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Preoperative Circulating Tumor DNA in Patients with Peritoneal Carcinomatosis Is an Independent Predictor of Progression-Free Survival

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Abstract

Background—Next-generation sequencing (NGS) is a useful tool for detecting genomic alterations in circulating tumor DNA (ctDNA). To date, most ctDNA tests have been performed on patients with widely metastatic disease. Patients with peritoneal carcinomatosis (metastases) present unique prognostic and therapeutic challenges. We therefore explored preoperative ctDNA in patients with peritoneal metastases undergoing surgery.

Methods—Patients referred for surgical resection of peritoneal metastases underwent preoperative blood-derived ctDNA analysis (clinical-grade NGS (68 to 73 genes)). ctDNA was quantified as the percentage of altered circulating cell-free DNA (% cfDNA).

Results—Eighty patients had ctDNA testing: 46 (57.5%) women; median age, 55.5 years. The following diagnoses were included: 59 patients (73.8%), appendix cancer; 11 (13.8%), colorectal; five (6.3%), peritoneal mesothelioma; two (2.5%), small bowel; one (1.3%), each of cholangiocarcinoma, ovarian, and testicular cancer. Thirty-one patients (38.8%) had detectable preoperative ctDNA alterations, most frequently in the following genes: *TP53* (25.8% of all alterations detected) and *KRAS* (11.3%). Among 15 patients with tissue DNA NGS, 33.3% also had ctDNA alterations (overall concordance = 96.7%). Patients with high ctDNA quantities (≥ 0.25% cfDNA, n=25) had a shorter progression-free survival (PFS) than those with lower ctDNA quantities (n=55; 7.8 vs. 15.0 months; hazard ratio (95% confidence interval), 3.23 (1.43 to 7.28), $P = 0.005$, univariate; (p = 0.044, multivariate)).

Conclusions—A significant proportion of patients with peritoneal metastases referred for surgical intervention have detectable ctDNA alterations preoperatively. Patients with high levels of ctDNA have a worse prognosis independent of histologic grade.

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INTRODUCTION

Detection and investigation of molecular alterations in cancer has led to advances in understanding of tumor biology, use of targeted cancer therapies, and provides potential assessment of response to therapy.[1–3] Molecular tests from tumor tissue require biopsies or resection, are often performed on archival tissue, and do not represent the heterogeneous genomic constitution of many malignancies.[4,5] Cell-free circulating tumor DNA (ctDNA) is measurable in the plasma using next-generation sequencing (NGS) techniques, and is less invasive than obtaining tumor-derived genetic material.[6] The half-life of cell-free DNA (cfDNA) in the bloodstream is between 16 minutes and 2.5 hours, such that it may serve as a 'real-time' snapshot of the of the genomic status of a patient's cancer. cfDNA is rapidly lysed in the circulation via nucleases, followed by primarily renal excretion as well as uptake by the liver and spleen, where it is ultimately degradaded by macrophages.[7] This reproducible, minimally invasive, and spatially unbiased technology has been used to identify targeted therapies, measure residual disease, and assess response to therapy.[8–10]

The peritoneum is a common site of tumor metastasis, with approximately 55,000 new cases of peritoneal metastasis occurring annually in the US.[11] Patients with peritoneal carcinomatosis (metastases) have an uncertain, but often poor prognosis, which is influenced by tumor histology and extent of disease.[12] Treatment options for peritoneal metastases are limited as many peritoneal tumors are poorly vascularized and can be surrounded by a viscous layer of mucin, thus limiting drug delivery.[13] Surgical approaches with complete cytoreductive surgery (CRS) and intraperitoneal chemotherapy in select patients and histologies (including appendiceal, colorectal, and ovarian cancers, and peritoneal mesothelioma) with disease confined to the peritoneal cavity have demonstrated improved outcomes versus treatment with systemic chemotherapy, or in some cases cytoreduction, alone.[14–19] Imaging of peritoneal metastases for operative candidacy and recurrence is imprecise and often underestimates the actual disease burden.[20,21] Given the difficulty in treatment, imaging, and estimation of prognosis in patients with peritoneal metastases, use of a reliable, non-invasive, molecular diagnostic test has potentially high utility in this disease; although it is not known whether DNA shed from peritoneal metastases reach the systemic circulation in sufficient quantity to serve as a potential predictive or prognostic biomarker.[22]

Herein, we sought to determine the rate of detection and type of ctDNA alterations in patients with peritoneal metastases undergoing surgical resection.

MATERIALS AND METHODS

Patients

This is a prospective study of ctDNA analysis in patients referred for surgical management of peritoneal metastases. All patients had potentially resectable peritoneal metastases, and were referred to our institution's peritoneal malignancy program for consideration for cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) from May 20th, 2015, to January 1st, 2017. This study was approved by the University of

California San Diego (UCSD) institutional review board (IRB) and patients gave consent prior to study enrollment in accordance with IRB guidelines.

Procedures and Follow-Up

Patients were taken to the operating room with the intent of removing peritoneal metastases. If all or nearly all visible disease could be removed, then patients typically underwent cytoreductive surgery and intraperitoneal chemotherapy, per standardized techniques at our institution;[23] which include resection of tumor nodules and/or involved viscera followed by 90 minute hyperthermic peritoneal chemoperfusion with mitomycin C (for colorectal or appendiceal primary tumors) or doxorubicin/cisplatin (for ovarian cancer and peritoneal mesothelioma). If complete cytoreduction was not possible, patients underwent palliative debulking procedures, with the goal of minimizing peritoneal metastasis-related symptoms and morbidity. Complete resection was defined as removal of all visible (gross) peritoneal metastases.

Histology of the resected specimen(s) was categorized by grade, with low-grade histology including patients with acellular mucin and low-grade mucinous carcinoma peritonei; and high-grade histology including patients with any grade of adenocarcinoma or high-grade mucinous carcinoma peritonei. Mesothelioma cases were not included in the grade analyses.

Postoperative treatment and surveillance were at the discretion of the referring oncologist; and due to referral patterns, complete information of all subsequent care and observation was not available for every patient. However, patients generally underwent surveillance imaging every three to six months after surgery. Postoperative systemic treatment was typically administered in those patients with high-grade malignancies with incomplete resections or without sufficient preoperative systemic treatment.

Next Generation Sequencing

Next generation digital sequencing of cell-free ctDNA extracted from plasma was performed by Guardant Health (Guardant360, www.guardanthealth.com/guardant360/), a Clinical Laboratory Improvement Amendments (CLIA)-certified, College of American Pathologists (CAP)-accredited, and New York State Department of Health-approved clinical laboratory (Guardant Health, Inc., Redwood City, CA). At the time of this study, this test identified potential tumor-related genomic alterations via exon sequencing of 68 to 73 cancer-related genes, 18 gene amplifications, 6 gene fusions, and 23 gene insertion/deletions. This assay has high analytic sensitivity (detects single molecules of somatic tumor DNA in 10 mL blood samples), high clinical sensitivity (detects 85%+ of the single nucleotide variants detected in tissue in advanced cancer patients), and high analytic specificity (>99.9999%).[24] All cell-free DNA (cfDNA) was sequenced, including the germline cell-free DNA that is derived from leukocyte lysis and the somatic cfDNA. Single nucleotide variants were quantitated as the mutant allele fraction which is the number of ctDNA fragments divided by the number of wild type DNA fragments that overlap the same mutated nucleotide base position. Gene amplifications were reported as absolute gene copy number in plasma. In each sequencing run, a normal control sample was included. Each ctDNA alteration was quantified as a percentage, or allele frequency, of circulating cell-free DNA (% cfDNA).

Variants of uncertain significance (VUS) were included in the ctDNA analysis in order to capture the most comprehensive DNA alteration profile. These alterations have uncertain functional consequences and clinical significance; but they may be reflective of tumor growth, turn-over, size, heterogeneity, vascularization, disease progression, or treatment. All samples were drawn prior to operative management of peritoneal metastases, typically 1–2 weeks before the operation.

Some patients also underwent tissue genomic analysis from resected peritoneal tumors, performed at the discretion of their treating clinician, which were compared with the ctDNA alterations. Tissue DNA analysis was performed from formalin-fixed, paraffin-embedded (FFPE) tumor samples in a CLIA-certified clinical laboratory for genomic profiling (Foundation Medicine, Cambridge, MA). At the time of this study, this test analyzes the entire coding sequence of 315 cancer-related genes and select introns from 28 genes.

Statistics

Continuous variables were reported as median and ranges, and dichotomous and categorical variables were reported as proportions in each group. Progression-free survival (PFS) was calculated from the time of operation to progression (as determined by RECIST on subsequent imaging, endoscopy, or operation) or death, and was analyzed by the Kaplan-Meier method. Univariate and multivariate Cox proportional hazards model were performed to identify predictors of PFS. A P-value of ≤ 0.05 was considered statistically significant. Concordance of ctDNA and tissue DNA alterations (by gene altered) was calculated as follows, considering variants reported on both assays: overall concordance was the sum of present and absent alterations found in tissue and blood among all possible concordant alterations; positive concordance was the number of present alterations in tissue and blood among the number of any present alterations in tissue or blood; and negative concordance was the number of absent alterations in tissue and blood among any absent alteration in tissue or blood. All statistical analyses were performed using SPSS Version 24 (IBM Corp., Armonk, NY) and R Version 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org/).

RESULTS

Patient and ctDNA Characteristics

This analysis included 80 consecutive patients who had ctDNA analyzed and underwent surgery for peritoneal metastases. Baseline and ctDNA characteristics are listed in Table 1. ctDNA assessments were made a median of 4.0 days prior to surgery (range 0–108 days).

Mutational Landscape

Altered genes by ctDNA among all cancers, and among the two most common primary sites are listed in Table 2. The quantity of ctDNA for each gene alteration is listed as the median percentage of altered cell-free DNA (% cfDNA). Taking into account the highest % cfDNA of any alteration in each patient (peak % cfDNA), the median value among all patients was 0% (61.2% had no ctDNA alteration), and the mean peak % cfDNA was 0.5%. Among patients with ctDNA detected (n = 31), the median peak % cfDNA was 0.30% (range 0.1 –

10.1). Dividing the entire cohort into high and low % cfDNA groups by comparing patients harboring the highest one-third of peak % cfDNA levels with others resulted in groups containing < or ≥ 0.25% cfDNA. There were 24 distinct genes altered in the entire cohort, including 59 distinct genomic alterations, with a median number of two alterations per patient. The two most common genes altered were *TP53* (n=16, 25.8% of all alterations) and *KRAS* (n=7, 11.3%).

Patients were stratified into high-grade (n = 39) versus low-grade (n = 36) tumors. The median number (range) of alterations per patient in high-grade versus low-grade tumors was 1 (0 to 9) versus 0 (0 to 3) ($P = 0.010$, student's t test). The median (range) peak % cfDNA was 0.20 (0 to 10.01) versus 0 (0 to 4.10) (P = 0.276, student's t test).

The extent of peritoneal disease (as measured by the peritoneal cancer index[25]), mucinous tumors, the presence of signet ring cells, and neoadjuvant chemotherapy within three months of surgery did not correlate with the level (< or ≥ 0.25% cfDNA) of ctDNA (by univariate logistic regression).

Concordance of Tissue and ctDNA Genetic Alterations

Fifteen patients underwent tissue DNA NGS from tumor removed during the index operation (procedure following ctDNA measurement). Results of tissue DNA and ctDNA alterations are shown in Table 3 by the gene(s) altered. Among the nine appendiceal primary patients that underwent tissue genomic profiling, three had low-grade disease (patients 4, 9, and 13 in Table 3). The median time between tissue acquisition and blood draw was 3.0 days (range, 1 to 108 days). Three patients were tested with a 68 gene ctDNA panel, one patient was tested with a 70 gene ctDNA panel, and one with a 73 gene ctDNA panel. Five patients with tissue alterations also had alterations in the blood (33.3% of 15 patients). All five patients with ctDNA alterations also had tissue alterations. There were a total of 17 variants identified that were covered by both the ctDNA panel and the tissue panel. An additional 15 variants were identified by tissue NGS, but these genes/regions were not covered in the ctDNA panel, and an additional one variant identified in ctDNA was not covered by the tissue NGS panel. Of the 17 alterations covered by both test types, 6 of 17 (35.3%) were concordant in both sample types, 7 of 17 (41.2%) were detected only in tissue, and 4 of 17 (23.5%) were detected only in ctDNA. Among patients with detectable ctDNA alterations (n = 5), the overall, positive, and negative concordance was 96.7%, 35.3%, and 96.6%, respectively, with tissue DNA alterations.

Correlation of ctDNA and Progression-Free Survival

Median follow-up for the entire cohort was 6.8 months (range 0.1–27.9 months). The median progression-free survival (PFS) in the entire cohort was 14.3 months (95% CI, 10.5 to 18.1 months). Median PFS was 15.0 months for those with low (< 0.25% cfDNA) ctDNA levels versus 7.8 months for those with high (≥ 0.25% cfDNA) ctDNA levels (HR 3.23, CI 95% 1.43 to 7.28, $P = 0.005$; Figure 1). PFS for high versus low ctDNA levels among subgroups (incomplete/incomplete resections, low/high-grade histology) is also shown in **Figure 1.**

Univariate and multivariate analyses by Cox proportional hazards model were performed to identify predictors of PFS (Table 4). On univariate analysis, high ctDNA (> 0.25% cfDNA) and high-grade histology were predictors of worse PFS (HR 3.23 for high vs. low levels ctDNA, $P = 0.005$; HR 1.99 for high- vs. low-grade, $P = 0.002$). These two factors remained independent predictors of PFS on multivariate analysis (HR 2.36 for high vs. low levels ctDNA, $P = 0.044$; HR 3.30 for high- vs. low-grade, $P = 0.009$). Excluding the 15 patients who had ctDNA drawn greater than two weeks prior to surgery, high ctDNA levels still correlated with worse PFS by univariate Cox proportional hazards model (HR 4.18, p=0.006). Analyzing the entire cohort by the presence/absence of ctDNA (n=31/49), we found the presence of ctDNA correlated with worse PFS (HR 2.32, p=0.035) by univariate analysis.

DISCUSSION

Repeatable, minimally invasive, cancer-specific biomarkers such as ctDNA allow potential opportunities for diagnosis, treatment, and surveillance of malignancy. In this study of patients with peritoneal metastases from a variety of primary tumors referred for surgical management, we found detectable ctDNA alterations in 38.8%. This is the first study evaluating ctDNA in patients with peritoneal metastases treated with surgical resection. The rate of detectable alterations and the quantity of ctDNA varied by histology, consistent with other studies.[26] Detectable ctDNA alterations also vary by stage of disease, and patients with peritoneal metastases are thought to have higher levels than those with localized disease.[27] The two most common alterations - in *TP53* and *KRAS* - are typical of gastrointestinal malignancies and represent the most commonly mutated genes in human cancer.[28] Furthermore, inactivation of TP53 is typically a truncal driver event, and shed from all tumor cells.[29] Thus, it would be expected to be at highest % cfDNA and most likely to be detectable. Overall, the levels of ctDNA in our patients were significantly lower than reported previously, possibly because prior studies focused on patients with widely metastatic disease and high tumor burden.[30,31]

We found high concordance rates with tissue DNA alterations (96.7% overall concordance), among those patients in whom tissue NGS was obtained, which has also been shown in other studies.[32] However, the positive concordance rates were overall much lower (35.3%) than the negative concordance rate (96.6%). Differences between tissue and circulating tumor-derived DNA alterations may be due, in part, to tumor heterogeneity, tumor cellularity, and the extent of tumor cell DNA shedding. Indeed, ctDNA analysis may not suffer from the sampling bias of tissue-based genomic analyses, since tumor DNA shed into the blood derives from multiple sites while tissue NGS reflects only the small tissue sample assayed.[5] Differences in the sensitivity of the assays may also explain these differences.

We found that high levels of ctDNA (≥ 0.25% cfDNA) correlated with worse PFS. This was true despite controlling for the grade of tumor, as high-grade tumors have higher rates and levels of ctDNA alterations. This finding may allow use of ctDNA to better predict prognosis, particularly in malignancies such as appendiceal cancer, which has a 3-fold variance in risk of recurrence among various subtypes.[33] The correlation between ctDNA

and PFS may also have implications for surveillance of patients using ctDNA (regardless of the genomic alteration), and investigation in this area is ongoing at our institution.

The primary limitations of ctDNA include the need for tumor-encoded mutations to be detected in the circulation. DNA in the circulation may also come from sources other than the tumor in question, such as a second primary or myeloid pre-malignant condition known as clonal hematopoiesis of indeterminate origin.[34] Other limitations of ctDNA, as it becomes more sensitive, may theoretically include the ability to discern genomic mutations derived from benign tissue.[35] However, the methods used herein are approaching a practical limit with sensitivity as low as 1–2 DNA molecular fragments in 10 mL of blood without evidence of ctDNA from benign lesions. Additional limitations of this project include modest sample size, single-institution setting, and incomplete follow-up due to referral patterns. Also, due to cost and complexity, not all patients had tissue molecular profiling completed from the index operation for comparison with the ctDNA results.

In summary, ctDNA alterations are detectable in many patients undergoing surgical treatment of peritoneal metastases. Patients with high preoperative levels of ctDNA have a worse prognosis. Liquid biopsy for ctDNA analysis in this population is a promising new method for prognostication that merits additional studies. Further investigations of longitudinal ctDNA analysis are ongoing, which may yield additional information regarding the role of ctDNA interrogation in diagnosis, treatment, and surveillance.

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Disclosures

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Ms. Raymond and **Dr. Lanman** are employees at Guardant Health, Inc.

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Synopsis

This paper examines preoperative circulating tumor DNA (ctDNA) in patients undergoing surgery for peritoneal metastasis at a single high-volume center; describing the ctDNA mutational landscape, concordance with tissue DNA alterations, and correlation with progression-free survival.

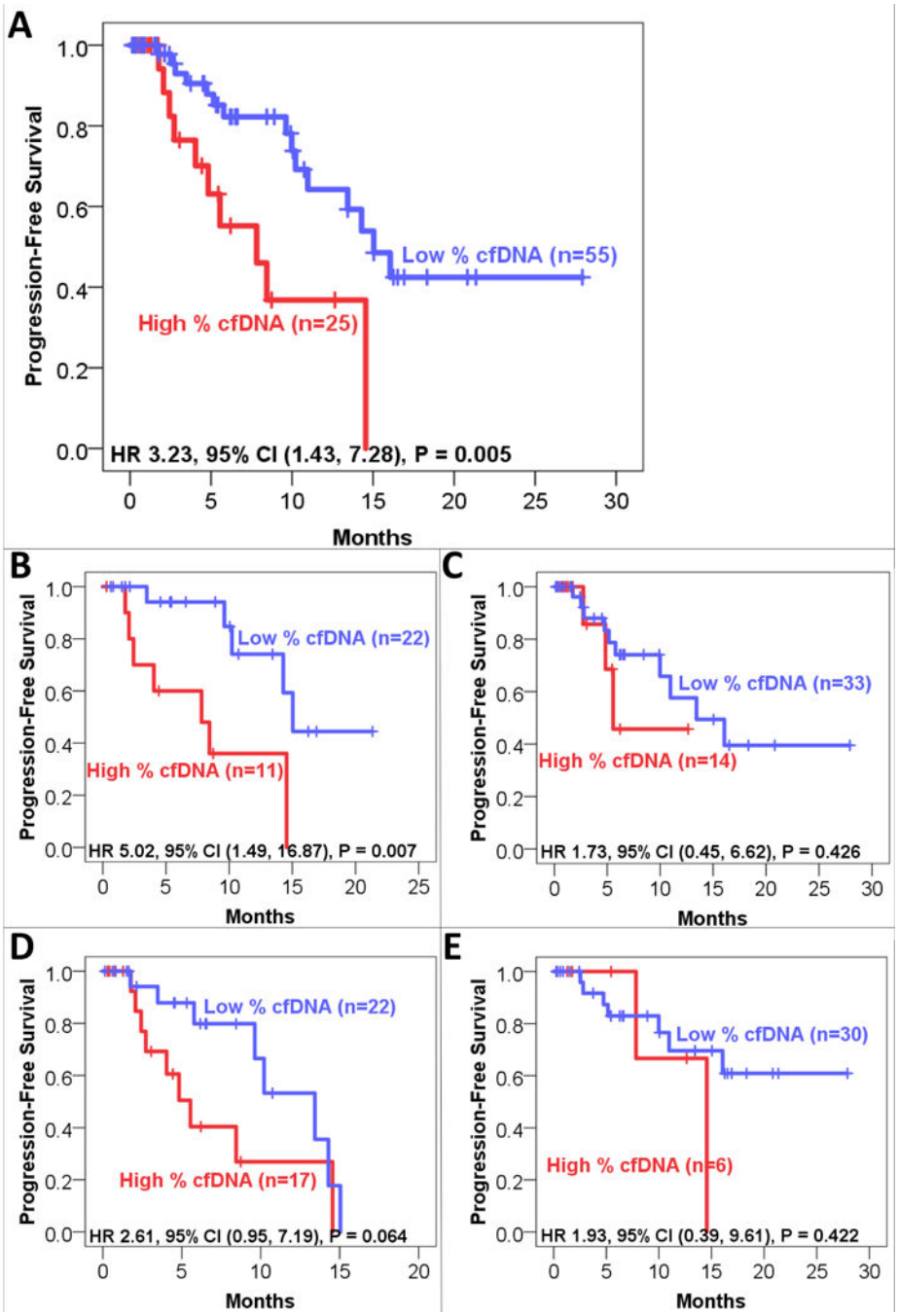

Figure 1. Progression-Free Survival (n=80 patients).

Kaplan-Meier analysis of progression-free survival (PFS) in patients with high (≥ 0.25%) or low (<0.25%) ctDNA level, measured as the alteration with highest percentage of altered cfDNA (peak % cfDNA). Patients who had not progressed at the time of last follow-up were censored (tick marks). **A**. All patients. **B.** Patients with complete resection. **C**. Patients in whom complete resection was not possible. **D**. Patients with high-grade histology. **E**. Patients with low-grade histology.

Table 1.

Baseline patient characteristics and ctDNA

Variable	**n (%)**
Total Patients	80 (100)
Age (yrs) (median, range)	55.5 (26–73)
Number of patients with alterations (includes variants of unknown significance) (%)	31 (38.8%)
Gender	
Men	34 (42.5)
Women	46 (57.5)
Procedure	
Colectomy	1 (1.3)
CRS/HIPEC	53 (66.3)
Palliative Debulking	26 (32.5)
Complete Resection[1]	
Yes	33 (41.3)
No	47 (58.8)
Detectable ctDNA Alteration[2]	
All Patients (n=80)	31 (38.8)[3]
Appendiceal Cancer (n=59)	21 (35.6)
Low-Grade Carcinomatosis (n=34)	9 (26.5)
High-Grade Carcinomatosis (n=25)	12 (48.0)
Colon Adenocarcinoma (n=11)	7 (63.6)
Malignant Mesothelioma (n=5)	2 (40.0)
Small Bowel Adenocarcinoma (n=2)	0 (0)
Cholangiocarcinoma (n=1)	0 (0)
Ovarian Cancer (n=1)	0 (0)
Testicular Cancer (n=1)	1 (100)

[1] Resection to no residual gross disease (R0/R1)

[2] ctDNA alterations include characterized alterations and variants of unknown significance

[3] % of patients with designated cancer with ctDNA alteration

Table 2.

Altered Genes and Proportion of Cell-Free DNA (n=31 patients with detectable alterations)

Gene	Alterations, n (%[1])			Median % cfDNA (range)
	All Cancers (n=31)	Appendiceal Cancer (n=21)	Colon Cancer (n=7)	
TP53	16 (25.8)	12 (32.4)	3 (15.0)	0.40 (0.10–4.00)
KRAS	7 (11.3)	3 (8.1)	4 (20.0)	0.52 (0.20–1.65)
EGFR	4 (6.5)	3 (8.1)	1 (5.0)	0.13 (0.10–0.30)
NF1	4 (6.5)	4 (10.8)		0.65 (0.40–4.10)
TERT	4 (6.5)	3 (8.1)	1 (5.0)	4.46 (0.30–10.10)
APC	3 (4.8)	1 (2.7)	1 (5.0)	0.10 (0.10–0.40)
GNAS	3 (4.8)	1 (2.7)	1 (5.0)	0.20 (0.20–3.60)
ARID1A	2 (3.2)	1 (2.7)	1 (5.0)	0.80 (0.20–1.40)
ATM	2 (3.2)	1 (2.7)	1 (5.0)	0.80 (0.30–1.30)
MET	2 (3.2)		1 (5.0)	0.40 (0.20–0.60)
PIK3CA	2 (3.2)	1 (2.7)	1 (5.0)	0.75 (0.10–1.40)
ARAF	1 (1.6)	1 (2.7)		0.3
CCND1	1 (1.6)	1 (2.7)		Amplification
CCND2	1 (1.6)		1 (5.0)	Amplification
ERBB2	1 (1.6)	1 (2.7)		0.2
FGFR2	1 (1.6)	1 (2.7)		0.2
HNF1A	1 (1.6)			0.3
JAK2	1 (1.6)	1 (2.7)		1.8
MYC	1 (1.6)		1 (5.0)	Amplification
NOTCH	1 (1.6)		1 (5.0)	1.42
PDGFRA	1 (1.6)	1 (2.7)		0.4
RAF1	1 (1.6)	1 (2.7)		0.1
RIT1	1 (1.6)		1 (5.0)	0.1
SMAD4	1 (1.6)		1 (5.0)	0.2
Total Alterations	**62**	**37**	**20**	**0.30 (0.10–10.10)**
Median Number Alterations/ Patient (range)	**1 (1–9)**	**1 (1–9)**	**2 (1–6)**	
Total Number Distinct Alterations	**59**	**36**	**19**	
Total Number Distinct Genes Altered	**24**	**17**	**15**	

[1]% of total alterations in designated cancer types; alterations include characterized alterations and VUS

Table 3.

Tumor Tissue Genomic Profiles (n=15 patients)*

Patient	Primary Cancer	Tissue DNA Alterations[1]		ctDNA Alterations[1]
		Not in ctDNA Panel	In ctDNA Panel	
1	Colon		***KRAS***, *APC*, *PIK3CA*	***KRAS***, *NOTCH*, *TP53*
2	Appendix		***GNAS***	***GNAS***
3	Colon	*PIK3R1*	***TP53*** *APC*, *KRAS*	***TP53*** *RIT1* (not in tissue panel)
4	Appendix		*GNAS*	
5	Appendix	*RBM10*	*KRAS*, *SMAD4*, *TP53*	
6	Small Bowel	*CHEK2*	*KRAS*	
7	Appendix	*SMAD2*	*KRAS*	
8	Appendix	*MAP2K4*, *SPTA1*, *TGFBR2*	*GNAS*, *KRAS*	
9	Appendix	*TGFBR2*	*GNAS*, *KRAS*, *PIK3CA*	
10	Appendix		*KRAS*, *SMAD4*, *TP53*	
11	Appendix	*TGFBR2*	*GNAS*, *KRAS*	
12	Colon	*AKT3, BARD1, FGF23, FGF6, KDM5A, KDM6A,*	***CCND2***, ***KRAS***, ***MYC***	***CCND2***, ***KRAS***, *MET*, ***MYC***
13	Appendix	*TGFBR2*	*GNAS*, *KRAS*	
14	Colon	*BTG1, CARD11, CREBBP, FAM123B, PIK3R2, PMS2, SOX9, TGFBR2*	***APC***, ***KRAS***, *TP53*	***APC***, ***KRAS***
15	Bile Duct		*CDKN2A*, *KRAS*, *MTOR*, *TP53*	

*Tissue NGS was performed at Foundation Medicine based on tissue obtained at the time of index surgery. ctDNA was generally obtained within two weeks prior to surgery.

[1]Include characterized alterations and VUS

Table 4.

Univariate and Multivariate Predictors of PFS (by Cox Proportional Hazards Model)*.

Variable		Univariate Analysis		Multivariate Analysis	
	Median PFS (months) (95% CI)	HR (95% CI)	P-value	HR (95% CI)	P-value
Preoperative ctDNA Alterations % cfDNA[1]					
< 0.25% (n=55)	15.0 (11.8, 18.3)	3.23 (1.43, 7.28)	**0.005**	2.36 (1.02, 5.45)	**0.044**
≥ 0.25% (n=25)	7.8 (3.6, 12.0)				
Complete Resection					
Yes (n=33)	14.5 (10.3, 18.8)	0.96 (0.65, 1.41)	0.837		
No (n=47)	13.4 (6.5, 20.4)				
Grade[2]					
Low (n=36)	Not reached	1.99 (1.29, 3.09)	**0.002**	3.30 (1.34, 8.11)	**0.009**
High (n=39)	9.6 (7.1, 12.1)				

*Multivariate model was developed using variables that were significant ($P \leq 0.05$) in univariate analysis. All HRs are given for ≥ 0.25% vs. < 0.25 peak % cfDNA, No vs. Yes Complete Resection, and High vs. Low Grade, respectively

[1]ctDNA as highest % of altered circulating cell-free DNA (peak % cfDNA)

[2]Excluding patients with mesothelioma

Abbreviations: cfDNA = cell free DNA; CI = confidence interval; HR = hazard ratio; PFS = progression-free survival