A prospective cohort study in colorectal cancer assessing the relationship between post-surgery detection of methylated *BCAT1* or *IKZF1* ctDNA and risk for residual disease and survival.

David H. Murray[1], Graeme P. Young[2], Susanne K. Pedersen[1], Philippa Rabbitt[4], Susan E. Byrne[2], Kathryn J. Cornthwaite[2], Amitesh Roy[5], Christos Karapetis[5], Erin L. Symonds[2,3].

[1]Clinical Genomics Technologies Pty Ltd., North Ryde, Australia; [2]Flinders Centre for Innovation in Cancer, Bedford Park, Australia; [3]Bowel Health Service, Flinders Medical Centre, Adelaide, Australia; [4]Colorectal Surgery, Flinders University Medical Centre, Adelaide, Australia; [5]Department of Oncology, Flinders Medical Centre, Daw Park, Australia

email: david.murray@clinicalgenomics.com

BACKGROUND

The methylated 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,[1] a prospective study was conducted to assess the relationship between their persistence post-surgery and presence of and risk for residual disease as well as survival.

STUDY SYNOPSIS

Aim To determine the relationship between detection of methylated *BCAT1* and *IKZF1* following surgery and risk for residual disease and recurrence.

Study Design An observational study collecting blood from CRC patients.
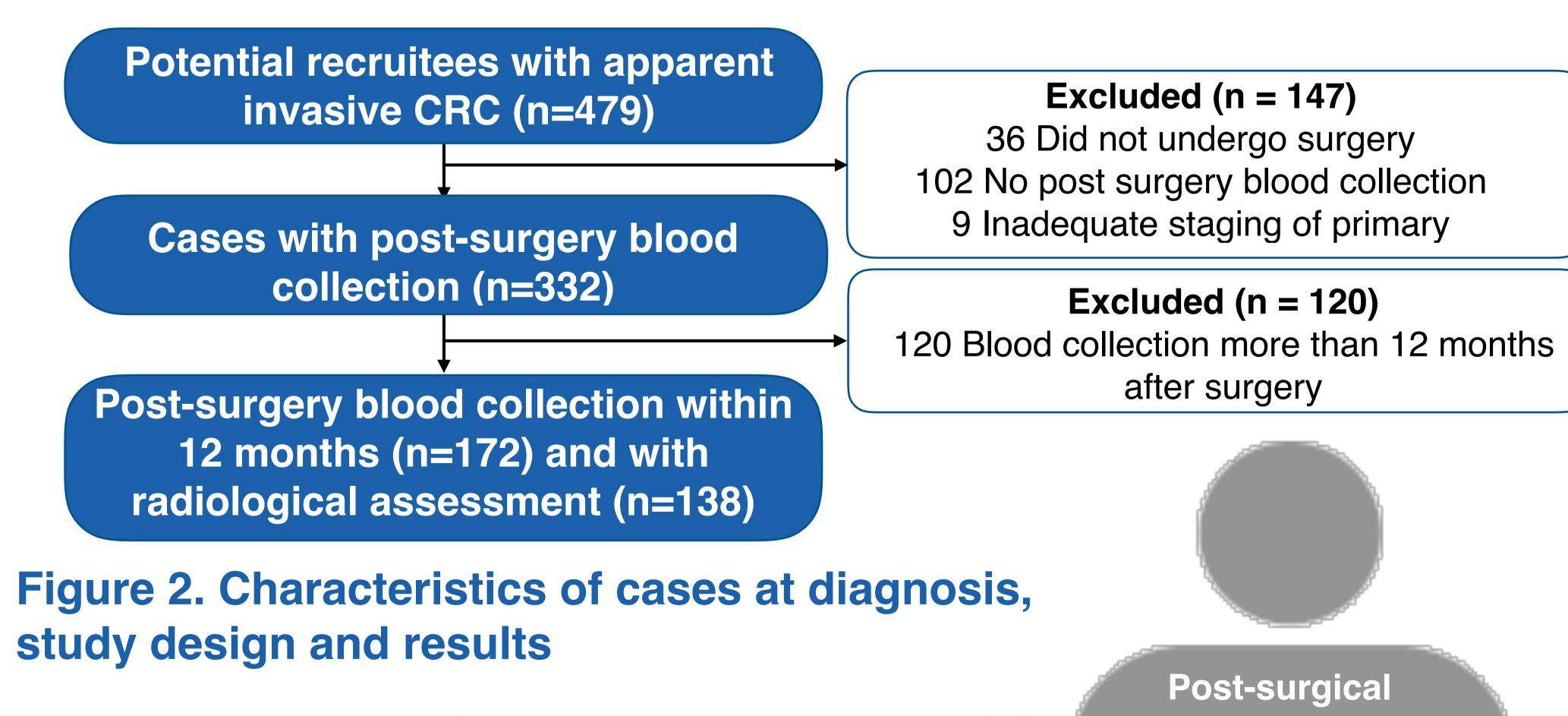
Study Cohort Adults diagnosed with invasive CRC and a blood sample collected within 12 months of surgery.

Methods DNA was extracted from at least 3.9mL of K_3EDTA-plasma collected within 12 months of initial surgical resection, bisulphite converted and assayed for methylated *BCAT1* and *IKZF1* as previously described.[2] 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.

Figure 1. Disposition and outcomes of study cohort


```
Potential recruitees with apparent invasive CRC (n=479)
        │                          Excluded (n = 147)
        │                          36 Did not undergo surgery
        ▼                          102 No post surgery blood collection
Cases with post-surgery blood       9 Inadequate staging of primary
collection (n=332)
        │                          Excluded (n = 120)
        │                          120 Blood collection more than 12 months
        ▼                          after surgery
Post-surgery blood collection within
12 months (n=172) and with
radiological assessment (n=138)
```

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

Characteristic	No. (%)	Post-surgical ctDNA status	
		-	+
Age, >65yo	100 (58)	62%	39%
Gender, Male	105 (61)	61%	61%
T stage, T3 or T4	124 (73)	70%	86%
M stage, M1	9 (5)	4%	86%
N stage, N1 or N2	72 (42)	38%	64%
Location, Distal	95 (56)	54%	70%
Tumor deposits found	11 (6)	3%	25%

Cases with margins involved, metastases present or apical node involvement are 5.3 times more likely to have a positive ctDNA test within 12 months of surgery

Cases with a positive ctDNA test within 12 months or surgery are 3.8 times more likely to have recurrence during follow up

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

Characteristic		No. (%)	Post-surgical ctDNA status, No. (%) Neg (n = 144)	Pos (n = 28)	OR (95%CI)[1]	P[2]
A. Resection margins involved		4 (2)	3 (2)	1 (4)	1.7 (0.2-17.4)	0.637
B. Number of nodes involved by tumor	0	112 (65)	100 (69)	12 (43)	reference	0.004
	1-3	41 (24)	34 (24)	7 (25)	1.7 (0.6-4.7)	
	4-6	11 (6)	6 (4)	5 (18)	6.9 (1.8-26.2)	
	*7 or more	8 (5)	4 (3)	4 (14)	8.3 (1.8-37.7)	
C. Apical node involved		7 (4)	2 (1)	5 (18)	15.4 (2.8-84.3)	0.002
D. Distant metastasis remaining after surgery		4 (2)	1 (1)	3 (10)	17.2 (1.7-171.6)	0.016
E. T4 Stage/peritoneal involvement		32 (19)	21 (15)	11 (39)	3.8 (1.6-9.2)	0.003
Less than 12 nodes sampled		32 (19)	26 (18)	6 (21)	1.2 (0.5-3.4)	0.675
[3]Incomplete non-surgical treatment at time of venesection		56 (33)	39 (27)	17 (61)	4.2 (1.8-9.7)	0.001
Any of A or D above		8 (5)	4 (3)	4 (14)	5.8 (1.4-24.9)	0.017
[3]Any of A, C or D above		13 (8)	6 (4)	7 (25)	7.7 (2.3-25.0)	0.001
Any of A, C, D or E above		36 (21)	23 (16)	13 (46)	4.6 (1.9-10.8)	0.001

[1]Odds Ratio determined by univariate logistic regression analysis; [2]Wald test, p-value; [3]Included in multivariate logistic regression.
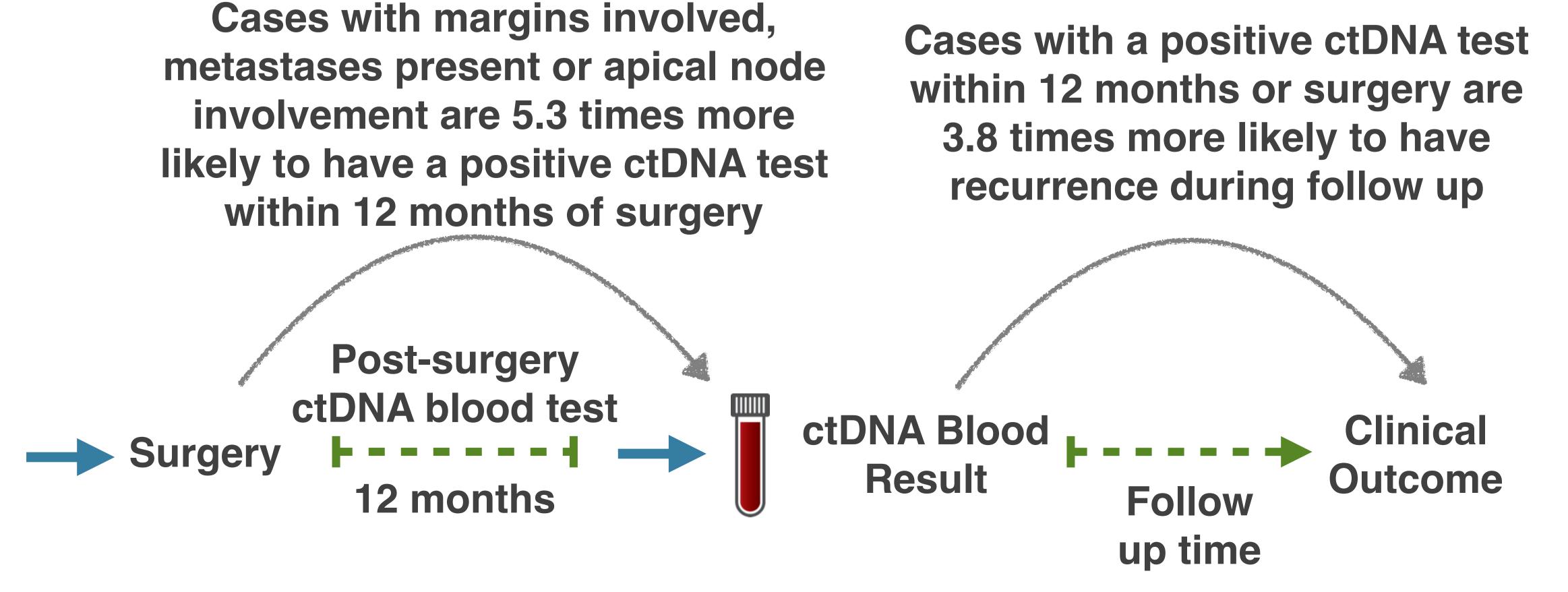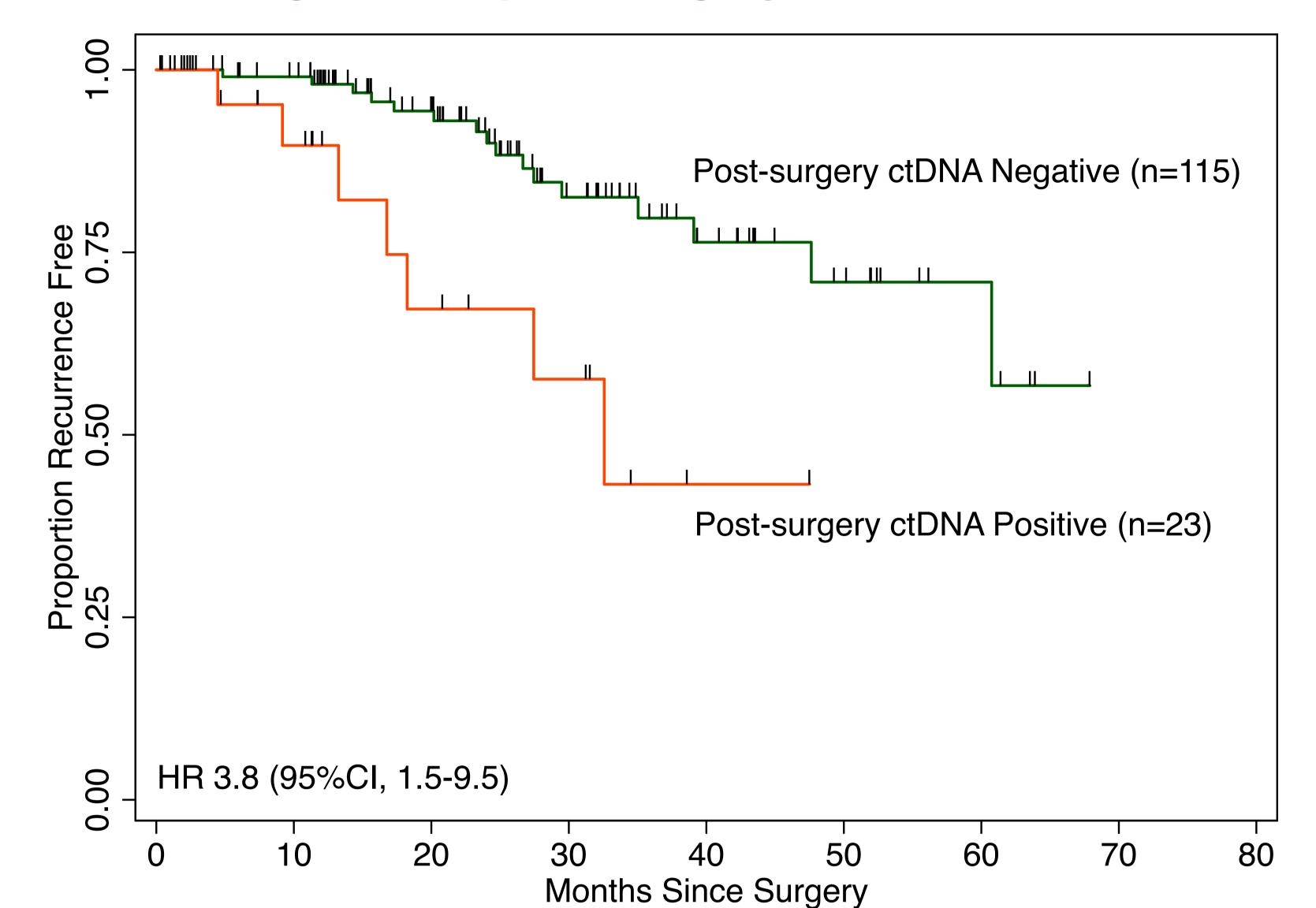
Funding

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

Post-surgery ctDNA Negative (n=115)
Post-surgery ctDNA Positive (n=23)
HR 3.8 (95%CI, 1.5-9.5)

Proportion Recurrence Free — Months Since Surgery

RESULTS

Study Cohort: Blood was collected from 172 CRC patients after surgery, tested for methylation of *BCAT1* and *IKZF1* and followed for a median 37.1mo (IQR 22.6-49.8) during which 23 experienced recurrence, 10 died from CRC and 28 (16%) were ctDNA positive post-surgery. Cases considered to warrant surveillance with radiographic examination for recurrence (n=138) were followed for a median 22.9 months (IQR 12.0-33.6).

Cases with presence or risk of residual disease more likely to be ctDNA positive: Multivariate logistic regression determined that cases with at least one of the 3-feature composites present (A, C or D as per table 2) were 5.3 times (95% CI: 1.5-18.4, p = 0.008) more likely to be ctDNA positive after surgery. Blood samples taken prior to completion of treatment (surgical resection of metastases and chemotherapy) were also more likely to return a positive ctDNA status (OR 3.4 (1.4-8.1), p = 0.007) compared to cases where the initial treatment had been completed.

Increased recurrence free survival for post-surgery ctDNA negative cases: The Cox PH multivariate modelling indicated that the ctDNA status determined within 12 months was the only significant predictor of recurrence and had an increased risk of recurrence (HR: 3.8, 95%CI: 1.5-9.5, p = 0.004) at any time compared to subjects with a negative result (figure 3).

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: (1) Pedersen et al. BMC Cancer 2015;15:654; (2) Symonds et al. Clin Transl Gastroenterol 2016;7:e137.

Post-surgery ctDNA blood test — 12 months — Surgery — ctDNA Blood Result — Follow up time — Clinical Outcome — CRC diagnosis