, 51.6% male) with invasive CRC, but without neoadjuvant therapy had blood collected prior to surgery and within 12 months after resection.
- Cancers were clinicopathologically staged and surgical pathology of relevance to surgical clearance was recorded.
- DNA extracted from plasma was analyzed for methylated *BCAT1* and *IKZF1* and the level of ctDNA was expressed as percentage of total mass of methylated DNA (*BCAT1+IKZF1*) measured in the total recovered DNA.

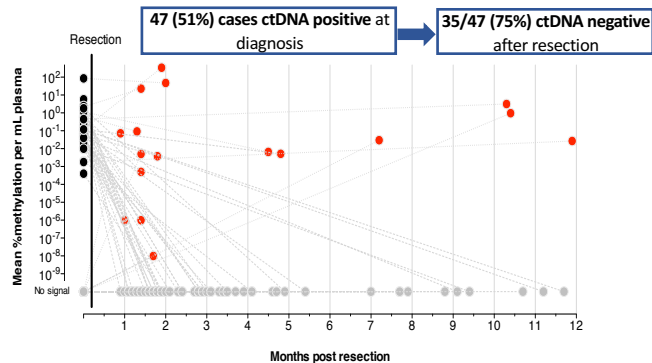


Figure 1: ctDNA levels in 93 cases before and within 12 months of resection. Black: Peri-diagnostic ctDNA positive cases (n=47). Red: Post resection ctDNA positive cases (n=17). Grey: 46 peri-diagnostic and 76 post-surgical ctDNA negative cases.

Results – cases with initial positive ctDNA (Figure 1)

- Of the 47 patients who were ctDNA positive prior to resection, 35 (74.5%) **became negative** after resection - most were noted to have done so within 3 months. None of these 35 had an involved surgical margin or residual metastatic tumor at resection.
- In the 12 cases who **remained positive** after surgery, incomplete resection was observed in 5/12 (41.7%) compared to 1/35 (2.9%) who became negative following surgery (p=0.003). Four subsequently developed metastatic disease.

Table 1. Case and cancer characteristics (n=93) ¹		ctDNA status after surgery		
		NEG	POS	P
All eligible cases, No. (%)		76 (82)	17 (18)	
ctDNA positive at diagnosis, No. (%)		35 (46)	12 (71)	0.106
<u>Tumor Characteristics</u>				
Stage, No. (%)	I	28 (37)	2 (12)	0.002
	II	27 (36)	3 (18)	
	III	21 (28)	10 (59)	
	IV	0 (-)	2 (12)	
<u>Histopathology</u>				
Perineural invasion, No. Present/Total (%)		5/74 (7)	6/16 (38)	0.003
Extramural venous invasion, No. Present/Total (%)		5/73 (7)	4/16 (25)	0.051
<u>Surgical pathology</u> Resection margin not clear, No. (%)		0 (-)	1 (6)	0.183
Number of nodes involved by tumor, No. (%)	0	51 (72)	8 (47)	0.029
	1-3	16 (23)	4 (24)	
	4-6	3 (4)	3 (18)	
	≥7	1 (1)	2 (12)	
	Not stated	5 (7)	-	
Apical node involved, No. Present/Total (%)		1/64 (2)	3/15 (20)	0.020
Distant metastases remaining after surgery, No. (%)		0	2 (12)	0.032
Tumor in adventitia (no nodal metastasis), No. (%)		2 (3)	4 (24)	0.010
Incomplete surgical resection ² , No. (%)		1 (1)	5 (29)	0.001

¹The following characteristics were not significantly different: Days elapsed between surgery and 1st post-surgical ctDNA test; tumor location, size), mucinous; differentiation; lymphovascular invasion; Intramural venous invasion, <12 nodes sampled.² Incomplete surgical resection (clear margins, absence of distant metastases and lack of apical node involvement) – Fisher Exact test.

Results – clinical significance of post-surgery result (Table 1)

- Significant **predictors of a positive ctDNA** after surgery were: 1) AJCC stage, 2) perineural invasion and 3) incomplete surgical resection (specifically: unresected metastases; risk of residual tumor at resection site - apical node involvement, 7 or more nodes involved).
- Given a **positive post-surgical ctDNA result**, modelling by logistic regression revealed that incomplete surgical resection was much more likely (Odds ratio, 31.2, 95%CI: 3.4-291.2)

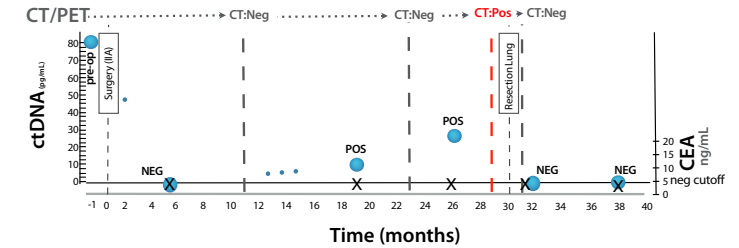


Figure 2: Example of serial ctDNA, CEA and CT results from diagnosis to post resection of lung metastasis. Blue: methylated *BCAT1/IKZF1* ctDNA results. Black cross: CEA results.

Summary and Conclusions

- Most patients become ctDNA negative soon after surgery.
- Following resection, a positive ctDNA result is associated with a higher likelihood of involved surgical margin, unresected metastases, apical node involvement, or 7 or more nodes involved, all features pointing to incomplete resection or likely residual tumor.
- Residual or recurrent disease (Fig. 2) should be suspected in cases who are ctDNA positive after surgery.
- Detection of methylated *BCAT1/IKZF1* in blood appears clinically useful for assessing adequacy of primary surgery and potential for recurrence.

