Predictors of circulating tumor-derived methylated *BCAT1* and *IKZF1* DNA in colorectal cancer patients

Erin L Symonds[1,2], Susanne Pedersen[3], David Murray[3], Rohan Baker[3], Graeme P Young[1]

[1]Flinders Centre for Innovation in Cancer, Bedford Park, SA, Australia; [2]Bowel Health Service, Repatriation General Hospital, Daw Park, SA, Australia; [3]Clinical Genomics Technologies Pty Ltd, North Ryde, NSW, Australia

BACKGROUND

Solid tumors release DNA into circulation and recent improvements in molecular technologies have demonstrated that circulating tumor DNA (ctDNA) can be detected in a robust and reproducible manner.[1] Aberrant DNA methylation is a characteristic of colorectal cancer (CRC), and the two genes, *BCAT1* and *IKZF1* are hypermethylated with a high frequency in colorectal adenomas and adenocarcinomas compared to non-neoplastic colon tissue.[2] A blood test targeting these two methylation markers for detection of ctDNA in CRC patients has previously been reported,[3-6] but the underlying tumor biology resulting in ctDNA is not well understood.

STUDY SYNOPSIS

Objectives To identify tumor features associated with detection of methylated *BCAT1* and *IKZF1* DNA in blood and to determine if any patient sociodemographic factors were associated with blood test positivity.

Study Design An observational study collecting blood from CRC patients.

Study Cohort Newly diagnosed CRC patients who did not receive neoadjuvant therapy prior to resection of primary tumor.

Methods K3EDTA-blood was collected prior to surgery, and '2-spin' plasma was isolated within 4 hours of blood collection. DNA was extracted and bisulphite converted as previously described,[4] and assayed for methylated *BCAT1* and *IKZF1* DNA. A multivariate logistic regression analysis (with Bonferroni correction) was used to compare blood test results with patient sociodemographic factors (age, gender, BMI, smoking status, ethnicity, alcohol intake, medication and heart comorbidity) and tumor features (AJCC staging, location, size, depth of invasion, degree of differentiation (well-, moderate-, poor-), vascularity, lymphatic and perineural invasion, lymph node involvement, tumor deposits and presence of metastasis).

Figure 1. Disposition and outcomes of study cohort

CRC diagnosis

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Pre-operative blood collection & patient interview (n = 188)

→ **Excluded (n = 45)**
- 14 received neoadjuvant therapy prior to resection
- 26 had no resection
- 5 had missing data in variables used in model

Resection (n = 143, 55.2% males, median age 68.6 years)

Staging	Total, n (%)	# Males (%)	Median age, years (range)
I	35 (24.5)	15 (42.9)	66.4 (44.6-83.4)
II	48 (33.6)	31 (64.6)	69.2 (31.6-85.4)
III	46 (32.2)	26 (56.5)	67.5 (42.6-85.9)
IV	14 (9.8)	7 (50.0)	64.8 (38.4-82.8)

Table 1. Appearance of circulating tumor-DNA (ctDNA) in CRC patients

143 cases		N	ctDNA Positivity			OR#	95%CI	*P value
			No. Pos	% Positive	95%CI			
CRC Staging	I	35	6	**17.4%**	7-34	1.0	-	-
	II	48	33	**68.8%**	54-81	6.6	2-23	**0.022****
	III	46	34	**73.9%**	59-86	9.9	3-34	**0.002****
	IV	14	13	**92.9%**	66-100	42.1	4-443	**0.014****

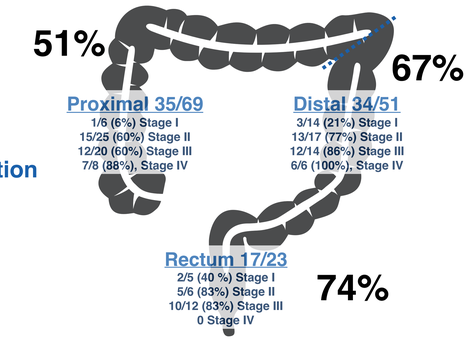
Table 2. Tumor features predicting appearance of ctDNA

Variable		N	ctDNA Positivity			OR#	95%CI	*P value
			No. Pos	% Positive	95%CI			
T stage	T1	21	1	**4.8%**	0.1-24	1.0	-	-
	T2	19	6	**31.6%**	13-57	8.1	1-93	**1**
	T3	77	56	**72.7%**	61-82	35.7	3-386	**0.039****
	T4	26	23	**88.5%**	70-98	84.2	6-1240	**0.013****
N Stage	N0	88	43	**48.9%**	38-60	1.0	-	-
	N1 & N2	55	43	**78.2%**	65-88	1.7	1-5	**1**
M Stage	M0	129	73	**56.6%**	48-65	1.0	-	-
	M1	14	13	**92.9%**	66-100	4.0	0.4- 36	**1**
Size (mm)	<20	12	2	**16.7%**	2-48	1.0	-	-
	20 to <50	76	40	**52.6%**	41-64	1.2	0.1-13	**1**
	≥ 50	55	44	**80.0%**	67-90	2.7	0.2-33	**1**
Location	Proximal	69	35	**50.7%**	38-63	1.0	-	-
	Distal	51	34	**66.7%**	52-79	3.9	1-12	**0.169**
	Rectal	23	17	**73.9%**	52-90	3.6	1-14	**0.819**
Extramural venous invasion	No	125	70	**56%**	47-65	1.0	-	-
	Yes	18	16	**88.9%**	65-99	2.7	0.5-14	**1**

#Odds Ratio; *Adjusted for multiplicity using the Bonferroni correction (k=13).

References: (1) Diehl et al. Nature Med 2008; (2) Mitchell et al. BMC Cancer 2014; (3) Pedersen et al. BMC Cancer 2015; (4) Symonds et al CTG 2016; (5) Symonds et al. APJCP 2015; (6) Young et al. Cancer Medicine 2016

Figure 2. Blood test positivity vs tumor location



RESULTS

Study cohort (Fig. 1): 143 CRC patients, median age 68.6 years, 55.2% male, CRC stages: 35 Stage 1, 48 Stage II, 46 Stage III and 14 Stage IV.

ctDNA status in stage I-IV CRC patients (Table 1): Of the 143 CRC patients, 86 (60.1%, 95%CI: 51-68) were positive for methylated *BCAT1/IKZF1* DNA in blood. Statistically significant higher positivity rates were measured in CRC stage II (68.8%), III (73.9%) and IV (92.9%) relative to stage I (17.4%).

Predictors of tumor DNA in blood (Table 2): Table 2 shows a selected number of the tumor features investigated for blood positivity association using multivariate logistic regression modelling. Only depth of invasion (T stage) was identified as a significant predictor of presence of ctDNA in blood. None of the assessed patient sociodemographic covariates had an effect on blood test positivity (data not shown).

Tumor location and blood test positivity (Fig. 2): Although not statistically significant, higher ctDNA positivity was observed for rectal tumors compared to cancers of the proximal colon: Proximal, n= 69, 35 positive (50.7%), 95%CI 38-63; Rectal, n= 23, 17 positive (73.9%), 95%CI 52-90; p value 0.567. This may be due to rectal tumors displaying a higher proportion of extramural venous invasion than proximal and distal colon tumors (rectal, 6/23 (26.1%, 95%CI 10-48); distal, 5/51 (9.8%), 95%CI 3.3-21.4; proximal 7/69 (10.1%), 95%CI 4.2-19.8).

CONCLUSION

In this study only tumor invasion depth was identified as a statistically significant predictor of presence of ctDNA in blood, although increased test positivity was observed for tumor features related to tumor aggressiveness (e.g. lymph node invasion, N0 vs N1-2: 49% vs 78%; M stage, M0 vs M1; 57% vs 93%) and tumor location. These tumor features may fail to reach significance as associations with appearance in blood due to case numbers (type-2 error). Demographic features did not influence the positivity of this ctDNA blood test.

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