

Appearance of tumour-associated methylated DNA in plasma correlates with colorectal tumour invasion

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Introduction

- Blood-based colorectal cancer (CRC) screening tests that detect circulating tumour-associated methylated DNA such as *SEPT9*, *BCAT1* or *IKZF1* provide an additional strategy for CRC screening (Figure 1).
- Acceptance of these new modalities by screening providers could be enhanced through a better understanding of the biology and mechanism(s) that result in the presence of these tumour biomarkers in blood
- We recently conducted a clinical evaluation of a 2-gene (methylated *IKZF1* and *BCAT1*) blood test in volunteers scheduled for colonoscopy or tumour resection.

Aim

- To determine whether the presence of methylated *BCAT1* and *IKZF1* in plasma correlates with morphological or biological features of the colorectal tumour.

Methods

- The study population consisted of volunteers undergoing colonoscopy for any indication (n = 1777).
- Blood was collected immediately prior to colonoscopy or prior to resection of the tumour
- Levels of methylated *BCAT1* and *IKZF1* in bisulphite-converted DNA isolated from 4mL of plasma were measured using a multiplexed methylation specific PCR assay.
- Any sample containing detectable levels of either methylated gene was considered positive.

Methods(cont..)

- Cancer histopathological assessment to determine cancer stage and features were undertaken by an expert pathologist
- Characteristics including T stage (TNM staging), nature of the tumor margin, degree of differentiation, perivascular or perineural invasion and metastasis were determined.
- Univariate (Chi² test) and multivariate logistic regression analysis were performed to determine the factors that are associated with test positivity. A Spearman Rank correlation was performed to assess the correlation of biological features to the levels of methylated *BCAT1* and *IKZF1* in the plasma. A p value less than 0.05 was considered statistically significant.

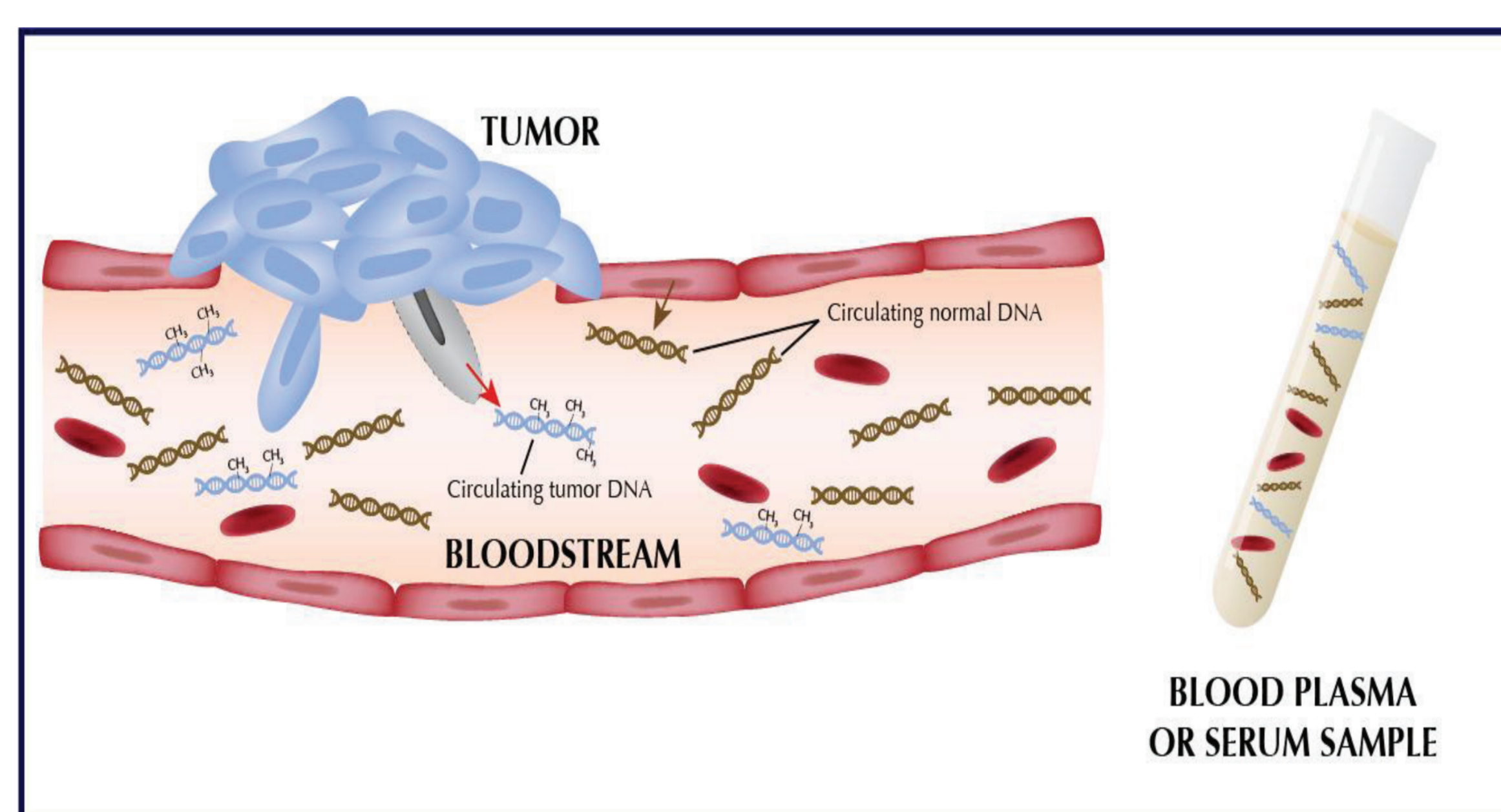


Figure 1. Proposed mechanism of genetic material from tumour entering into circulation

<http://healthcare.utah.edu/huntsmancancerinstitute/research/labs/varley/#dna>

Results

- A total of 111 patients with cancer were identified (median age 67yrs, 61% male)
- Distribution of cancer stages (AJCC) were stage I (n=29), Stage II (n=32), Stage III (n=27), Stage IV (n=20) with three cancers unstaged.
- Univariate analysis revealed advanced cancer stage (AJCC), presence of vascular invasion, presence of nodal metastasis and distant metastatic stage were significantly associated with presence of markers in the blood (p < 0.05, Table 1 and Figure 2).

Results (Cont..)

- Following adjustment for the biological features, multivariate logistic regression analysis revealed depth of invasion (T stage), tumour size and advanced cancer stage (AJCC) were significantly associated with presence of markers in the blood (Table 2)
- Concentration of both methylation markers in the blood were strongly associated with depth of invasion (*BCAT1* r = 0.396, p=0.00003; *IKZF1* r = 0.406, p=0.00002) and overall tumour size (*BCAT1* r = 0.331, p=0.0008; *IKZF1* r = 0.436, p=0.000006).

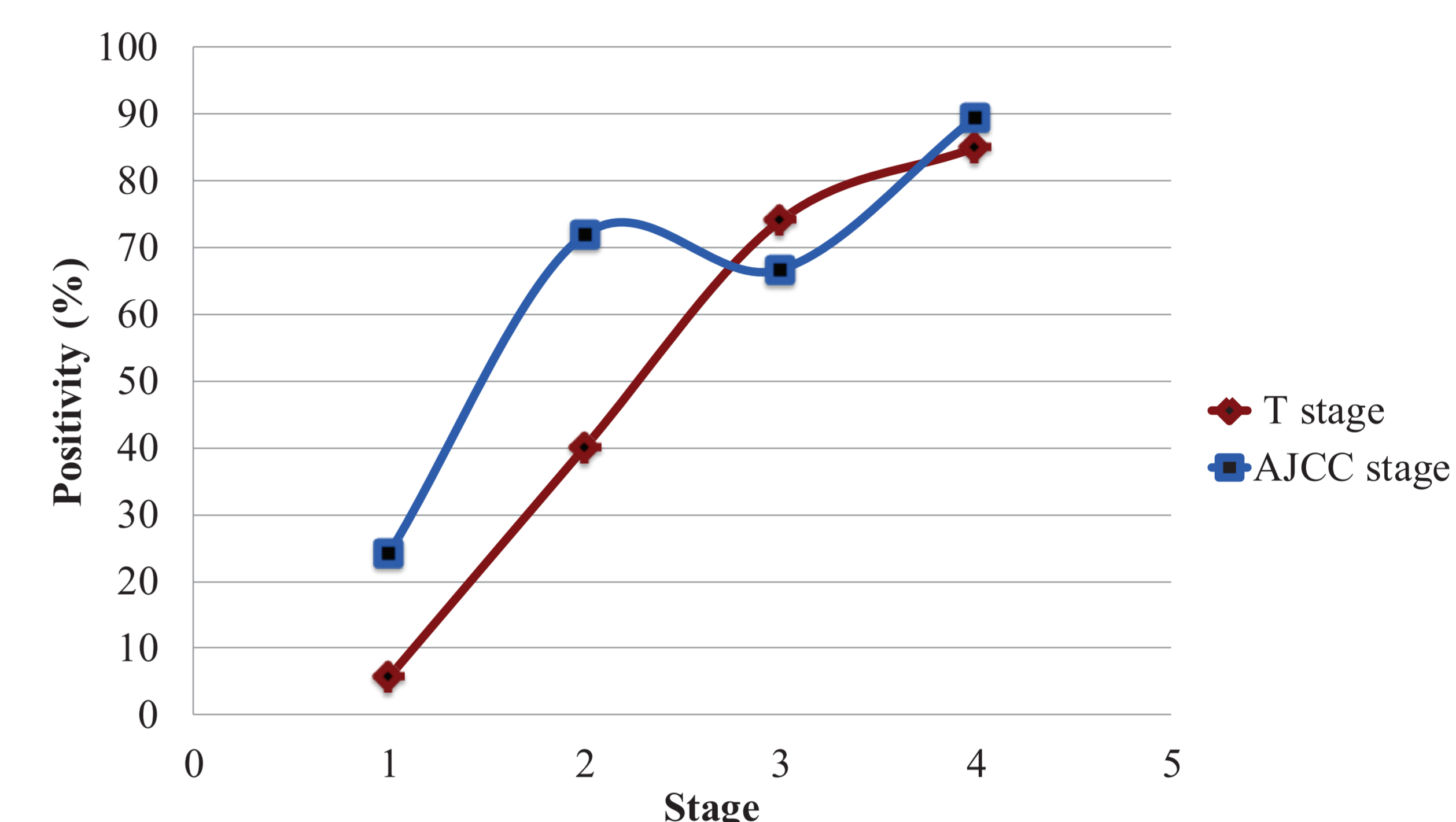


Figure 2. Positivity rate at the different T and AJCC stages

Table 1- Significant biological variables on univariate analysis

Biological variable	Total number	% Positive
T stage:		
T1	18	5.6%
T2	15	40.0%
T3	50	74.0%
T4	20	85.0%
AJCC stage:		
I	29	24.1%
II	32	71.9%
III	27	66.7%
IV	20	90.0%
Nodal metastasis:		
N0	60	51.7%
N1	25	64.0%
N2	12	100.0%
Distant metastasis:		
M0	61	49.2%
M1	17	88.2%
Vascular invasion:		
No	72	50.0%
Yes	27	81.5%

Table 2 -Significant biological factors on multivariate logistic regression analysis

Biological variable	Odds ratio	95% Confidence Interval	P value
T stage:			
T1	1.00		
T2	2.51	0.16 – 38.61	0.509
T3	53.1	3.77 – 748.76	0.003
T4	118.9	4.97 – 2841.72	0.003
AJCC stage:			
I	1.00		
II	8.16	2.48 – 26.82	0.001
III	6.54	2.00 – 21.36	0.002
IV	26.70	4.86 – 146.79	0.000
Tumour size (mm)	1.05	1.03 – 1.08	0.000

Conclusion

- Two-gene (methylated *IKZF1* and *BCAT1*) DNA blood test methylation concentrations positively correlated with depth of cancer invasion, tumour size, advanced stage, presence of nodal and distant metastasis.
- These results shed some light on likely mechanisms involved in the appearance of tumour-associated DNA in circulation.