

CANCER

Detection of Circulating Tumor DNA in Early- and Late-Stage Human Malignancies

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The development of noninvasive methods to detect and monitor tumors continues to be a major challenge in oncology. We used digital polymerase chain reaction–based technologies to evaluate the ability of circulating tumor DNA (ctDNA) to detect tumors in 640 patients with various cancer types. We found that ctDNA was detectable in >75% of patients with advanced pancreatic, ovarian, colorectal, bladder, gastroesophageal, breast, melanoma, hepatocellular, and head and neck cancers, but in less than 50% of primary brain, renal, prostate, or thyroid cancers. In patients with localized tumors, ctDNA was detected in 73, 57, 48, and 50% of patients with colorectal cancer, gastroesophageal cancer, pancreatic cancer, and breast adenocarcinoma, respectively. ctDNA was often present in patients without detectable circulating tumor cells, suggesting that these two biomarkers are distinct entities. In a separate panel of 206 patients with metastatic colorectal cancers, we showed that the sensitivity of ctDNA for detection of clinically relevant *KRAS* gene mutations was 87.2% and its specificity was 99.2%. Finally, we assessed whether ctDNA could provide clues into the mechanisms underlying resistance to epidermal growth factor receptor blockade in 24 patients who objectively responded to therapy but subsequently relapsed. Twenty-three (96%) of these patients developed one or more mutations in genes involved in the mitogen-activated protein kinase pathway. Together, these data suggest that ctDNA is a broadly applicable, sensitive, and specific biomarker that can be used for a variety of clinical and research purposes in patients with multiple different types of cancer.

INTRODUCTION

Cancer will occur in more than 1.6 million individuals this year in the United States alone, but a clinically proven circulating biomarker that can be used to help guide patient management will be available for only a minority of them, even in the setting of widespread metastasis (1–6). Although serum-based protein biomarkers such as carcinoma antigen-125 (CA-125), carcinoembryonic antigen (CEA), and prostate-specific antigen (PSA) are commonly used for this purpose, these proteins are also found in the serum of individuals without cancer, albeit in lower concentrations (2–4). Additionally, these markers are not found to be elevated in a substantial portion of patients with advanced cancers (5, 6).

A new generation of biomarkers has become available with the discovery of the genetic alterations that are responsible for the initiation and progression of human cancers (7–11). With the influx of genomic information from recent cancer genome sequencing studies, it is now known that virtually all cancers of every type harbor somatic genetic alterations. These alterations include single-base substitutions, insertions, deletions, and translocations (the latter including those asso-

ciated with the creation of gene fusions, gene amplifications, or losses of heterozygosity). These somatic mutations occur at negligible frequencies in normal cell populations and therefore provide exquisitely specific biomarkers from a biological perspective (9).

There are two sources of tumor DNA that can be noninvasively assessed in the circulation: cell-free circulating tumor DNA (ctDNA) and circulating tumor cells (CTCs) (12, 13). ctDNA is composed of small fragments of nucleic acid that are not associated with cells or cell fragments (14). In contrast, CTCs represent intact, often viable, cells that can be purified from blood by virtue of physicochemical characteristics or cell surface molecules that distinguish them from normal blood cells (15). Many studies have shown that both ctDNA and CTCs are present in advanced neoplasia, although only a few studies have compared the amounts of CTCs and ctDNA templates in the same patients (16–19). The studies comparing the two approaches have reached opposing conclusions, likely because of technical issues that limited interpretation of either the ctDNA or CTC content. Furthermore, the mechanism by which CTCs or ctDNA is released into the circulation

is unclear, although it is possible that ctDNA actually comes from CTCs. One of the purposes of the current study was to compare the quantities of ctDNA and CTCs in the circulation of the same patients using an unbiased approach.

Most studies of ctDNA published to date have each evaluated patients with a single tumor type. In light of considerable differences in DNA preparation and analytic techniques in these studies, it has been difficult to directly compare the amounts of ctDNA among tumor types (16, 20–26). Comparisons of studies are also challenging because of differences in the types of data that are reported. For example, it is often impossible to compare real-time polymerase chain reaction (PCR) results with those reporting the fraction of mutant template molecules assessed, or to compare results based on the analysis of serum with those based on plasma. To directly compare different tumor types and to determine the spectrum of cancers in which ctDNA measurements could prove clinically useful, we evaluated a large number of tumor types in the current study. We purified plasma and tumor DNA using regimented protocols for all samples and used digital technologies to evaluate ctDNA levels from each tumor so that we could report the number of mutant templates per milliliter of plasma in each case (Fig. 1). This approach also allowed us to directly compare the two most commonly used types of tumor-specific genetic alterations found in the circulation: single-base substitutions and rearrangements.

One of the most immediate applications of ctDNA has been termed the “liquid biopsy” (20). In research studies as well as in clinical practice, it is often difficult to obtain tumor samples for genetic analyses. Some tumors are only accessible through fine-needle aspirates (lung cancer, for example) with insufficient material available for genotyping, whereas in other cases it can be challenging or time-consuming to

acquire samples from different medical centers (27). Additionally, once a targeted therapy is initiated in a patient with multiple metastases, clinicians frequently search for early evidence of recurrence or mechanisms underlying resistance, a scenario in which liquid biopsies are particularly valuable. For example, they can provide temporal measurements of the total tumor burden as well as identify specific mutations that arise during therapy (16, 20, 21, 23, 28, 52). Although the liquid biopsy approach has been shown to be promising, its sensitivity and specificity with respect to conventional tumor biopsies have not been evaluated in a large, clinically relevant cohort. Here, we evaluated the sensitivity and specificity of this approach in patients with colorectal cancers (CRCs) who were candidates for epidermal growth factor receptor (EGFR) blockade. We also used liquid biopsies to identify mutations that were responsible for recurrence in patients who initially responded to EGFR blockade. In aggregate, these studies provide a wealth of information on the potential utility, as well as the limitations, of ctDNA measurements for the assessment of patients with various cancers.

RESULTS

Patients with metastatic cancers

We began this study with an evaluation of 136 metastatic tumors originating from 14 different tissue types, as well as of 41 patients with primary brain tumors (glioma and medulloblastoma). Primary brain tumors were included in this evaluation because they are generally lethal even though they rarely metastasize. We also included 10 additional cases, composed of stage III ovarian ($n = 7$) and hepatocellular carcinomas ($n = 3$), in this particular evaluation because stage IV cases are rare and stage III disease is more representative of advanced disease in these two tumor types. The clinical characteristics of these patients are summarized in Table 1. Targeted sequencing, exomic sequencing, or whole-genome sequencing was used to identify mutations in the tumors, as described in Supplementary Materials and Methods. In these advanced cases, at least one genetic alteration—a point mutation (151 cases) or genetic rearrangement (36 cases)—was found in each of the tumors studied (table S1). Except for a subset of mutations at the known hotspots of the *KRAS*, *NRAS*, *PIK3CA*, and *BRAF* genes (which are well known to be somatic), all other genetic alterations were demonstrated to be somatic through evaluation of DNA from non-neoplastic cells of the same patients. ctDNA was assessed by one of three digital methods (see Supplementary Materials and Methods). These methods yielded comparable results when applied to the same plasma samples (fig. S1) and all were able to detect one mutant template in the DNA purified from up to 5 ml of plasma. The amounts of plasma available from each patient are listed in table S1.

ctDNA was detected in most of the studied patients with solid tumors outside the brain (112 of 136; 82%). However, the fraction of patients with detectable ctDNA varied with tumor type (likelihood ratio test, $P < 0.001$). As shown in Fig. 2A and fig. S2, most patients with stage III ovarian and liver cancers and metastatic cancers of the pancreas, bladder, colon, stomach, breast, liver, esophagus, and head and neck, as well as patients with neuroblastoma and melanoma, harbored detectable levels of ctDNA. In contrast, less than 50% of patients with medulloblastomas or metastatic cancers of the kidney, prostate, or thyroid, and less than 10% of patients with gliomas, harbored detectable ctDNA. The number of patients with some of the tumor types depicted in Fig. 2A was small, limiting the statistical

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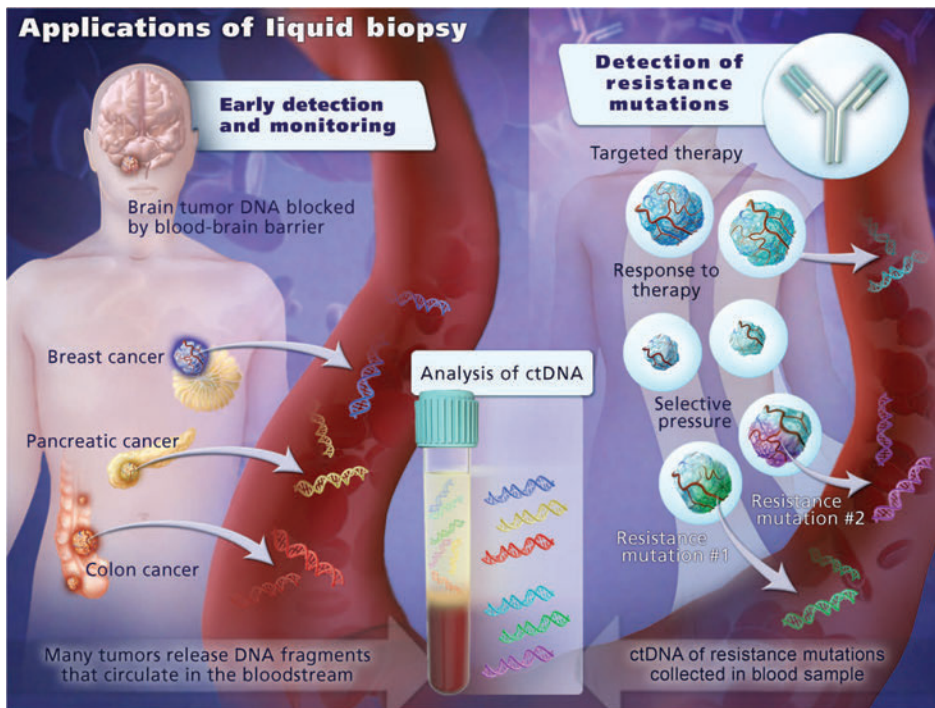


Fig. 1. Potential applications of ctDNA.

significance of comparisons among tumor types, but patients with gliomas (low or high grade; table S1) were less likely to harbor ctDNA than those with metastatic cancers of the pancreas, colon, breast, esophagus/stomach, or ovary (Fig. 2A and fig. S2).

Although ctDNA was detectable in most patients with metastatic cancers, the concentration of ctDNA varied among patients, even those with the same tumor type (Fig. 2B and table S1). Some of this variability was due to differences in copy number of the genes assayed in different tumors. For example, if the queried gene was amplified 50-fold in the tumor of patient A, whereas the queried gene in the tumor of patient B was present at normal copy number, the amount of ctDNA would be expected to be 50-fold higher in patient A than in patient B (see “Comparison of rearrangements with single-base substitutions in ctDNA”). However, great variability was also observed among cancers in which only nonamplified genes (such as *TP53*) were assessed.

Patients with localized disease

We next evaluated ctDNA in patients with localized disease, that is, no clinical or radiographic evidence of distant metastasis at the time of sample collection. Among 223 patients with localized cancers of all types evaluated, detectable levels of ctDNA were found in 55% (122 of 223 patients; table S1). This fraction was lower than observed in patients with metastatic disease from all tumor types in which a sufficient number of samples were available (breast, colon, pancreas, and gastroesophageal; Fig. 3A; Cochran-Mantel-Haenszel χ^2 test, $P < 0.001$). Detectable levels of ctDNA were present in 49 to 78% of patients with localized tumors and in 86 to 100% of patients with metastatic tumors of these four types (Fig. 3A).

Differences in the fraction of patients with detectable levels of ctDNA also correlated with stage: 47% of patients with stage I cancers

of any type had detectable ctDNA, whereas the fraction of patients with detectable ctDNA was 55, 69, and 82% for patients with stage II, III, and IV cancers, respectively (Fig. 3B; Somers' Dxy rank correlation = 0.337). The concentration of ctDNA in the plasma similarly increased with stage (Fig. 3C).

Comparison of ctDNA with CTCs

For these experiments, DNA was isolated from the cellular compartment of blood obtained after centrifugation; these pellets contained CTCs as well as white blood cells (WBCs), platelets, and other cellular fragments. In each case, whole-genome sequencing of tumor DNA was used to identify somatic rearrangements. PCR-based assays were then used to identify these rearrangements in blood pellets (CTCs) or in the blood supernatants (plasma) of the same patients. This experiment could be performed with tumor-specific rearrangements, but not with tumor-specific point mutations, for the reasons given in the Discussion. We did not identify any cases in which CTCs were detected but in which ctDNA was absent.

However, in many cases in which ctDNA was detected (13 of 16; 81%), no CTCs were detectable with the identical assay (Table 2). Moreover, in the three cases wherein both CTC and ctDNA levels were detectable, the average number of mutant fragments in the plasma was >50-fold higher than analogous levels in CTCs (Table 2).

Comparison of rearrangements with single-base substitutions in ctDNA

We were also interested in comparing the quantity of two different types of genetically altered DNA fragments in the circulation of the same patients. Although practical issues precluded us from identifying a rearrangement in all patients in this study (see Discussion), tumor-specific rearrangements as well as tumor-specific point mutations were identified in 19 patients (table S2). The rearrangements were identified by whole-genome sequencing of tumor DNA, and the point mutations identified by targeted sequencing. In each case, the alteration was shown to be somatic via evaluation of normal DNA from the same patients. In 18 of the 19 patients harboring a circulating point mutation, a circulating rearrangement was also detectable (table S2). The one exception was a patient (CRC 37) with a circulating point mutation in *TP53* in which the rearrangement identified in that patient's tumor could not be identified in her plasma (table S2). The absolute number of circulating DNA fragments with point mutations versus rearrangements was highly correlated (Fig. 4; correlation coefficient = 0.96). However, in four patients, the number of circulating fragments containing rearrangements was >10-fold that of the queried point mutation (table S2). The reason for this was that the rearrangements we chose for analysis often arose as a result of gene amplification in the tumor, whereas the point mutations were generally present only once per tumor genome.

Table 1. Summary of clinical characteristics of 410 patients with various malignancies.

	Parameter value
Age, years	
Mean (SD)	63.0 (13.6)
Median (range)	64 (23 95)
No. unknown (%)	67 (16.3)
Gender, <i>n</i> (%)	
Female	163 (39.8)
Male	181 (44.1)
No. unknown (%)	66 (16.1)
Tumor type, <i>n</i> (%)	
Bladder	10 (2.4)
Breast	33 (8.0)
Colorectal	64 (15.6)
Endometrial	12 (2.9)
Gastroesophageal	21 (5.1)
Glioma	27 (6.6)
Head and neck	12 (2.9)
Hepatocellular	4 (1.0)
Medulloblastoma	14 (3.4)
Melanoma	20 (4.9)
Neuroblastoma	9 (2.2)
Non small cell lung cancer	5 (1.2)
Ovary	9 (2.2)
Pancreas	155 (37.8)
Prostate	5 (1.2)
Renal cell carcinoma	5 (1.2)
Small cell lung cancer	1 (0.2)
Thyroid	4 (1.0)
Clinical stage*	
1	49 (13.3)
2	133 (36.0)
3	51 (13.8)
4	136 (36.9)

*Excludes 41 primary brain tumor patients.

The sensitivity and specificity of liquid biopsy

The results described above were obtained by first identifying a mutation in a tumor and then determining whether that same mutation was detectable in the plasma. For certain liquid biopsy applications, the mutation in the tumor is not known a priori and all mutations of interest are queried at once. To determine the sensitivity of the liquid biopsy approach, we evaluated the plasma and tumors of 206 patients with metastatic CRC in a blinded fashion (table S3). This cohort of patients was completely distinct from the 410 patients described above and in tables S1 and S2. For each case, we determined whether mutations at codon 12 or 13 of *KRAS* were present in either

the primary tumor or in 2 ml of plasma drawn before treatment. The *KRAS* gene was chosen for this study because of its clinical relevance; the absence of a *KRAS* gene mutation in the primary tumor is a prerequisite for treatment of metastatic CRC patients with antibodies that block EGFR (29). We identified 69 patients (33% of the 206) who harbored circulating mutant *KRAS* in their plasma. Circulating *KRAS* mutations were not detected in 127 of 128 patients with *KRAS* wild-type tumors, yielding an uncorrected specificity of 99.2%. The mutation identified in the 69 plasma samples was always identical to that identified in the tumors, further emphasizing the specificity of the liquid biopsy. In addition to these 69 tumors, we identified 10 cases (of 206) in which mutations were present in the primary tumors but not in the plasma, yielding a sensitivity of 87.2%. Percent concordance between *KRAS* mutation status in the plasma and tumor tissue was 95%, and the agreement was highly significant (κ statistic = 0.88, $P < 0.0001$).

We next evaluated 26 clinical and pathologic characteristics to better understand the observed false-negative results (tables S3 and S4). The factors associated with a false-negative ctDNA result (mutant *KRAS* in the tumor but no mutants detectable in the plasma) were low CEA level, mucinous histology, low alanine aminotransferase levels, low WBC count, and younger age (tables S4 and S5). CEA levels were also positively correlated with the concentration of mutant *KRAS* fragments in the plasma (tables S6 and S7). These observations are consistent with the idea that lower tumor burdens (reflected by normal CEA levels) are associated with lower ctDNA levels.

We next examined the relationship between the concentration of ctDNA and survival. Beginning with a model of known prognostic factors [age, Eastern Cooperative Oncology Group (ECOG) performance status (PS), and CEA], and assuming linearity for these adjustment variables, we found that ctDNA concentration provided added value in predicting survival (likelihood ratio test, $P = 0.00253$, $df = 3$). We then estimated the 2-year survival rate for differing concentration of ctDNA, holding the other predictors constant (Fig. 5). We observed a steady decrease in survival rate as ctDNA concentration increased.

Monitoring patients for resistance-conferring mutations

Liquid biopsies can also be used to monitor patients being treated with targeted agents, providing an early warning of recurrence and information about the genetic basis of resistance. For example, *KRAS* codon 12 and 13 mutations were shown to develop in 38% of 24 patients who first responded to EGFR blockade and then progressed (20). In each case, the *KRAS* gene mutation was not present in the primary tumor but had presumably arisen in a small population of cells within a metastatic lesion and expanded under the influence of the EGFR blockade. Here, we wished to determine whether other resistance mutations, besides those at *KRAS* codons 12 and 13, could be identified in liquid biopsies of patients treated with EGFR blockade. We therefore designed a multiplexed, sequencing-based assay to query known mutated hotspots of several genes in the EGFR pathway: the regions within and surrounding *KRAS* codons 12, 13, 59, 60, and 61; *NRAS* codons 12, 13, 59, 60, and 61; *BRAF* codons 599 and 600; *EGFR* codons 712 to 721, 738 to 748, 790 to 800, and 847 to 859; and *PIK3CA* codons 538 to 549 and 1039 to 1050. The 24 cases assessed included 17 of those previously assessed for *KRAS* mutations (20) plus 7 additional cases of patients who had first responded, then progressed, while being treated with blocking antibodies to EGFR (panitumumab or cetuximab). The primary tumors of nine of these cases were unavailable, so we used pretreatment DNA from plasma to assess whether any of the

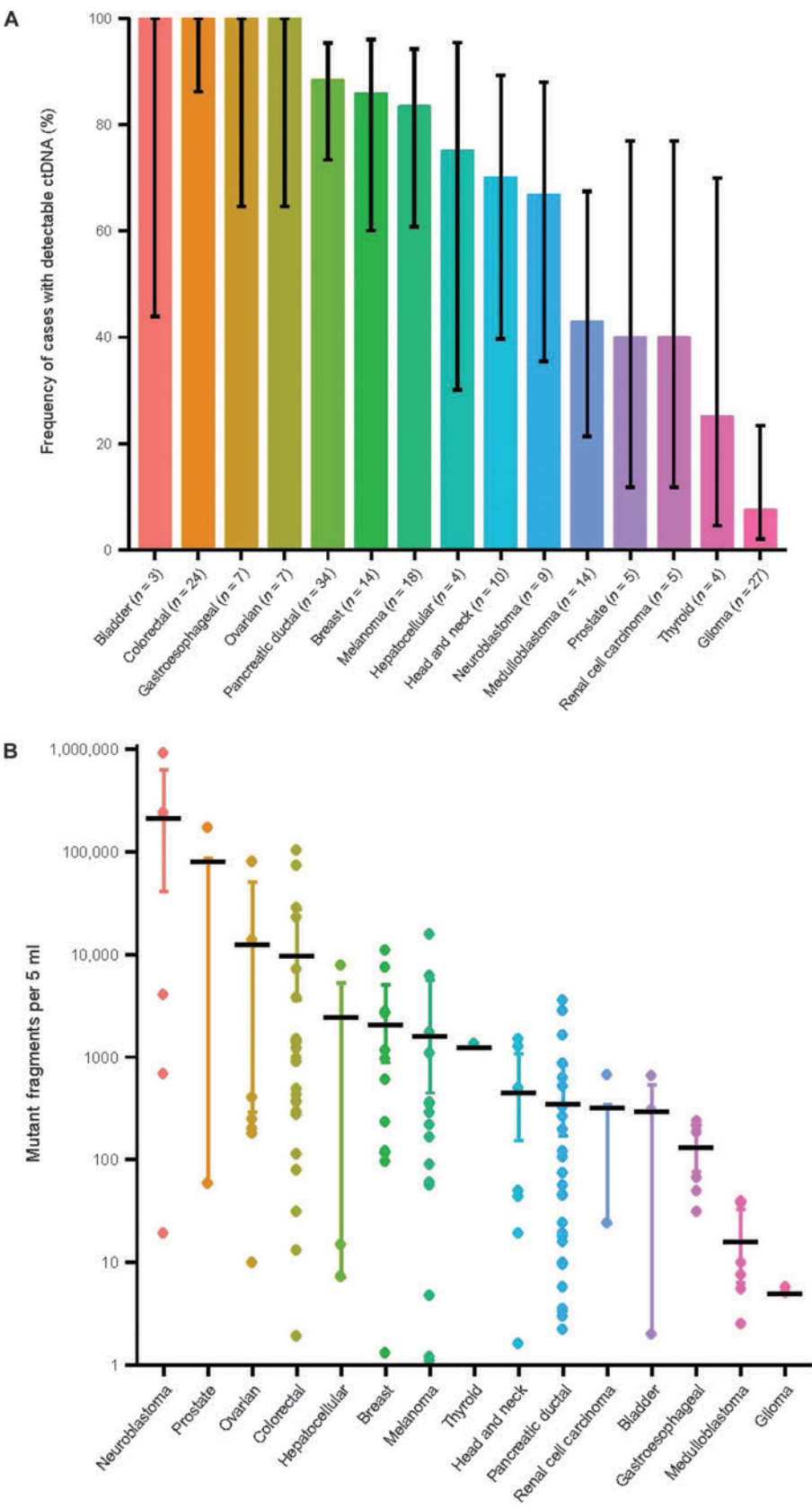
Fig. 2. ctDNA in advanced malignancies.
(A) Fraction of patients with detectable ctDNA.
(B) Quantification of mutant fragments. Error bars represent the 95% bootstrapped confidence interval of the mean (tumor types with <4 samples were excluded from this figure).

queried mutations were detected before administration of EGFR antibodies; none of the mutations listed in Fig. 6 were found before antibody treatment.

We identified emergent circulating mutations of at least one mitogen-activated protein kinase pathway gene in 23 of the 24 patients (96%). The number of different mutations identified in the circulation of individual patients averaged 2.9 (range, 0 to 12). The development of different mutations in the same patient is not surprising given that each of these patients had multiple lesions; each lesion that responds to EGFR blockade and then progresses is expected to harbor at least one resistance mutation (20, 30).

In total, we observed 70 somatic mutations that were not detected in the tumor or in the plasma before EGFR blockade and only appeared after therapy was initiated (table S8 and Fig. 6). Half of the mutations (34 of 70) occurred in *KRAS* codon 12. These mutations are known to cause resistance to EGFR blockade when present in the primary tumor, and have been observed to arise after EGFR blockade in vitro as well as in vivo (20, 30). One mutation in *BRAF* was observed. Several previous studies have shown that *BRAF* V600E mutations, when present in primary tumors, are associated with failure to achieve a response to EGFR blockade (31–33). Two other patients developed mutations in the kinase domain of EGFR (codons 714 and 794; table S8 and Fig. 6). Mutations at these residues have been previously observed in primary CRC, albeit infrequently, and resistance to EGFR blockade has been shown to result from genetic alterations in the *EGFR* gene (34, 35). We did not identify treatment-related mutations in the known PIK3CA gene hotspots (exons 9 and 20) (36).

The most surprising observation in the EGFR blockade component of our study was the large number of mutations in codon 61 of either the *KRAS* or *NRAS* gene (table S6 and Fig. 6). Fifteen of the



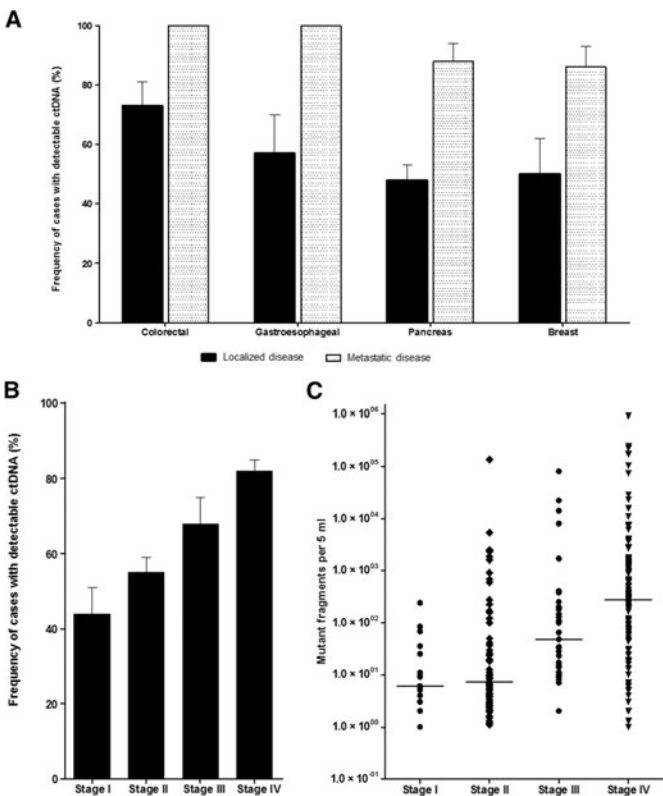


Fig. 3. ctDNA in localized and nonlocalized malignancies. (A) Fraction of patients with detectable ctDNA in localized (stages I to III) and metastatic (stage IV) colorectal, gastroesophageal, pancreatic, and breast cancers. (B) Fraction of patients with detectable ctDNA. (C) Quantification of mutant fragments in cancer cases categorized by stage. Error bars represent SEM.

24 patients (62.5%) harbored at least one codon 61 mutation, and the 31 mutations in these 15 patients comprised 45% of the total (69) mutations observed. Forty-eight percent of the codon 61 mutations were in *NRAS* and the remainder were in *KRAS* (table S6 and Fig. 6).

DISCUSSION

Through the study of 640 patients, we have learned that mutant DNA fragments are found at relatively high concentrations in the circulation of most patients with metastatic cancer and at lower but detectable concentrations in a substantial fraction of patients with localized cancers. These results have several translational implications and suggest important avenues of future research.

Monitoring disease in advanced cancer patients

A genetic alteration could be identified in the tumor of all 410 patients evaluated in this part of study, making ctDNA a widely applicable biomarker for cancer patients. Moreover, >80% of patients with metastatic disease had detectable levels of ctDNA, higher than that reported for most conventional biomarkers (37). Unlike proteins such as CEA or CA19-9, which are expressed in normal cells as well as in neoplastic cells, genetic alterations of a clonal nature are only found in neoplasms.

Table 2. Comparison of CTCs with ctDNA.

Sample ID	Tumor type	Clinical stage	Cellular DNA (mutant fragments per 5 ml)	Plasma DNA (mutant fragments per 5 ml)
BLD 21	Bladder cancer	2	0	226
BLD 24	Bladder cancer	2	0	4
CRC 12	Colorectal cancer	4	0	79
CRC 14	Colorectal cancer	4	0	31
CRC 31	Colorectal cancer	1	0	35
CRC 32	Colorectal cancer	2	0	37
CRC 35	Colorectal cancer	2	0	5
CRC 40	Colorectal cancer	1	0	25
CRC 60	Colorectal cancer	4	680	73,000
CRC BIO 23a*	Colorectal cancer	4	370	21,000
CRC BIO 23b*	Colorectal cancer	4	400	28,000
BR 833	Breast cancer	2	0	2,500
BR 834	Breast cancer	2	0	41
BR 837	Breast cancer	2	0	3
BR 841	Breast cancer	2	0	690
BR 848	Breast cancer	2	0	9,900

*Two independent blood samples from the same patient, drawn 2 months apart, were separately analyzed.

Our data indicate that measurements of ctDNA can also provide therapeutic, predictive, and prognostic information in patients with metastatic disease. As shown in Fig. 5, metastatic CRC patients with relatively low levels of ctDNA lived significantly longer than patients with higher levels, and there was a marked correlation between ctDNA concentration and survival. A similar association between survival and ctDNA concentration has recently been reported in patients with advanced breast cancers (16).

Although these advantages of ctDNA render it promising for monitoring patients, there are potential limitations. The specific mutations are defined by evaluation of the primary tumor, adding both time and expense to patient management. This may be less of an obstacle in the future because more cancer patients will have their tumors genetically analyzed to guide therapeutic decisions. The genetic alterations used to

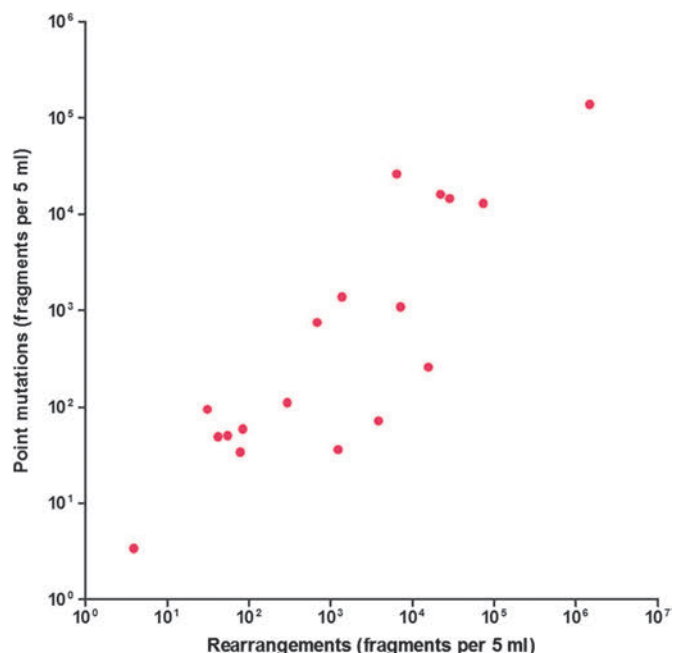


Fig. 4. Scatter plot correlating point mutations with rearrangements in the same plasma specimens.

guide therapies can also be used for ctDNA analysis. A more serious issue relates to the utility of monitoring patients with advanced cancers, either with ctDNA or with other biomarkers (38, 39). On one hand, patients and their physicians are anxious to know, as soon as possible, whether disease has progressed. Imaging studies are often noninformative or slow to reflect progression. Repeated imaging also subjects patients to radiation, whereas monitoring ctDNA is noninvasive. On the other hand, it has not yet been shown that monitoring patients with advanced disease with any biomarker provides clinical as opposed to psychological benefits. Knowing that progression (or response) has occurred prior to changes in clinical symptoms may not prolong survival or improve quality of life.

Methodological comparisons

There are two sources of tumor DNA accessible in the blood (CTCs and ctDNA), and two types of genetic alterations that can be most easily assessed in either source (point mutations and translocations). Previous studies that compared ctDNA with CTCs reached mixed conclusions. For example, one group concluded that ctDNA was present less often than CTCs (17); this group used state-of-the-art methods to detect CTCs but did not use a highly sensitive method to detect ctDNA. The second group concluded that ctDNA was present more often than CTCs (16); this group used a sensitive method for analyzing ctDNA but used a relatively insensitive method for analyzing CTCs. More recently, much higher levels of ctDNA than CTCs were found in two of three pediatric patients with neuroblastomas (19).

To investigate this issue further, we assessed both ctDNA and CTCs in the same blood sample from patients with typical solid tumors. We simply separated the cellular component from plasma and determined the fraction of cells or cell equivalents, respectively, in which tumor-specific rearrangements could be identified. Because

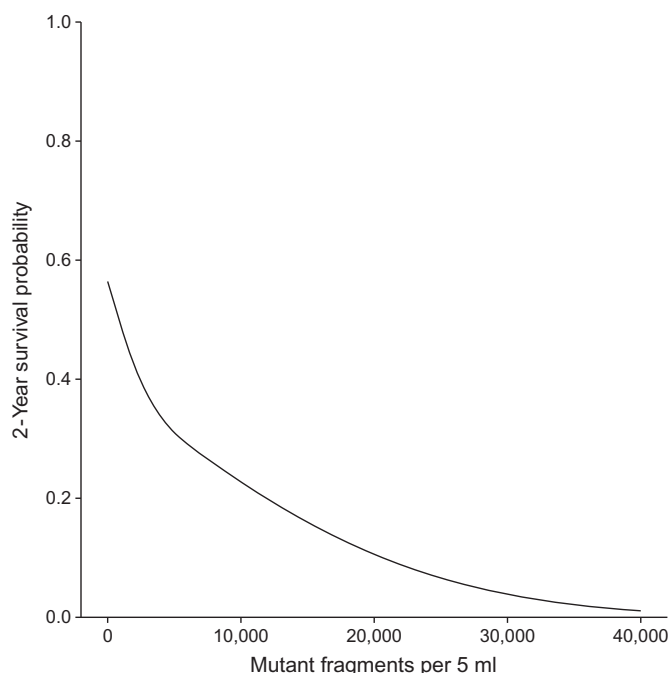


Fig. 5. The relationship between ctDNA concentration (mutant fragments per milliliter) and 2-year survival. The association between survival and ctDNA concentration was assessed, holding known prognostic factors (age, ECOG PS, and CEA) constant. The 2-year survival was estimated on the basis of a multivariable Cox regression model, in which ctDNA concentration level was transformed with a natural spline function.

we did not attempt to physically separate tumor cells from normal WBCs, technical issues related to the efficiency of CTC purification were eliminated. The comparison between DNA from CTCs and ctDNA cannot easily be performed with point mutations because the background level of point mutations in PCR-based assays is too high, even with the sensitive methods used in our study. This background precludes the detection of point mutations at levels less than 1 in 100,000 cells (40, 41). Because several million normal cells but only a few CTCs are present per milliliter of blood, a technology that is more sensitive is required. The detection of rearrangements is well suited for this task because it has been shown that one mutation can be reliably detected among millions of wild-type template molecules; PCR errors do not generate specific rearrangements (42).

Using patient-specific rearrangements as a tool, we were able to show that the level of ctDNA was always higher than that of CTCs. In 13 of 16 patients, ctDNA levels were relatively high, whereas no CTCs at all could be detected. This does not mean that ctDNA is preferable to CTCs for the detection or monitoring of cancer. Rather, the optimal technology depends on many other factors, including cost and throughput, for which CTC detection has advantages. However, this comparison does suggest that the vast majority of ctDNA is not derived directly from CTCs. Because the half-life of ctDNA is short (<1.5 hours) (21), in fact shorter than that of CTCs (43), our work suggests that the mutant molecules in the plasma are generally not derived from the CTCs.

Another comparison of interest concerns translocations and point mutations. Our results (table S2) show that the number of ctDNA

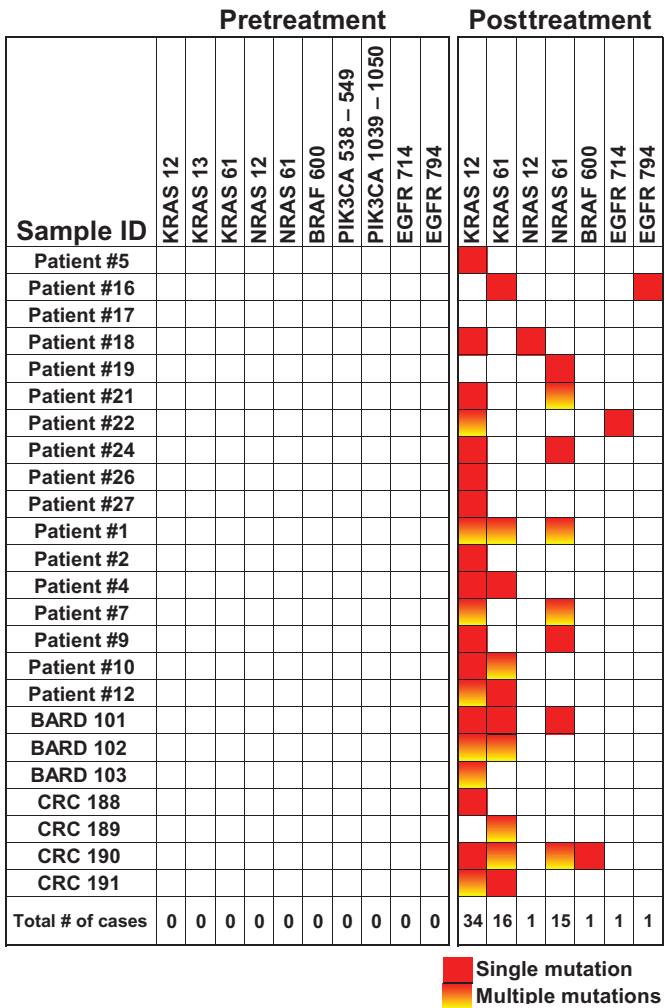


Fig. 6. Heat map of acquired resistance mutations to EGFR blockade in ctDNA from patients with metastatic CRC.

fragments per milliliter of plasma for translocations and point mutations was similar in most of the cases studied. However, in 1 of 19 cases, a point mutation was detected in a plasma sample in which the studied rearrangement was absent. The likely reason for this was that the point mutation was in a driver gene that occurred relatively early in tumorigenesis, whereas the rearrangement was subclonal, perhaps not contributing to the development of the tumor. In four other cases, rearrangements were detected at 10-fold higher levels than the point mutations (table S2). In these cases, the rearrangements were found to be components of somatically amplified genes.

From a practical perspective, these data suggest the following conclusions: Maximal sensitivity for detecting a genetic alteration can be achieved by using a rearrangement present within an amplicon. Many tumors, particularly advanced ones, contain such amplifications, making them relatively easy to detect with low-coverage (10×) genome sequencing. As with the comparison between CTCs and ctDNA, however, this greater sensitivity does not mean that rearrangements are preferred over point mutations for clinical use. The discovery of a rearrangement in a patient's tumor, and the work and time required to

develop and test primer pairs that can efficiently detect the rearrangement(s) in the degraded DNA characteristic of plasma, is considerable. In contrast, a panel of assays detecting the most commonly mutated point mutations is currently simpler and less expensive to implement in the clinical setting.

Early detection of localized cancers

Until therapeutic agents with much greater potency and minimal side effects are developed, the current best hope for reducing cancer morbidity and mortality is early detection of neoplastic disease (9). Prior to metastasis, most solid tumors can be cured by extant surgical methods, and even when occult metastasis has occurred, adjuvant therapy or additional surgery can lead to cure in some patients. One of the encouraging results of our study is that ctDNA was found in most of the patients with localized disease, when their chances of a favorable outcome are highest (Fig. 3). Even in patients with stage I disease, who are nearly always curable by surgery alone, 47% of patients were shown to have detectable levels of ctDNA in their plasma. In stage III disease, which is curable in many patients with certain forms of cancer, more than two-thirds of patients had detectable ctDNA.

Although early detection strategies based on ctDNA are promising, numerous obstacles must be overcome before they can be applied clinically. The fraction of patients with detectable ctDNA represents the maximum obtainable with the amount of plasma collected in this study (table S1). In a screening setting, with the exception of pancreatic ductal adenocarcinomas [where one gene, KRAS, is mutated in almost all cases (44)], the mutation of interest would not be known a priori and a panel of genes would have to be assessed. Our study on the EGFR blockade cohort shows that it is indeed possible to assess several genes at once for the detection of relatively rare mutations in plasma (table S6).

In addition to these technical challenges, biomedical issues will have to be addressed by any ctDNA-based screening test. False-positive findings can be problematic for any screening assay (45). Experience thus far suggests that benign tumors and nonneoplastic conditions do not generally give rise to ctDNA (46), so the “overdiagnosis” of benign tumors is not likely to pose a major problem. However, other studies suggest that a tumor containing ~50 million malignant (rather than benign) cells releases sufficient DNA for detection in the circulation (20). A cancer of this size is far below that required for definitive imaging at present. How would a patient who had a positive ctDNA test be managed if follow-up imaging tests were negative? A related issue is the fact that the type of mutation does not provide many clues to the tumor type. For example, a patient with a circulating TP53 mutation, in the absence of other mutations, could have a cancer in any of several organs. Another question concerns the value of detecting early cancers. In pancreatic ductal adenocarcinomas, for example, it might be argued that most patients with a positive ctDNA test will die from their disease anyway, given the aggressive nature of this form of cancer. Although these obstacles are formidable, we would argue that the presence of detectable amount of a mutant driver gene is a cause for serious concern given the known causal relationships between such mutations and cancer. Indeed, this point distinguishes mutation-based biomarkers from all other types of biomarkers yet described.

Liquid biopsies

Our studies demonstrate two uses for liquid biopsies. The first—assessing plasma for the presence of specific mutations that can direct patient

management—is clinically actionable. We show here that the sensitivity of the liquid biopsy for testing *KRAS* codon 12 is 88.2% in patients with metastatic CRC. Although conventional tumor biopsies are preferable, these often cannot be obtained for logistic or medical reasons. When tumor tissue specimens from metastatic cancer patients are unavailable, liquid biopsies offer an alternative that can be rapidly implemented without the pain, risk, and expense entailed by a biopsy of one of the metastatic lesions. Of note is the fact that ctDNA from neoplasms confined to the central nervous system (Fig. 2A) and those with mucinous features (table S4) was infrequently detectable. This suggests that physical obstacles such as the blood-brain barrier and mucin could prevent ctDNA from entering the circulation.

Tracking resistance

A second use of liquid biopsies is for identifying resistance mutations that occur when patients first respond to therapy and then progress. The detection of ctDNA requires tumor cells to die, and even tumor cells that are resistant to therapy turn over rapidly; they die almost as frequently as they are born (20). Thus, it is expected, and in fact observed, that the DNA fragments from drug-resistant cancer cells are found in the plasma. Although this approach is mainly of interest for research purposes at present, the obtained information can be clinically informative. A good example of this principle is provided by our discovery of remarkably frequent mutations at codon 61 of *NRAS* and of *KRAS*, representing 46% of the detected mutations in patients resistant to EGFR blockade. Codon 61 mutations of *KRAS* and *NRAS* have previously been observed to occur in primary CRCs, but very infrequently compared to the prevalence at which we found them in patients progressing after EGFR blockade (33). *KRAS* codon 61 mutations have been observed to be associated with primary resistance to EGFR blockade when they occur in primary CRCs (32, 33, 47). There are no previous studies indicating that *NRAS* codon 61 mutations are associated with acquired resistance, but the results in Fig. 6 leave little doubt as to their role. This finding provides unequivocal evidence that these mutations confer resistance to therapy—the probability that recurrent mutations at these positions occurred by chance alone is essentially nil (20). It also supports studies showing that *KRAS*, *BRAF*, *NRAS*, and *EGFR* mutations compromise the efficacy of EGFR blockade in patients with CRC (47, 48).

Collectively, codon 600 mutations of *BRAF*, codon 61 mutations of *KRAS*, and codon 12 or 61 mutations of *NRAS* occur about half as often as mutations in *KRAS* codons 12 or 13 in primary CRCs (49). These data therefore strongly suggest that patients being considered for treatment with EGFR blocking agents should be tested for these additional mutations. This conclusion was independently supported by a clinical study reported during the review of our manuscript (50). Patients harboring mutations at these positions are unlikely to benefit from these agents and would be better served by other therapeutic approaches.

SUMMARY

In summary, we demonstrate that ctDNA can be used as a feasible biomarker for a variety of different solid tumor types and clinical indications. The clinical utility of this biomarker, and the risks and benefits accruing from knowledge of ctDNA levels, can only be addressed through longitudinal studies of ctDNA in appropriate populations of

patients, as is currently under way for CTCs (51). The studies reported here lay the groundwork for such future studies.

SUPPLEMENTARY MATERIALS

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Materials and Methods

Fig. S1. Comparison of methods for analysis of point mutations in plasma DNA.

Fig. S2. ctDNA in advanced malignancies, ranking of the fraction of patients with detectable ctDNA.

Fig. S3. Diagram of the assay used to confirm rearrangements in plasma DNA.

Table S1. Summary of tissue mutations, plasma mutations, and clinical information for 410 early- and advanced-stage subjects.

Table S2. Comparison of ctDNA levels assessing point mutations and rearrangements in the same specimens.

Table S3. Comparison between plasma and tumor tissue *KRAS* status in 206 patients with metastatic CRC.

Table S4. Clinical characteristics of patients with discordant tissue and plasma *KRAS* mutation data.

Table S5. Clinical characteristics of patients with false negatives in plasma *KRAS* mutation compared with tissue samples.

Table S6. Association between clinical characteristics and absolute levels of ctDNA.

Table S7. Association between clinical characteristics and ctDNA concentration (log scale) in metastatic CRC patients.

Table S8. Patient characteristics and plasma mutations detected after EGFR blockade.

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Supplementary Materials for Detection of Circulating Tumor DNA in Early- and Late-Stage Human Malignancies

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This PDF file includes:

Materials and Methods

Fig. S1. Comparison of methods for analysis of point mutations in plasma DNA.

Fig. S2. ctDNA in advanced malignancies, ranking of the fraction of patients with detectable ctDNA.

Fig. S3. Diagram of the assay used to confirm rearrangements in plasma DNA.

References (52–58)

Other Supplementary Material for this manuscript includes the following:

(available at www.sciencetranslationalmedicine.org/cgi/content/full/6/224/224ra24/DC1)

Table S1 (Microsoft Excel format). Summary of tissue mutations, plasma mutations, and clinical information for 410 early- and advanced-stage subjects.

Table S2 (Microsoft Excel format). Comparison of ctDNA levels assessing point mutations and rearrangements in the same specimens.

Table S3 (Microsoft Excel format). Comparison between plasma and tumor tissue *KRAS* status in 206 patients with metastatic CRC.

Table S4 (Microsoft Excel format). Clinical characteristics of patients with discordant tissue and plasma *KRAS* mutation data.

Table S5 (Microsoft Excel format). Clinical characteristics of patients with false negatives in plasma *KRAS* mutation compared with tissue samples.

Table S6 (Microsoft Excel format). Association between clinical characteristics and absolute levels of ctDNA.

Table S7 (Microsoft Excel format). Association between clinical characteristics and ctDNA concentration (log scale) in metastatic CRC patients.

Table S8 (Microsoft Excel format). Patient characteristics and plasma mutations detected after EGFR blockade.

Filename: generate.table.r

Filename: Table_S1_analysis.r

Filename: Table_S3_analysis.r

Materials and Methods

Samples

All samples were collected after Institutional Review Board (IRB) approval at participating institutions, under full compliance with HIPAA guidelines. Tumors and adjacent normal tissues were either frozen at a minimum of -80 C or formalin-fixed and paraffin- embedded (FFPE) according to standard histopathologic procedures. Tumors were macro-dissected under a dissecting microscope to ensure a neoplastic cellularity of >60%. DNA was purified from the macrodissected frozen tumors using AllPrep (Qiagen, cat #80204) and from macrodissected paraffin-embedded tumors with a Qiagen FFPE Kit (Qiagen cat #56494). Translocations, but not point mutations, were previously reported for three of the CRCs (42, 52). Translocation data, but not all clinical correlatives, were previously reported for eight of the nine neuroblastomas recorded in table S1 (53); these cases were included in the current study for comparative purposes only. For white blood cell DNA extraction, cells were pelleted at 1000 g prior to the preparation of plasma. DNA from these cells was purified using AllPrep (Qiagen, cat #80204). Plasma was used for ctDNA measurements in all experiments except in 17 of the 24 cases described in table S6, in which serum was used. DNA from plasma or serum was purified using QIAamp Circulating Nucleic Acid kit (Qiagen cat# 55114). Total plasma DNA concentration was measured using quantitative PCR as described (54). The amounts of plasma available from all patients except those used for the liquid biopsy studies are listed in table S1; for the liquid biopsy study in table S3, 2 ml plasma was available and for the liquid biopsy study in table S6, 1 ml serum or 2 ml plasma was used. One plasma draw was used for each patient except those described in table S6, in which two plasma draws were obtained: one prior to initiating EGFR blockade and one sample when the tumors had recurred after a clinical response.

Tumor Mutational Profiling

A tiered approach was used to identify somatic mutations in tumors. For pancreatic cancers, genomic regions encompassing *KRAS* codons 12,13, 59, 60 and 61 were amplified and the sequence of the PCR products determined via ligation of mutant-specific probes (53) or via SafeSeqS (40), as it is well known that nearly all pancreatic ductal adenocarcinomas harbor mutations in the *KRAS* gene (44), (55). For colorectal cancers, PCR was used to amplify the *KRAS*, *BRAF*, *TP53*, *SMAD4*, *PIK3CA*, and *APC* genes and the sequence of the PCR products was determined, generally using SafeSeqS, as described below. For all other cancers, paired-end libraries were generated and regions encompassing 100 genes commonly mutated in cancers were captured as described previously (53). For tumors that did not contain

detectable mutations of these genes, exomic sequencing was performed after capture of the same libraries via SureSelect (Agilent), as previously described (56, 57). In cases in which rearrangements were analyzed using PARE (personalized analysis of rearranged ends), (42, 52), genomic libraries were constructed for whole genome sequencing with a physical coverage of ~10x. Whenever possible, we selected rearrangements within amplified segments of the genome. Such rearrangements would be represented more often in tumor DNA than in DNA from normal cells, theoretically increasing the sensitivity of detection of the altered fragment in plasma. Once putative rearrangements were identified on the basis of sequencing data, PCR primers were designed to amplify PCR products of 100 bp that spanned the rearrangement. The rearrangements were confirmed to be somatic by demonstrating that PCR products were generated from the DNA of the tumor but not from DNA of non-neoplastic cells of the same patient.

Mutation Detection in ctDNA or CTCs

In the early phases of this study, single base substitutions and small insertions or deletions (indels) were assessed either by BEAMing or (21, 53) or by a PCR/ligation method (21, 53). For the latter method, 25% of the plasma DNA was aliquotted into wells of a 384-well plate so that an average of 1 ng was contained in each well. After PCR and ligation as described (21, 53), all wells were individually evaluated via gel electrophoresis and fluorescence imaging. If all wells contained a mutation, the plasma DNA was re-diluted for more precise quantification. If no wells contained a mutation, then a further 65% of the plasma was aliquotted and the assay repeated; ~10% of the plasma was used to determine DNA concentration and to confirm that the plasma and tumor were derived from the same patient via single nucleotide polymorphism analysis. In the latter stages of this study, mutations were assessed by SafeSeqS, an approach in which template molecules are individually assessed via massively parallel sequencing (40). For SafeSeqS, 25% of the plasma DNA was aliquotted into wells of a 96-well plate so that an average of 3 ng DNA was contained in each well. The DNA from each well was then amplified using well-specific index primers, and the DNA from all wells was pooled and subjected to massively parallel sequencing and analysis as described (40). If no mutations were detected, a further 65% of the plasma was aliquotted and the assay repeated; ~10% of the plasma was used to determine DNA concentration and to confirm that the plasma and tumor were derived from the same patient via single nucleotide polymorphism analysis. In each experiment, equivalent amounts of DNA from non-neoplastic cells were included in adjacent wells performed to ensure that the identified mutations were not the result of errors generated during PCR or other steps of the procedures (40). SafeSeqS was used to assess all of the 206 metastatic colorectal cancer patients assayed for mutations in the liquid biopsy

study (table S3) as well as to assess the 24 patients assayed for resistance mutations after EGFR blockade (table S6).

To assess differences in assay performance among the methods used to assess ctDNA, we quantified the amount of mutations in 20 plasma samples that had been evaluated by all three methods used for detecting point mutations (BEAMing (58), PCR-Ligation (53), or Safe-SeqS (40)). We found that the results were comparable, as evident from the data in fig. S1. All three methods could detect one mutant template in the DNA from 5 ml plasma, as determined by spiking known amounts of mutant KRAS DNA in plasma DNA from normal individuals.

Rearrangements in ctDNA or CTCs were detected and quantified by digital PCR, using PARE (Paired Analysis of Rearranged Ends) as described previously (40, 42) with the following modifications. First, 25% of the plasma DNA was aliquotted into wells of a 384-well plate so that an average of 3 ng (plasma) or 300 ng (WBCs containing CTCs) were contained in each well. After amplification, a portion of each well was evaluated by polyacrylamide gel electrophoresis to determine whether a PCR product of the predicted size was present. If all wells contained a mutation, the DNA was re-diluted for more precise quantification. If no wells contained a mutation, then a further 65% of the plasma was aliquotted and the assay repeated. To further verify that the PCR fragments of the expected size contained the intended rearrangement, ligation reactions were performed on each PCR fragment as described (21, 53). The two oligonucleotides used in the ligation reaction spanned the breakpoint so that ligation only occurred if the PCR products assessed contained the rearrangement (fig. S3). Control experiments with DNA from non-neoplastic cells of the same patients showed that each rearrangement reported in this study was not found in the germ line of that patient.

Statistical Analyses

Proportions of patients with detectable ctDNA, with 95% Wilson confidence intervals, the rank of the proportion and ctDNA concentration by cancer type, with 95% bootstrapped confidence intervals, are listed. Proportions of patients with detectable ctDNA were compared across cancer types with a likelihood ratio chi-square test from a logistic model of detectable ctDNA, across stage of disease with Somers' Dxy rank correlation, and across both cancer type and stage of disease for breast, colon, pancreas, and gastroesophageal cancers using a Cochran-Mantel-Haenszel chi-squared test.

For the liquid biopsy cohort of metastatic colorectal cancer patients, the sensitivity and specificity, along with 95% confidence intervals, for detecting a plasma KRAS mutation compared to the detection of a

tissue KRAS mutation were calculated. We also report the percent concordance and kappa statistic for the agreement between liquid biopsy and tissue samples.

Clinical characteristics of the false negative and true negative groups were compared with Pearson's chi-square test for categorical variables and Wilcoxon Mann Whitney tests for continuous variables. Those variables which had <20% missingness of the dependent variable were included in a multivariable logistic regression model of true negative status using lasso penalties.

In cases with detectable levels of mutant KRAS fragments in the plasma, the association of clinical characteristics with the log ctDNA levels was evaluated using univariable linear regression models. Logarithm transformations were made for the dependent variable and some continuous predictor variables to correct for skewness. Those variables which had <20% missingness of the dependent variable were included in a multivariable linear regression using lasso penalties.

Overall survival was calculated from the time of ctDNA measurement to the date of death or last follow-up. The known prognostic factors (age, ECOG PS and CEA), linearity assumed, were included in a multivariable Cox proportional hazards model with ctDNA concentration level transformed with a natural spline function. The 2-year survival probability estimates were plotted against ctDNA concentration levels, fixing the other covariates at the mean (continuous variables) or mode (categorical variables). The other prognostic factors, MSI and BRAF status, had more than 20% missing values and were not adjusted in the multivariable model. The variables were selected based on their clinical relevance, and none were removed by statistical significance testing.

Statistical analyses were performed using the R statistical package (version 2.15.1). A complete self-contained software script that can be used to reproduce all data manipulation, transformation, and analysis steps reported in this paper is available as part of the supplementary materials.

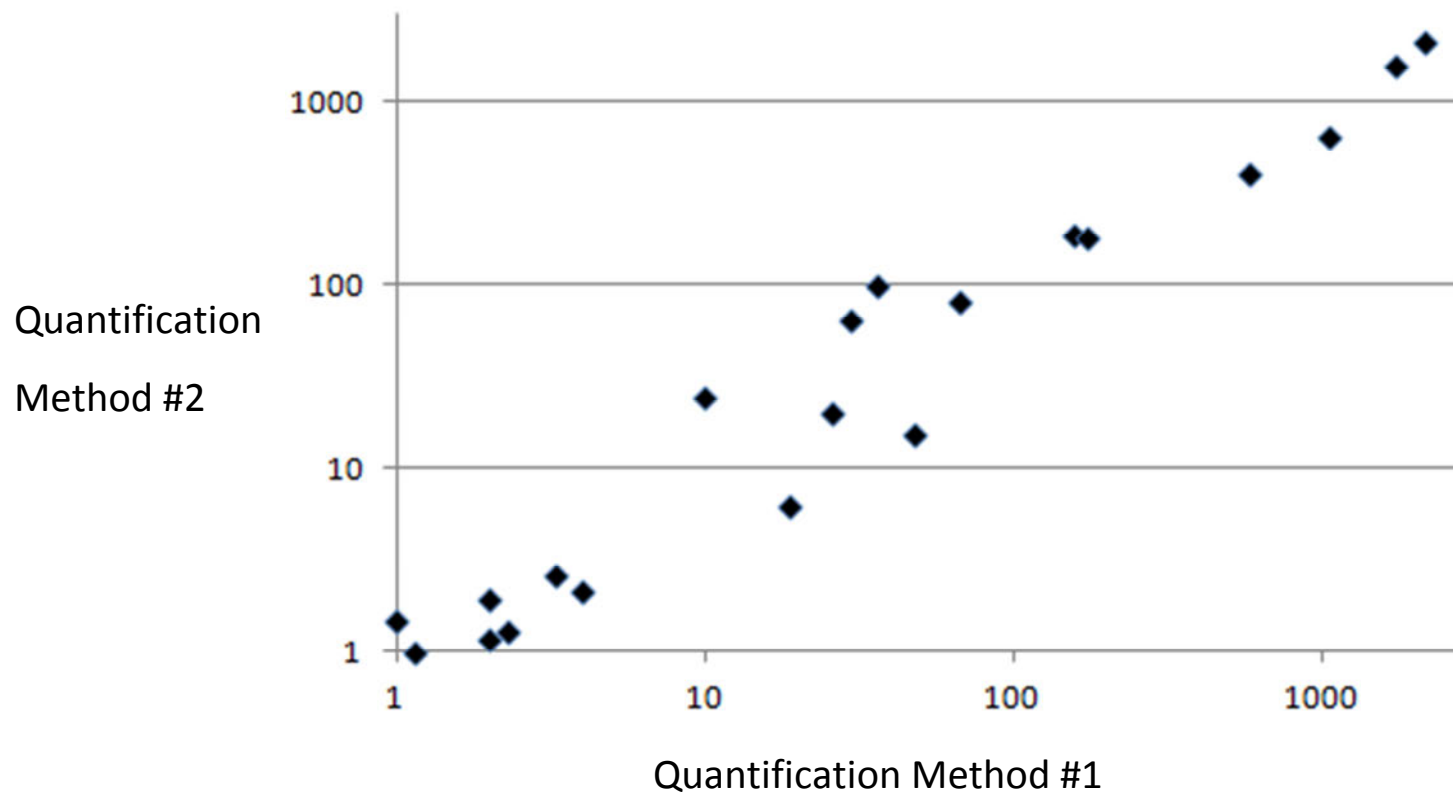


Fig. S1. *Comparison of methods for analysis of point mutations in plasma DNA.* Duplicate aliquots of plasma or serum from 20 different patients were collected. A mutation present in the corresponding tumors was quantified in the first aliquot by Quantification Method #1 (PCR-ligation) and in the second aliquot by Quantification Method #2 (BEAMing in 11 patients and SafeSeqS in 9 patients). Mutant templates per 5 ml plasma or serum are plotted on both the x- and y-axes. In addition to the 20 samples displayed in this graph, each of which yielded at least one mutation with both quantification methods, we tested 10 other duplicate samples containing low amounts of mutant DNA. In five of these cases, neither of the aliquots tested by the two methods revealed any mutations. In five other cases, one method revealed a single mutant template molecule while the other method revealed zero. These results are consistent with expectations based on a Poisson distribution of mutant templates in the circulation.

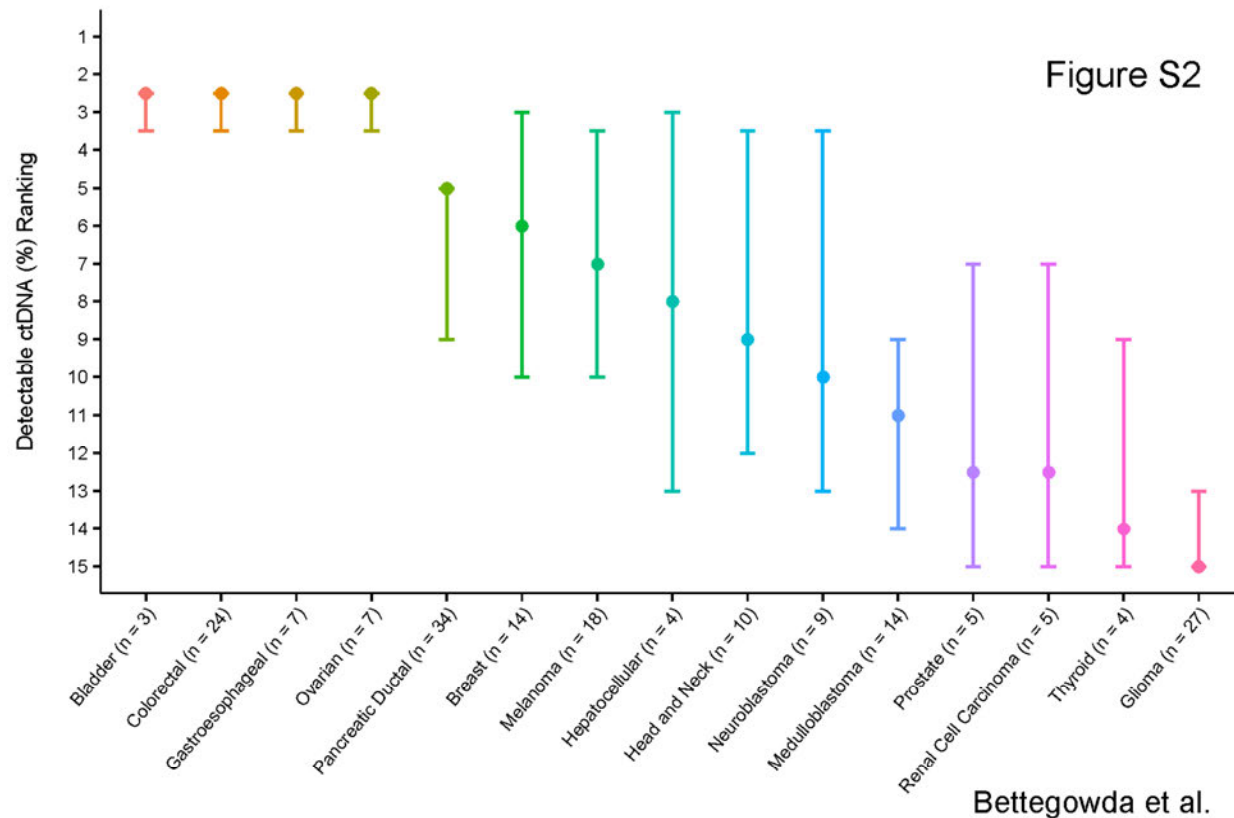


Fig. S2. *ctDNA in advanced malignancies, ranking of the fraction of patients with detectable ctDNA.* For each tumor type, the rank of the proportion of detectable ctDNA is reported, where a rank of 1 means having the highest proportion and a rank of 15 means having the lowest proportion among the 15 tumor types. Ties were handled by taking the average ranking. The error bars represent the 95% bootstrap confidence interval of the rank of each tumor type.

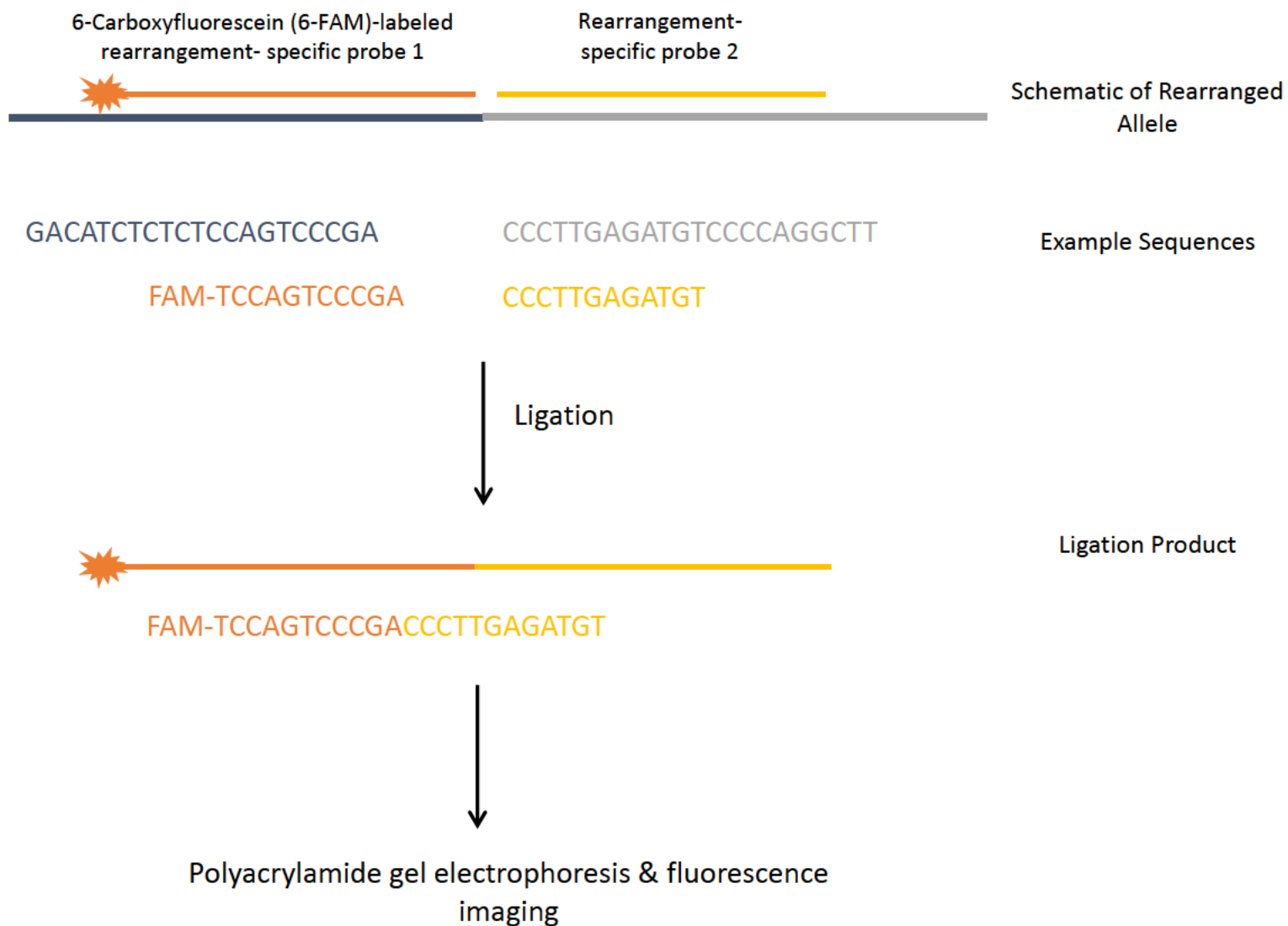


Fig. S3. *Diagram of the assay used to confirm rearrangements in plasma DNA.*

Table S1. Summary of tissue mutations, plasma mutations and clinical information for 410 early and advanced stage subjects

Sample ID #	Tumor type (adenocarcinoma unless otherwise indicated)	Plasma volume (µl)	Mutation - nucleotide alteration	Mutation - amino acid alteration	Mutant fragments/5 mL plasma	Method used for mutation detection in plasma	Clinical Stage	Age	Sex	Evaluated as part of the metastatic cohort (fig. 2A and fig. 2B)
BLD 41	Bladder	5000	TP53 c.841G>A	TP53 p.D281N	655	SafeSeqS	4	62	M	X
BLD 46	Bladder	2500	TP53 c.853G>A	TP53 p.E285K	2.0	SafeSeqS	4	82	M	X
BLD 47	Bladder	2000	TP53 c.991C>T	TP53 p.Q331X	308	SafeSeqS	4	77	M	X
BREAST10-1	Breast	3000	TP53 c.332T>A	TP53 p.L1111Q	1,170	PCR-Ligation	4	56	F	X
BREAST2-1	Breast	3000	FBXL4 c.1187T>A	FBXL4 p.I396N	0.0	SafeSeqS	4	82	F	X
BREAST3-1	Breast	3000	TP53 c.536A>G	TP53 p.H179R	7,500	PCR-Ligation	4	76	F	X
BREAST4-1	Breast	3000	PIK3CA c.3140A>G	PIK3CA p.H1047R	0.0	PCR-Ligation	4	71	F	X
BREAST5-1	Breast	3000	EIF4B c.1756-1G>C	Not applicable	122	SafeSeqS	4	80	F	X
CP10	Breast	4000	TP53 c.637C>T	TP53 p.R213X	2,660	SafeSeqS	4			X
CRC 13	Colorectal	1000	TP53 c.743G>A	TP53 p.R248Q	13	SafeSeqS	4	87	F	X
CRC 59	Colorectal	5000	TP53 c.35G>A	KRAS p.G12D	277	SafeSeqS	4	48	F	X
CRC 61	Colorectal	5000	TP53 c.818G>A	TP53 p.R273H	427	SafeSeqS	4	35	F	X
CRC 62	Colorectal	5000	TP53 c.455C>T	TP53 p.P152L	361	SafeSeqS	4	63	M	X
CRC 63	Colorectal	5000	TP53 c.844C>T	TP53 p.R282W	1,490	SafeSeqS	4	69	M	X
CRC 64	Colorectal	4000	KRAS c.38G>A	KRAS p.G13D	113	SafeSeqS	4	37	F	X
CRC 65	Colorectal	5000	KRAS c.38G>A	KRAS p.G13D	973	SafeSeqS	4	58	M	X
CRC 66	Colorectal	5000	KRAS c.35G>C	KRAS p.G12A	1,220	SafeSeqS	4	64	F	X
CRC 67	Colorectal	5000	TP53 c.396G>C	TP53 p.K132N	1.9	SafeSeqS	4	43	F	X
CRC Bio 162	Colorectal	1000	APC c.3927_3931delAAAGA	APC p.E1309_I1311fs	909	BEAMing	4	48	M	X
CRC Bio 168	Colorectal	1000	KRAS c.35G>A	KRAS p.G12D	374	BEAMing	4	38	M	X
CRC Bio 180	Colorectal	1000	BRAF c.1799T>A	BRAF p.V600E	22,900	SafeSeqS	4	60	M	X
CRC Bio 203	Colorectal	1000	KRAS c.35G>A	KRAS p.G12D	485	SafeSeqS	4	86	F	X
CRC Bio 204	Colorectal	3000	APC c.4348C>T	APC p.R1450X	1,440	SafeSeqS	4	36	M	X
PAP 35	Endometrial	5000	KRAS c.35G>A	KRAS p.G12D	30	SafeSeqS	4	49	F	X
CBGP1-1	Gastroesophageal	3000	CDH1 c.1569T>A	CDH1 p.Y523X	185	PCR-Ligation	4	71	F	X
CBGP3-1	Gastroesophageal	3000	TP53 c.817C>T	TP53 p.R273C	67	PCR-Ligation	4	57	M	X
CBGP5-1	Gastroesophageal	3000	CDH1 c.563T>A	CDH1 p.V188D	220	PCR-Ligation	4	67	F	X
CBGP9-1	Gastroesophageal	3000	TP53 c.559+1C>T	Not applicable	50	PCR-Ligation	4	95	M	X
ESOPL1-1	Gastroesophageal	5000	TP53 c.818G>A	TP53 p.R273H	31	PCR-Ligation	4	57	M	X
ESOPL2-1	Gastroesophageal	5000	TP53 c.1010G>A	TP53 p.R337H	215	PCR-Ligation	4	66	M	X
G804	Gastroesophageal	2000	RELN c.2005T>C	RELN p.Y669H	239	SafeSeqS	4	39	F	X
CB GLIOMA 22	Glioma	2000	IDH1 c.395G>A	IDH1 p.R132H	0.0	PCR-Ligation	II oligodendrc	23	M	X
CB GLIOMA 29	Glioma	5000	IDH1 c.395G>A	IDH1 p.R132H	0.0	PCR-Ligation	II oligodendrc	35	M	X
CB GLIOMA 30	Glioma	5000	IDH1 c.395G>A	IDH1 p.R132H	0.0	PCR-Ligation	de II astrocyt	42	M	X
CB GLIOMA 31	Glioma	5000	IDH1 c.395G>A	IDH1 p.R132H	0.0	PCR-Ligation	de II astrocyt	24	M	X
CB GLIOMA10	Glioma	2000	IDH1 c.395G>A	IDH1 p.R132H	0.0	PCR-Ligation	II oligodendrc	43	F	X
CB GLIOMA4	Glioma	2000	IDH1 c.395G>A	IDH1 p.R132H	0.0	PCR-Ligation	II oligodendrc	31	M	X
CB GLIOMA5	Glioma	3000	TP53 c.856G>A	TP53 p.E286K	0.0	SafeSeqS	Glioblastoma	44	M	X
CBBRP11	Glioma	5000	IDH1 c.395G>A	IDH1 p.R132H	0.0	PCR-Ligation	Glioblastoma	58	M	X
CBBRP12	Glioma	5000	TP53 c.481G>A	TP53 p.A161T	0.0	PCR-Ligation	de II astrocyt	53	F	X
CBBRP2	Glioma	2400	TP53 c.419C>T	TP53 p.T140I	0.0	PCR-Ligation	Glioblastoma	64	M	X
CBBRP23	Glioma	4500	IDH1 c.395G>A	IDH1 p.R132H	0.0	PCR-Ligation	II oligodendrc	35	F	X
CBBRP24	Glioma	4800	IDH1 c.394C>A	IDH1 p.R132S	0.0	PCR-Ligation	grade astrocy	32	F	X
CBBRP25	Glioma	4800	IDH1 c.395G>A	IDH1 p.R132H	0.0	PCR-Ligation	II oligodendrc	24	F	X
CBBRP27	Glioma	4800	IDH1 c.394C>A	IDH1 p.R132S	0.0	PCR-Ligation	de II astrocyt	34	F	X
CBBRP28	Glioma	3840	IDH1 c.394C>A	IDH1 p.R132S	0.0	PCR-Ligation	de II astrocyt	36	M	X
CBBRP32	Glioma	1920	IDH1 c.395G>A	IDH1 p.R132H	0.0	PCR-Ligation	Gliosarcoma			X
CBBRP9	Glioma	5000	TP53 c.452C>A	TP53 p.P151H	0.0	PCR-Ligation	de III astrocyt	55	M	X

GLI 101	Glioma	5000	TP53 c.731G>A	TP53 p.G244D	0.0	SafeSeqS	Glioblastoma	59	M	X	
GLIOMA 102	Glioma	5000	EGFR c.2156G>A	EGFR p.G719D	0.0	SafeSeqS	Glioblastoma	62	M	X	
GLIOMA 105	Glioma	5000	PIK3CA c.263G>A	PIK3CA p.R88Q	0.0	SafeSeqS	Glioblastoma	46	M	X	
GLIOMA 106	Glioma	5000	TP53 c.569_570CT>TC	TP53 p.P190L	0.0	SafeSeqS	Glioblastoma	62	M	X	
GLIOMA 109	Glioma	5000	PTEN c.799A>T	PTEN p.K267X	0.0	SafeSeqS	Glioblastoma	78	F	X	
GLIOMA 110	Glioma	3500	TP53 c.569C>T	TP53 p.P190L	0.0	SafeSeqS	Glioblastoma	84	F	X	
CB HN 9	Head and Neck Squamous Cel	5000	PIK3CA c.1624G>C	PIK3CA p.E542Q	1.6	SafeSeqS		4	79	F	X
CBHNP1-1	Head and Neck Squamous Cel	5000	PIK3CA c.112C>T	PIK3CA p.R38C	44	PCR-Ligation		4	38	M	X
CBHNP2-1	Head and Neck Squamous Cel	5000	TP53 c.1010G>T	TP53 p.R337L	1,500	PCR-Ligation		4	59	F	X
CBHNP3-1	Head and Neck Squamous Cel	3200	BRAF c.1801A>G	BRAF p.K601E	0.0	PCR-Ligation		4	78	M	X
CBHNP4-1	Head and Neck Squamous Cel	4000	TP53 c.536A>G	TP53 p.H179R	50	PCR-Ligation		4	58	M	X
CBHNP6-1	Head and Neck Squamous Cel	5000	TP53 c.579_580TC>AT	TP53 p.H193_L194QF	500	PCR-Ligation		4	52	F	X
CBHNP7-1	Head and Neck Squamous Cel	5000	AKAP9 c.3395G>T	AKAP9 p.R1132L	1,280	SafeSeqS		4	68	M	X
HN 14	Head and Neck Squamous Cel	1500	TP53 c.817C>T	TP53 p.R273C	19	PCR-Ligation		4	44	F	X
HN16	Head and Neck Squamous Cel	1500	TP53 c.378C>G	TP53 p.Y126X	0.0	PCR-Ligation		4	68	M	X
HN41	Head and Neck Squamous Cel	1500	PIK3CA c.3140A>T	PIK3CA p.H1047L	0.0	PCR-Ligation		4	42	M	X
HCC 103	Hepatocellular	5000	TP53 c.536A>G	TP53 p.H179R	7.2	SafeSeqS		4	55	F	X
HCC 105	Hepatocellular	2000	PALB2 c.1620C>G	PALB2 p.N540K	15	SafeSeqS		3	87	F	X
HCC 106	Hepatocellular	2000	EGFR c.2014C>G	EGFR p.H672D	7,910	SafeSeqS		3	67	F	X
HCC 107	Hepatocellular	2000	SLC17A9 c.799G>C	SLC17A9 p.E267Q	0.0	SafeSeqS		3	62	M	X
CBMB10-1	Medulloblastoma	1000	CTNNB1 c.98C>G	CTNNB1 p.S33C	38	PCR-Ligation	edulloblastoma				X
CBMB1-1	Medulloblastoma	2000	CTNNB1 c.98C>A	CTNNB1 p.S33Y	2.5	PCR-Ligation	edulloblastoma				X
CBMB11-1	Medulloblastoma	1000	PTCH1 c.2778G>C	PTCH1 p.W926C	0.0	PCR-Ligation	edulloblastoma				X
CBMB12-1	Medulloblastoma	1000	PTCH1 c.707G>A	PTCH1 p.W236X	0.0	PCR-Ligation	edulloblastoma				X
CBMB13-1	Medulloblastoma	2000	CTNNB1 c.94G>T	CTNNB1 p.D32Y	0.0	PCR-Ligation	edulloblastoma				X
CBMB2-1	Medulloblastoma	1000	PTCH1 c.3154_3155insCGGC	PTCH1 p.T1052fs	5.5	PCR-Ligation	edulloblastoma				X
CBMB24-1	Medulloblastoma	5000	KDM6A c.4153C>T	KDM6A p.Q1385X	39	SafeSeqS	edulloblastoma				X
CBMB3-1	Medulloblastoma	1000	MLL2 c.1652C>T	MLL2 p.P551L	10.0	PCR-Ligation	edulloblastoma				X
CBMB4-1	Medulloblastoma	2000	CTNNB1 c.98C>T	CTNNB1 p.S33F	0.0	PCR-Ligation	edulloblastoma				X
CBMB5-1	Medulloblastoma	1000	PTCH1 c.981T>A	PTCH1 p.C327X	0.0	PCR-Ligation	edulloblastoma				X
CBMB6-1	Medulloblastoma	1000	PTEN c.633delCinsGCG	PTEN p.C211fs	0.0	PCR-Ligation	edulloblastoma				X
CBMB7-1	Medulloblastoma	1000	CTNNB1 c.110C>G	CTNNB1 p.S37C	7.5	PCR-Ligation	edulloblastoma				X
CBMB8-1	Medulloblastoma	1000	TP53 c.376-2T>C	Not applicable	0.0	PCR-Ligation	edulloblastoma				X
CBMB9-1	Medulloblastoma	1000	PTCH1 c.3119_3120insT	PTCH1 p.F1040fs	0.0	PCR-Ligation	edulloblastoma				X
CB MEL 09	Melanoma	2000	NRAS c.182A>G	NRAS p.Q61R	0.0	SafeSeqS		4	57	F	X
CB MEL 10	Melanoma	2000	BRAF c.1799T>A	BRAF p.V600E	165	SafeSeqS		4	52	F	X
CB MEL 11	Melanoma	5000	ALK c.4732C>T	ALK p.P1578S	1.1	SafeSeqS		4	40	F	X
CB MEL2	Melanoma	5000	BRAF c.1799T>A	BRAF p.V600E	1.2	PCR-Ligation		4	32	F	X
MEL 23	Melanoma	4000	BRAF c.1799T>A	BRAF p.V600E	1,750	SafeSeqS		4	52	F	X
MEL 24	Melanoma	4000	BRAF c.1799T>A	BRAF p.V600E	6,140	SafeSeqS		4	61	M	X
MEL 30	Melanoma	5000	TERT promoter, chr5 g.1295250G>A	Not applicable	288	SafeSeqS		4			X
MELP1-1	Melanoma	5000	BRAF c.1799T>A	BRAF p.V600E	345	PCR-Ligation		4	48	F	X
MELP4-1	Melanoma	2000	BRAF c.1799T>A	BRAF p.V600E	0.0	PCR-Ligation		4	35	M	X
MELP5-1	Melanoma	5000	BRAF c.1799T>A	BRAF p.V600E	220	SafeSeqS		4	68	M	X
MELP6-1	Melanoma	5000	BRAF c.1799T>A	BRAF p.V600E	57	PCR-Ligation		4	43	F	X
MELP7-1	Melanoma	5000	BRAF c.1799T>A	BRAF p.V600E	4.7	PCR-Ligation		4	54	M	X
MELP8-1	Melanoma	5000	BRAF c.1405G>C	BRAF p.G469R	0.0	SafeSeqS		4			X
1110	Ovarian	2000	TP53 c.524G>A	TP53 p.R175H	14,000	SafeSeqS		3	47	F	X
CB01-1	Ovarian	2000	TP53 c.614A>G	TP53 p.Y205C	180	PCR-Ligation		3	67	F	X
CB05-1	Ovarian	2000	TP53 c.725G>T	TP53 p.C242F	80,000	PCR-Ligation		3	53	F	X
CB06-1	Ovarian	2000	TP53 c.487_488insGAT	TP53 p.Y163X	405	PCR-Ligation		3	54	F	X
CB09-1	Ovarian	2000	TP53 c.818G>A	TP53 p.R273H	200	PCR-Ligation		3	44	F	X
CB10-1	Ovarian	2000	TP53 c.843C>A	TP53 p.D281E	250	PCR-Ligation		3	74	F	X
CB11-1	Ovarian	2000	TP53 c.742C>T	TP53 p.R248W	10.0	PCR-Ligation		3	64	F	X
CBPANC 0	Pancreatic Ductal	1000	KRAS c.35G>A	KRAS p.G12D	625	BEAMing		4	53	M	X

CBPP12-1	Pancreatic Ductal	4500	KRAS c.35G>A	KRAS p.G12D	10.0	PCR-Ligation	4	63	F	X
CBPP15-1	Pancreatic Ductal	4500	KRAS c.35G>T	KRAS p.G12V	2.2	PCR-Ligation	4	62	M	X
CBPP31-1	Pancreatic Ductal	4800	KRAS c.35G>T	KRAS p.G12V	16	PCR-Ligation	4	78	M	X
CBPP32-1	Pancreatic Ductal	4800	KRAS c.34G>C	KRAS p.G12R	265	PCR-Ligation	4	68	M	X
CBPP50-1	Pancreatic Ductal	5000	KRAS c.35G>A	KRAS p.G12D	10.0	PCR-Ligation	4	66	M	X
CBPP55-1	Pancreatic Ductal	5000	KRAS c.35G>A	KRAS p.G12D	19	PCR-Ligation	4	73	F	X
CBPP57	Pancreatic Ductal	5000	KRAS c.34G>C	KRAS p.G12R	3.3	PCR-Ligation	4	46	M	X
CBPP60	Pancreatic Ductal	5000	KRAS c.35G>T	KRAS p.G12V	16	PCR-Ligation	4	66	F	X
CBPP64	Pancreatic Ductal	5000	KRAS c.35G>A	KRAS p.G12D	19	PCR-Ligation	4	73	F	X
CBPP67	Pancreatic Ductal	2368	KRAS c.35G>T	KRAS p.G12V	46	PCR-Ligation	4	75	F	X
CBPP7-1	Pancreatic Ductal	4050	KRAS c.35G>T	KRAS p.G12V	3.0	PCR-Ligation	4	77	M	X
CBPP73	Pancreatic Ductal	5000	KRAS c.34G>T	KRAS p.G12C	57	PCR-Ligation	4	66	F	X
PANC 10	Pancreatic Ductal	5000	KRAS c.34G>C	KRAS p.G12R	24	PCR-Ligation	4	74	M	X
PANC 13	Pancreatic Ductal	3000	KRAS c.35G>A	KRAS p.G12D	9.5	SafeSeqS	4	66	M	X
PANC 14	Pancreatic Ductal	5000	KRAS c.35G>A	KRAS p.G12D	0.0	SafeSeqS	4	59	M	X
PANC 147	Pancreatic Ductal	3000	KRAS c.35G>T	KRAS p.G12V	10	SafeSeqS	4	81	M	X
PANC 155	Pancreatic Ductal	5000	KRAS c.34G>C	KRAS p.G12R	517	SafeSeqS	4	53	M	X
PANC 156	Pancreatic Ductal	5000	KRAS c.35G>A	KRAS p.G12D	2,820	SafeSeqS	4	63	F	X
PANC 157	Pancreatic Ductal	5000	KRAS c.35G>A	KRAS p.G12D	18	SafeSeqS	4	79	F	X
PANC 158	Pancreatic Ductal	5000	KRAS c.35G>A	KRAS p.G12D	197	SafeSeqS	4	79	M	X
PANC 161	Pancreatic Ductal	5000	KRAS c.34G>C	KRAS p.G12R	0.0	SafeSeqS	4	65	M	X
PANC 162	Pancreatic Ductal	5000	KRAS c.35G>T	KRAS p.G12V	3,560	SafeSeqS	4	75	M	X
PANC 163	Pancreatic Ductal	5000	KRAS c.35G>T	KRAS p.G12V	1,630	SafeSeqS	4	59	F	X
PANC 164	Pancreatic Ductal	5000	KRAS c.35G>A	KRAS p.G12D	326	SafeSeqS	4	55	M	X
PANC 17	Pancreatic Ductal	5000	KRAS c.35G>A	KRAS p.G12D	74	PCR-Ligation	4	66	M	X
PANC 85	Pancreatic Ductal	5000	KRAS c.35G>A	KRAS p.G12D	0.0	SafeSeqS	4	60	F	X
PANC 9	Pancreatic Ductal	3000	KRAS c.35G>T	KRAS p.G12V	875	PCR-Ligation	4	80	M	X
PANC 93	Pancreatic Ductal	5000	KRAS c.35G>A	KRAS p.G12D	3.5	SafeSeqS	4	56	M	X
PANC104	Pancreatic Ductal	3000	KRAS c.35G>T	KRAS p.G12V	45	SafeSeqS	4	75	M	X
PANC124	Pancreatic Ductal	3000	KRAS c.34G>C	KRAS p.G12R	0.0	SafeSeqS	4	59	M	X
PANC132	Pancreatic Ductal	4000	KRAS c.35G>A	KRAS p.G12D	107	SafeSeqS	4	73	F	X
PANC150	Pancreatic Ductal	5000	KRAS c.35G>T	KRAS p.G12V	122	SafeSeqS	4	77	M	X
PANC152	Pancreatic Ductal	5000	KRAS c.35G>T	KRAS p.G12V	5.7	SafeSeqS	4	83	M	X
CBPP11-1	Prostate	4500	KRAS c.35G>A	KRAS p.G12D	0.0	PCR-Ligation	4	62	M	X
CBPRP1	Prostate	4800	BRCA2 c.9281C>G	BRCA2 p.S3094X	173,000	PCR-Ligation	4	63	M	X
CBPRP2	Prostate	4800	TP53 c.743G>T	TP53 p.R248L	59	PCR-Ligation	4	63	M	X
CBPRP3	Prostate	4800	SPOP c.304T>G	SPOP p.F102V	0.0	SafeSeqS	4	57	M	X
CBPRP5-1	Prostate	5000	RPP30 c.905G>A	RPP30 p.R302K	0.0	SafeSeqS	4	68	M	X
RCC 10	Renal Cell Carcinoma	5000	VHL c.263G>T	VHL p.W88L	0.0	SafeSeqS	4	70	M	X
RCC 11	Renal Cell Carcinoma	5000	VHL c.263G>T	VHL p.W88L	0.0	SafeSeqS	4	73	M	X
RCC 7	Renal Cell Carcinoma	5000	HOOK2 c.1165G>T	HOOK2 p.E389X	0.0	SafeSeqS	4	77	M	X
RCC PL1	Renal Cell Carcinoma	5000	VHL c.388G>C	VHL p.V130L	24	PCR-Ligation	4	49	M	X
RCC PL2	Renal Cell Carcinoma	5000	MET c.3687_3688GT>TA	MET p.M1229_Y1230IN	665	PCR-Ligation	4	86	M	X
CB LUNG 24	Small Cell Lung Cancer	5000	TP53 c.832C>G	TP53 p.P278A	320	SafeSeqS	4			X
CB THY 4	Thyroid	5000	NRAS c.182A>G	NRAS p.Q61R	0.0	SafeSeqS	4	79	M	X
CB THY 5	Thyroid	5000	NF2 c.20C>G	NF2 p.S7C	0.0	SafeSeqS	4	54	M	X
CB THY 7	Thyroid	5000	TP53 c.743G>A	TP53 p.R248Q	1,350	SafeSeqS	4	56	M	X
THY 1	Thyroid	5000	BRAF c.1799T>A	BRAF p.V600E	0.0	SafeSeqS	4	64	F	X

Table S2. Comparison of ctDNA levels assessing point mutations and rearrangements in the same specimens

ID#	Tumor Type	Clinical Stage	Rearrangement	Rearrangement (mutant fragments per 5 mL)	Point Mutation (nucleotide)	Point Mutation (codon)	Point mutation (mutant fragments per 5 mL)	Rearrangement fragments/Point mutation fragments (ratio)
BLD 24	Bladder	2	Inter-chromosomal; chr2:204396710-chr1:154286405	3.9	BRAF c.1801A>G	BRAF p.K601E	3.4	1.1
CRC 02	Colorectal	1	Inter-chromosomal; chr12:73097777-chr1:63099083	22,000	TP53 c.730G>A	TP53 p.G244S	16,115	1.4
CRC 03	Colorectal	1	Intra-chromosomal; chr11:98914172-chr11:98993460	6,415	APC c. 4012C>T	APC p.Q1338X	26,155	0.2
CRC 06	Colorectal	1	Intra-chromosomal; chr13:27679603-chr13:25685009	85	KRAS c.182_183AA>CC	KRAS p.Q61P	59	1.4
CRC 07	Colorectal	1	Intra-chromosomal; chr4:85109657-chr4:85408635	42	KRAS c.35G>A	KRAS p.G12D	49	0.9
CRC 12	Colorectal	4	Intra-chromosomal; chr6:58080933-chr6:67229823	79	KRAS c.35G>A	KRAS p.G12D	34	2.3
CRC 14	Colorectal	4	Intra-chromosomal; chr8:141375810-chr8:141405769	31	TP53 c.818G>A	TP53 p.R273H	95	0.3
CRC 21	Colorectal	2	Intra-chromosomal; chr1:237741691-chr1:244145093	685	KRAS c.35G>C	KRAS p.G12A	745	0.9
CRC 23	Colorectal	4	Intra-chromosomal; chr16:6343641-chr16:6727736	28,500	TP53 c.817C>T	TP53 p.R273C	14,500	2.0
CRC 27	Colorectal	2	Intra-chromosomal; chr13:102839339-chr13:105470344	55	KRAS c.35G>A	KRAS p.G12D	51	1.1
CRC 30	Colorectal	3	Intra-chromosomal; chr20:2658058-chr20:2770031	1,466,665	KRAS c.35G>A	KRAS p.G12D	138,445	10.6
CRC 37	Colorectal	1	Intra-chromosomal; Duplication - chr3:170782510-chr3:170870975	0.0	TP53 c.844C>T	TP53 p.R282W	38	0.0
CRC 51	Colorectal	4	Inter-chromosomal; chr8:30060122-chr1:190065347	3,850	APC c.4012C>T	APC p.Q1338X	72	53.5
CRC 53	Colorectal	4	Intra-chromosomal; chr16:52977177-chr16:52965488	7,150	TP53 c.452C>A	TP53 p.P151H	1,085	6.6
CRC 54	Colorectal	4	Intra-chromosomal; chr11:7250750-chr11:19457296	1,240	KRAS c.35G>T	KRAS p.G12V	36	34.4
CRC 55	Colorectal	4	Intra-chromosomal; chr8:38281631-chr8:39225849	295	TP53 c.637C>T	TP53 p.R213X	112	2.6
CRC 58	Colorectal	4	Intra-chromosomal; chr20:14839816-chr20:14863644	1,370	KRAS c.35G>A	KRAS p.G12D	1,380	1.0
CRC 60	Colorectal	4	Intra-chromosomal; chr17:35277642-chr17:35163714	73,335	TP53 c.817C>T	TP53 p.R273C	12,905	5.7
MEL 27	Melanoma	4	Intra-chromosomal; chr2:137699753-chr2:137700957	15,600	BRAF c.1799T>A	BRAF p.V600E	260	60.0

Table S3. Comparison between plasma and tumor tissueKRAS status in 206 patients with metastatic colorectal cancer

Sample ID	Plasma Mutation	mutant fragments per 5 ml	Tissue Mutation	On therapy during blood draw?	Age	Gender	Race	ECOG PS	Met Site - Liver	Met Site - Lung	Met Site - Peritoneum	Met Site - Pelvis	Met Site - Bone	Met Site - Brain	# of Met Sites	Histological subtype	Mucinous	Smoking Status	Family History	MSI Status	BRAF Status	Vital Status (1=deceased)	OS from cDNA measurement (days)	OS from diagnosis (days)	Response to 1st line therapy	Prior Surgery	Prior chemotherapy	Prior Radiation	CEA (+/- weeks)	WBC	ALT	Bilirubin	Albumin	Creatinine		
BARD 001	None Detected	Not applicable	WT	Yes	54	M	W	0	1	0	0	0	0	0	1	Poorly	No	No	No	Unknown	WT	0	933	1112.00	Partial	No	Yes	Yes	39	3800	8	0.7	3.7	0.9		
BARD 002	None Detected	Not applicable	WT	Yes	58	M	W	0	1	0	0	0	0	0	1	Moderately	No	No	No	Unknown	WT	0	672	706.00	Complete	Yes	Yes	No	1	6500	41	0.7	4.1	0.9		
BARD 003	KRAS p.G13D	9,339	KRAS p.G13D	Yes	48	M	W	0	1	1	0	0	0	0	2	Poorly	No	No	No	Unknown	WT	0	118	308.00	Progression	Yes	Yes	No	3	6500	35	0.7	4.7	0.9		
BARD 004	None Detected	Not applicable	WT	Yes	67	F	W	0	1	1	0	0	0	0	2	Moderately	No	No	No	Unknown	Mutation found	0	318	498.00	Partial	Yes	Yes	No	2	4900	14	0.5	3.9	0.7		
BARD 005	None Detected	Not applicable	WT	Yes	59	M	W	0	0	0	1	1	0	0	2	Moderately	No	No	No	Unknown	WT	0	298	1600.00	Stable Disease	Yes	Yes	Yes	6	7400	26	0.8	4.4	1.0		
BARD 006	None Detected	Not applicable	WT	Yes	79	M	W	0	1	1	1	0	0	0	3	Moderately	No	No	No	Unknown	WT	0	199	2723.00	Partial	Yes	Yes	No	2	8100	15	Unknown	Unknown	4.2	1.1	
BARD 007	None Detected	Not applicable	WT	Yes	69	M	W	0	1	0	1	0	0	0	2	Poorly	Yes	No	No	Unknown	Mutation found	1	291	956.00	Progression	Yes	Yes	No	Unknown	4300	17	Unknown	Unknown	4.0	1.0	
BARD 008	None Detected	Not applicable	WT	Yes	72	M	W	0	1	0	0	0	0	0	1	Moderately	No	No	No	Unknown	WT	1	95	108.00	Unknown	No	Yes	No	25	1300	17	Unknown	3.6	2.8		
BARD 009	None Detected	Not applicable	WT	Yes	76	M	W	0	1	1	0	0	0	0	2	Moderately	No	Yes	No	Unknown	WT	0	195	418.00	Progression	Yes	Yes	Yes	No	28	4400	20	0.9	4.1	0.5	
BARD 010	None Detected	Not applicable	WT	No	61	M	W	0	1	0	0	0	0	0	1	Moderately	Yes	No	No	Unknown	WT	0	180	215.00	Stable Disease	No	Yes	No	107	5900	15	Unknown	Unknown	4.3	0.9	
BARD 011	None Detected	Not applicable	WT	No	69	M	W	0	1	0	0	0	0	0	1	Moderately	No	No	No	Unknown	WT	0	733	783.00	Partial	No	No	No	5	5300	22	Unknown	Unknown	3.7	0.7	
BARD 012	KRAS p.G12D	93	KRAS p.G12D	Yes	83	F	W	0	0	0	1	0	0	0	1	Moderately	Yes	No	No	Unknown	WT	0	350	1498.00	Unknown	Yes	No	No	1	5600	16	1.0	3.7	0.7		
BARD 013	None Detected	Not applicable	WT	Yes	85	F	W	0	1	1	0	0	0	0	2	Moderately	No	No	No	Unknown	WT	1	290	2097.00	Progression	Yes	Yes	No	52	9600	13	0.6	4.4	0.9		
BARD 014	KRAS p.G13D	2,400	KRAS p.G13D	Yes	60	M	W	0	0	1	0	0	0	0	1	Moderately	No	No	No	Unknown	WT	1	208	1809.00	Partial	Yes	Yes	No	34	7200	10	0.7	4.1	1.0		
BARD 015	None Detected	Not applicable	KRAS p.G12D	Yes	60	M	W	0	0	1	0	0	0	0	1	Poorly	No	No	No	Unknown	WT	0	428	888.00	Partial	Yes	Yes	No	1	3900	16	0.6	4.7	1.0		
BARD 016	None Detected	Not applicable	WT	No	61	M	W	0	1	0	1	0	0	0	1	Moderately	No	No	No	Unknown	WT	0	56	864.00	Progression	Yes	Yes	No	335	2600	156	1.8	3.4	0.8		
BARD 017	KRAS p.G12D	1,050	KRAS p.G12D	Yes	82	M	W	0	0	0	1	0	0	0	1	Moderately	No	No	No	Unknown	WT	0	149	520.00	Progression	Yes	Yes	No	4	4800	9	Unknown	Unknown	4.1	0.8	
BARD 018	None Detected	Not applicable	WT	Yes	49	M	W	0	1	1	0	0	0	0	2	Moderately	No	No	No	Unknown	WT	0	523	1279.00	Stable Disease	Yes	Yes	No	5	8900	19	1.0	4.2	0.9		
BARD 019	None Detected	Not applicable	WT	Yes	71	M	W	0	1	1	0	0	0	0	2	Moderately	Yes	No	No	Unknown	WT	0	910	2211.00	Stable Disease	Yes	Yes	No	6	3800	15	1.7	4.3	1.1		
BARD 020	None Detected	Not applicable	WT	Yes	71	M	W	0	1	0	0	0	0	0	1	Moderately	No	No	No	Unknown	WT	1	717	798.00	Stable Disease	Yes	Yes	No	2	7600	16	Unknown	Unknown	4.6	0.7	
BARD 021	None Detected	Not applicable	WT	Yes	69	F	W	0	1	0	0	0	0	0	1	Moderately	Yes	No	No	Unknown	Mutation found	0	383	1171.00	Unknown	Yes	Yes	No	1	10800	16	0.6	4.2	0.5		
BARD 022	None Detected	Not applicable	WT	Yes	75	M	W	0	1	1	1	0	0	0	3	Unknown	Unknown	No	No	Unknown	WT	0	489	914.00	Stable Disease	No	Yes	No	5	10500	20	0.5	4.5	1.0		
BARD 023	None Detected	Not applicable	WT	Yes	71	M	W	0	1	1	1	0	0	0	3	Moderately	No	No	No	Unknown	WT	0	616	1992.00	Progression	Yes	Yes	No	145	7400	9	Unknown	Unknown	3.2	2.7	
BARD 024	KRAS p.G13D	2,330	KRAS p.G13D	Yes	80	M	W	0	0	1	1	1	1	0	4	Poorly	Yes	No	No	Unknown	WT	1	149	828.00	Progression	Yes	Yes	Yes	83	8200	Unknown	0.5	4.2	0.8		
BARD 026	None Detected	Not applicable	WT	No	61	M	W	0	1	0	0	0	0	0	1	Moderately	Unknown	No	No	Unknown	WT	0	73	158.00	Unknown	Yes	No	No	2	7300	11	Unknown	Unknown	4.2	0.8	
BARD 027	None Detected	Not applicable	WT	No	25	F	W	0	0	0	1	0	0	0	1	Moderately	No	No	No	Unknown	WT	1	330	831.00	Stable Disease	Yes	Yes	No	25	4200	45	Unknown	Unknown	4.9	0.7	
BARD 028	KRAS p.G12S	17,300	KRAS p.G12S	Yes	61	F	W	0	1	1	0	0	0	0	3	Moderately	Unknown	No	No	Unknown	WT	1	293	329.00	Progression	Yes	Yes	No	1	1084	3700	27	Unknown	Unknown	4.1	0.5
BARD 029	KRAS p.G12D	13,700	KRAS p.G12D	Yes	51	F	W	0	1	1	1	0	0	0	4	Moderately	No	No	No	Unknown	WT	0	159	145.00	Partial	Yes	No	No	529	10900	7	Unknown	Unknown	3.8	0.9	
BARD 030	None Detected	Not applicable	KRAS p.G12V	No	69	M	W	0	0	0	0	0	1	0	1	Moderately	No	No	No	Unknown	WT	0	380	418.00	Unknown	Yes	No	No	2	6600	9	Unknown	Unknown	4.1	1.1	
BARD 031	None Detected	Not applicable	WT	No	49	M	W	0	1	0	0	0	0	0	1	Moderately	No	No	No	Unknown	WT	0	297	330.00	Partial	Yes	No	No	743	9700	19	Unknown	Unknown	3.8	0.9	
BARD 032	None Detected	Not applicable	WT	No	82	M	W	0	1	0	0	0	0	0	1	Moderately	No	No	No	Unknown	WT	0	14	29.00	Unknown	No	Yes	No	27	7900	14	Unknown	Unknown	3.8	1.0	
BARD 033	KRAS p.G12D	5,260	KRAS p.G12D	No	38	F	W	0	1	1	1	0	0	0	3	Moderately	Yes	No	No	Unknown	WT	1	368	666.00	Progression	Yes	Yes	No	7	10800	17	Unknown	Unknown	4.5	0.6	
BARD 034	KRAS p.G12A	158,000	WT	Yes	74	M	W	0	1	0	0	0	0	0	1	Moderately	No	No	No	Unknown	WT	0	202	773.00	Partial	Yes	Yes	No	4	8600	15	Unknown	Unknown	4.1	1.4	
BARD 035	KRAS p.G13D	381	KRAS p.G13D	No	61	M	W	0	1	0	1	1	0	0	3	Well to moderately	No	No	No	Unknown	WT	0	586	1210.00	Partial	Yes	Yes	Yes	Unknown	5700	40	Unknown	Unknown	4.5	1.0	
BARD 036	None Detected	Not applicable	WT	Yes	60	F	W	0	1	1	1	0	0	0	3	Moderately	No	No	No	Unknown	WT	1	41	1329.00	Partial	Yes	Yes	No	65	3700	19	0.7	4.3	0.7		
BARD 037	KRAS p.G12C	60,800	KRAS p.G12V	Yes	62	F	W	0	1	0	1	0	0	0	2	Poorly	Unknown	No	No	Unknown	WT	1	113	137.00	Unknown	No	Yes	Yes	2	12900	22	0.7	3.7	0.5		
BARD 038	None Detected	Not applicable	WT	No	60	M	W	0	1	0	0	0	0	0	1	Moderately	No	No	No	Unknown	WT	0	616	1328.00	Partial	No	Yes	No	100	4300	16	Unknown	Unknown	4.7	0.9	
BARD 039	None Detected	Not applicable	WT	Yes	38	F	W	0	0	1	0	0	0	0	1	Moderately	No	No	No	Unknown	WT	0	255	1007.00	Partial	Yes	Yes	No	72	4800	39	Unknown	Unknown	3.5	0.6	
BARD 040	None Detected	Not applicable	WT	Yes	74	M	W	0	1	0	1	0	0	0	2	Well to moderately	No	No	No	Unknown	WT	0	202	773.00	Partial	Yes	Yes	No	4	8600	15	Unknown	Unknown	4.1	1.4	
BARD 041	None Detected	Not applicable	WT	Yes	76	M	W	0	1	1	0	0	0	0	3	Moderately	No	No	No	Unknown	WT	0	851	1606.00	Partial	Yes	Yes	No	25	8500	Unknown	1.2	4.0	1.1		
BARD 042	None Detected	Not applicable	WT	Yes	66	M	W	0	1	0	0	0	0	0	1	Moderately	No	No	No	Unknown	WT	0	988	1624.00	Stable Disease	Yes	Yes	No	3	8200	56	Unknown	Unknown	4.8	1.0	
BARD 043	None Detected	Not applicable	WT	Yes	63	F	W	0	1	1	0	0	0	0	2	Poorly	No	No	No	Unknown	WT	0	105	333.00	Partial	Yes	Yes	No	8	7400	8	Unknown	Unknown	0.6		
BARD 044	None Detected	Not applicable	WT	No	71	M	W	1	1	1	0	0	0	0	2	Moderately	No	No	No	Unknown	WT	1	199	1265.00	Partial	Yes	Yes	Yes	21	5700	25	0.6	3.6	0.6		
BARD 045	KRAS p.G12D	224	KRAS p.G12D	No	71	M	W	1	1	1	0	0	0	0	2	Moderately	No	No	No	Unknown	WT	1	585	2106.00	Stable Disease	Yes	Yes	Yes	2	7800	26	0.8	4.4	1.0		
BARD 046	None Detected	Not applicable	WT	Yes	73	M	W	0	0	1	0	0	0	0	1	Poorly	No	No	No	Unknown	WT	1	90	1424.00	Partial	Yes	Yes	No	4	8300	25	0.7	4.4	0.8		
BARD 047	None Detected	Not applicable	WT	Yes	61	M	W	0	1	0	0	0	0	0	1	Moderately	No	No	No	Unknown	WT	0	289	1980.00	Stable Disease	Yes	Yes	No	4	7000	17	Unknown	Unknown	4.9	0.8	
BARD 048	None Detected	Not applicable	WT	Yes	72	M	W	0	1	0	0	0	0	0	2	Moderately	No	No	No	Unknown	WT	0	1025	2458.00	Stable Disease	Yes	Yes	No	3	4200	Unknown	0.7	4.0	0.9		
BARD 049	None Detected	Not applicable	WT	Yes	58	M	W	0	1	1	0	0	0	0	2	Moderately	No	No	No	Unknown	WT	0	507	931.00	Partial	No	Yes	Yes	Unknown	8100	17	Unknown	Unknown	Unknown		
BARD 050	KRAS p.G12D	485	KRAS p.G12D	Yes	51	F	W	1	0	1	0	0	0	0	1	Unknown	No	No	No	Unknown	WT	0	1025	931.00	Partial	No	Yes	Yes	Unknown	8100	17	Unknown	Unknown	Unknown		
BARD 051	None Detected	Not applicable	WT	Yes	38	F	W	0	1	0	0	0	0	0	1	Moderately	No	No	No	Unknown	Unknown	0	288	433.00	Partial	Yes										

CR102	None Detected	Not applicable	WT	No	82	M	W	0	0	1	0	0	0	0	1	Well to moderately	No	Distant	Yes	Unknown	Unknown	1	120	136.00	No Therapy	No	No	No	6	5670	20	0.3	3.2	1.0		
CR104	KRAS p.G12C	1,200	WT	No	57	F	W	0	1	0	0	0	0	0	1	Unknown	Unknown	No	Yes	Unknown	Unknown	WT	1	170	64.00	Partial	Yes	Yes	No	5	7770	854	0.9	3.3	0.6	
CR105	KRAS p.G13D	218	WT	No	68	M	W	0	1	0	0	0	0	0	1	Moderately	Yes	Unknown	Unknown	Unknown	Unknown	WT	0	934	1174.00	Partial	Yes	Yes	No	5	6660	724	1.6	2.6	1.7	
CR106	KRAS p.G12D	37,800	WT	No	71	M	W	0	1	0	0	0	0	0	1	Moderately	No	No	No	Unknown	Unknown	WT	1	217	438.00	Progression	Yes	Yes	No	7	6360	45	0.6	4.1	0.8	
CR107	None Detected	Not applicable	WT	Yes	64	F	W	0	1	0	0	0	0	0	1	Well to moderately	Yes	No	No	Unknown	Unknown	WT	0	1064	1381.00	No Therapy	Yes	Yes	No	3	6120	31	0.7	4.6	0.7	
CR108	None Detected	Not applicable	WT	No	74	F	W	1	0	0	0	0	0	0	1	Moderately	No	No	No	Unknown	Mutation found	WT	0	1001	1091.00	No Therapy	Yes	No	Yes	7	6290	24	0.3	4.4	0.6	
CR110	None Detected	Not applicable	WT	No	62	F	A	0	1	0	0	0	0	0	0	1	Well to moderately	No	No	No	Unknown	Unknown	WT	1	774	3504.00	Stable	Yes	Yes	No	141	7180	14	0.4	4.7	0.5
CR111	None Detected	Not applicable	WT	No	38	F	W	0	1	0	0	0	0	0	1	Moderately	No	No	Yes	Unknown	Unknown	WT	0	845	859.00	No Therapy	No	No	No	272	6520	345	1.0	2.7	0.6	
CR112	None Detected	Not applicable	WT	No	52	F	W	0	1	0	0	0	0	0	1	Unknown	Unknown	No	No	Unknown	Unknown	WT	0	335	863.00	Stable	Yes	Yes	No	6	5010.0	36.0	Unknown	Unknown	Unknown	
CR113	KRAS p.G12V	638	WT	No	35	M	W	0	1	0	0	0	0	0	1	Unknown	Unknown	No	Yes	Unknown	Unknown	WT	1	494	539.00	No Therapy	No	No	No	2	5830	26	0.3	4.3	0.8	
CR114	None Detected	Not applicable	WT	No	66	M	W	1	1	1	0	0	0	0	2	Moderately	No	Distant	No	Unknown	Unknown	WT	0	532	562.00	No Therapy	No	No	No	6	8160	9	0.4	3.7	0.0	
CR115	None Detected	Not applicable	WT	No	60	M	B	1	0	0	0	0	0	0	1	Moderately	No	Distant	No	Unknown	Unknown	WT	0	335	6410	No Therapy	No	No	No	335	6410	17	0.4	3.5	0.4	
CR116	None Detected	Not applicable	WT	No	49	M	W	0	0	0	0	0	0	0	0	Poorly	Yes	Distant	Yes	Unknown	Unknown	WT	0	645	668.00	No Therapy	No	No	No	Unknown	9840	18	0.3	4.4	0.8	
CR117	None Detected	Not applicable	WT	No	39	F	W	0	0	0	1	0	0	0	1	Poorly	Yes	No	Yes	MSS	WT	0	428	439.00	No Therapy	No	No	No	55	4410	11	0.1	3.8	0.5		
CR118	None Detected	Not applicable	WT	No	66	F	B	2	1	0	0	0	0	0	1	Unknown	Unknown	No	Yes	Unknown	Unknown	WT	0	333	337.00	No Therapy	No	No	No	Unknown	13864	31.0	0.7	4.1	1.1	
CR119	None Detected	Not applicable	WT	No	62	M	W	0	1	0	0	0	0	0	1	Moderately	No	No	No	Unknown	Unknown	WT	0	984	39365.00	No Therapy	Yes	No	No	3	3270	36	1.4	4.3	1.0	
CR120	None Detected	Not applicable	WT	No	42	F	W	0	1	0	0	0	0	0	1	Moderately	No	No	Yes	MSS	WT	0	949	984.00	No Therapy	No	No	No	3	8520	17	0.3	4.0	0.7		
CR121	None Detected	Not applicable	WT	No	68	M	W	0	1	0	0	0	0	0	1	Moderately	No	No	Yes	Unknown	Unknown	WT	1	39	233.00	Partial	Yes	No	Yes	No	Unknown	7450	17	0.4	4.5	0.9
CR122	None Detected	Not applicable	WT	No	70	F	W	0	1	0	0	0	0	0	1	Moderately	No	No	Yes	Unknown	Unknown	WT	0	217	723.00	Partial	Yes	Yes	No	Unknown	4870	31	0.6	4.2	0.6	
CR123	None Detected	Not applicable	WT	No	47	M	W	0	1	0	0	0	0	0	1	Moderately	No	No	Yes	MSS	Unknown	WT	0	342	809.00	Stable	Yes	Yes	No	Unknown	7490	54	0.7	4.5	0.6	
CR124	None Detected	Not applicable	WT	No	50	F	B	0	1	0	0	0	0	0	1	Well to moderately	Yes	No	Yes	MSS	WT	0	819	534.00	Complete	No	No	No	Unknown	3910	62	0.6	3.9	0.8		
CR125	None Detected	Not applicable	WT	No	56	M	W	0	1	1	0	0	0	0	2	Moderately	No	No	Yes	Unknown	Unknown	WT	0	823	856.00	No Therapy	No	No	No	3	4170	23	0.7	4.7	0.8	
CR126	None Detected	Not applicable	WT	No	54	M	W	0	1	0	0	0	0	0	1	Well to moderately	No	Distant	No	Unknown	Unknown	WT	0	777	1171.00	No Therapy	No	No	No	5	10820	178	1.0	3.0	0.9	
CR127	KRAS p.G12V	28,430	WT	No	39	F	W	0	1	1	0	0	0	0	2	Moderately	No	No	Yes	Unknown	Unknown	WT	0	818	869.00	No Therapy	No	No	No	Unknown	7130	33	0.5	4.2	0.7	
CR128	None Detected	Not applicable	WT	Yes	18	M	W	0	1	0	0	0	0	0	1	Moderately	No	No	No	Unknown	Unknown	WT	1	816	841.00	Partial	Yes	No	Yes	No	Unknown	4800	22	0.5	5.0	0.7
CR129	KRAS p.G12V	211	WT	No	73	M	W	0	1	0	0	0	0	0	0	1	Moderately	No	No	No	Unknown	Unknown	WT	1	468	1012.00	Mixed	Yes	Yes	No	Unknown	5620	20	1.2	4.1	0.8
CR130	KRAS p.G12V	2,220	WT	No	45	M	B	0	1	0	0	0	0	0	1	Well to moderately	No	No	Yes	Unknown	Unknown	WT	0	738	790.00	No Therapy	No	No	No	3	3180	23	0.7	4.4	0.9	
CR131	None Detected	Not applicable	WT	No	44	M	W	0	1	0	0	0	0	0	1	Moderately	Yes	Distant	No	MSS	WT	0	427	537.00	Partial	No	Yes	No	Unknown	4600	33	0.4	4.9	1.0		
CR132	None Detected	Not applicable	WT	No	54	F	W	0	1	0	0	0	0	0	1	Moderately	No	No	Yes	Unknown	Unknown	WT	0	477	575.00	No Therapy	No	No	No	Unknown	8840	23	0.5	3.6	0.6	
CR133	None Detected	Not applicable	WT	No	69	M	W	0	1	0	0	0	0	0	1	Moderately	No	No	No	Unknown	Unknown	WT	1	32	612.00	Partial	Yes	Yes	No	96	8970	17	0.4	3.1	1.5	
CR134	KRAS p.G12V	1,280	WT	No	56	F	W	0	1	0	0	0	0	0	2	Moderately	No	No	Yes	Unknown	Unknown	WT	1	42	1060.00	Unknown	Yes	No	Yes	7	8680	43	0.5	4.6	1.0	
CR135	None Detected	Not applicable	WT	Yes	56	M	W	0	1	0	0	0	0	0	1	Poorly	Yes	Yes	No	Unknown	Unknown	WT	1	74	270.00	Partial	Yes	Yes	No	10	5480	31	0.5	3.4	0.8	
CR136	None Detected	Not applicable	WT	No	44	F	W	0	1	0	0	0	0	0	1	Moderately	No	Unknown	Yes	Unknown	Unknown	WT	0	548	853.00	Partial	Yes	Yes	Yes	No	146	10270	610	0.6	3.3	0.5
CR137	None Detected	Not applicable	WT	No	50	F	W	0	1	0	0	0	0	0	1	Moderately	No	No	No	Unknown	Unknown	WT	1	173	703.00	Unknown	Yes	Yes	Yes	6	5850	22	0.4	4.3	0.6	
CR139	KRAS p.G12D	267	WT	No	69	M	W	1	1	0	0	0	0	0	1	Moderately	Yes	No	Yes	Unknown	Unknown	WT	1	45	419.00	Progression	Yes	Yes	No	27	4580	33	0.4	4.3	0.9	
CR140	None Detected	Not applicable	WT	No	51	F	B	0	1	0	0	0	0	0	1	Unknown	Unknown	No	Yes	Unknown	Unknown	WT	0	1606	1677.00	Partial	Yes	Yes	No	55	1692	861	0.9	3.1	0.6	
CR141	KRAS p.G12A	9,040	WT	No	64	F	W	0	1	0	0	0	0	0	1	Moderately	No	No	Yes	Unknown	Unknown	WT	1	816	841.00	Partial	Yes	Yes	No	Unknown	7090	19	0.4	4.6	0.9	
CR142	None Detected	Not applicable	WT	Yes	53	M	W	0	1	0	0	0	0	0	1	Moderately	No	Yes	Yes	Unknown	Unknown	WT	1	140	822.00	Partial	Yes	Yes	No	118	6910	41	1.0	4.2	1.0	
CR143	None Detected	Not applicable	WT	No	63	M	W	0	1	0	0	0	0	0	1	Moderately	No	No	No	Unknown	Unknown	WT	1	2130	2543.00	Stable	Yes	Yes	No	5	5820	31	0.6	4.3	1.1	
CR144	None Detected	Not applicable	WT	Yes	43	F	W	0	1	0	0	0	0	0	1	Moderately	No	No	No	Unknown	Unknown	WT	1	30	184.00	Partial	Yes	Yes	No	30	4510	31	0.6	4.9	0.7	
CR145	None Detected	Not applicable	WT	No	58	F	W	0	1	0	0	0	0	0	1	Moderately	No	No	Yes	Unknown	Unknown	WT	0	1636	38810.00	No Therapy	Yes	No	No	Unknown	6550	51	0.2	4.1	0.7	
CR146	None Detected	Not applicable	WT	Yes	50	M	W	0	1	0	0	0	0	0	1	Moderately	Unknown	No	Yes	Unknown	Unknown	WT	1	489	711.00	Partial	Yes	Yes	No	4	5800	39	0.6	4.6	0.9	
CR147	None Detected	Not applicable	WT	No	63	F	W	0	1	0	0	0	0	0	1	Moderately	Unknown	No	Yes	Unknown	Unknown	WT	1	2083	2720.00	Partial	Yes	Yes	No	3	10880	922	0.9	2.6	1.0	
CR148	None Detected	Not applicable	WT	Yes	36	F	W	0	1	0	0	0	0	0	1	Moderately	Yes	No	Yes	Unknown	Unknown	WT	1	15	400.00	Partial	No	Yes	No	5	19290	504	2.3	2.2	0.8	
CR150	KRAS p.G13D	539	WT	No	41	M	W	0	1	0	0	0	0	0	1	Moderately	No	No	Yes	Unknown	Unknown	WT	0	1756	2242.00	Partial	Yes	Yes	Yes	3	11090	617	2.0	3.3	0.7	
CR151	None Detected	Not applicable	WT	No	59	M	W	nkown	1	0	0	0	0	0	1	Moderately	No	No	Yes	Unknown	Unknown	WT	1	207	988.00	Complete	Yes	Yes	No	12	13420	428	2.1	3.5	0.9	
CR152	KRAS p.G12S	32,900	WT	No	59	F	W	1	1	1	0	0	0	0	2	Well	Unknown	No	No	Yes	Unknown	Unknown	WT	1	0	1453.00	Progressed	Yes	Yes	No	Unknown	11810	8	0.3	2.6	0.4
CR153	None Detected	Not applicable	WT	No	63	M	W	3	0	0	1	0	0	0	1	Poorly	Yes	No	No	Yes	Unknown	Unknown	WT	1	482	539.00	No Therapy	Yes	No	Yes	3	3980	11	0.3	3.9	1.9
CR154	None Detected	Not applicable	WT	Yes	64	F	W	1	1	1	1	0	0	0	3	F	Unknown	Unknown	Unknown	Unknown	Unknown	WT	1	67	765.00	Progressed	Yes	Yes	Yes	7	5700	25	0.1	4.3	0.8	
CR155	None Detected	Not applicable	WT	No	68	M	W	nkown	0	1	0	0	0	0	1	Moderately	No	No	No	Unknown	Unknown	WT	1	217	1236.00	Stable	Yes	Yes	Yes	546	8250	14	0.7	4.2	0.7	
CR156	KRAS p.G12D	2,560	WT	No	38	M	W	nkown	1	1	0	0	0	0	2	Moderately	Unknown	No	Yes	Unknown	Unknown	WT	1	967	1085.00	No Therapy	Yes	Yes	No	54	5530					

LCR 081	None Detected	Not applicable	WT	No	55	F	nkno	0	0	1	1	0	0	0	2	Moderately	No	No	No	Unknown	Mutation found	0	335	342.00	SD	No	No	No	18	6800	9	1.0	2.8	0.4
LCR 082	None Detected	Not applicable	WT	No	60	M	nkno	0	1	0	1	0	0	0	2	Moderately	No	No	No	Unknown	Mutation found	0	262	800.00	SD	Yes	Yes	No	7	4300	40	0.6	3.8	0.7
LCR 083	KRAS p.G12S	500	KRAS p.G12S	No	55	M	nkno	0	0	1	0	0	0	0	1	Moderately	No	Distant	No	Unknown	WT	0	321	698.00	SD	Yes	Yes	No	31	8800	21	1.0	2.9	0.7
LCR 084	KRAS p.G12D	139,000	KRAS p.G12D	Yes	58	M	nkno	1	1	1	1	0	0	0	3	Moderately	No	No	Yes	MSS	WT	1	136	185.00	SD	No	Yes	No	26	Unknown	Unknown	Unknown	Unknown	Unknown
LCR 088	None Detected	Not applicable	WT	No	64	F	nkno	0	0	1	1	0	1	0	3	Moderately	No	Distant	No	MSS	WT	0	234	425.00	Partial	Yes	No	No	55	4500	15	1.3	3.3	0.6
LCR 099	None Detected	Not applicable	WT	No	71	M	nkno	0	1	1	0	0	0	0	2	Moderately	No	Distant	No	MSS	WT	1	46	77.00	Partial	Yes	No	No	17	8900	16	1.7	3.2	1.2
LCR 110	KRAS p.G12D	628	KRAS p.G12D	No	54	F	nkno	0	1	0	0	0	0	0	1	Moderately	No	Yes	No	MSS	WT	0	208	273.00	Partial	Yes	No	No	90	5200	27	0.4	4.2	0.6
LCR 113	None Detected	Not applicable	WT	No	57	M	nkno	1	1	0	1	0	0	0	2	Poorly	No	Yes	No	MSI	Mutation found	0	194	254.00	Partial	Yes	No	No	2	11200	15	0.5	3.7	0.6
LCR 116	KRAS p.G12C	768	KRAS p.G12C	No	75	M	nkno	0	1	0	1	0	0	0	2	Moderately	No	No	No	Unknown	WT	0	189	202.00	Partial	No	No	No	10	6900	13	1.2	3.8	0.9
LCR 117	None Detected	Not applicable	WT	No	71	M	nkno	1	1	1	1	0	0	0	3	Moderately	No	Unknown	Unknown	Unknown	WT	0	189	257.00	SD	Yes	No	No	963	Unknown	Unknown	Unknown	Unknown	Unknown
LCR 118	None Detected	Not applicable	WT	No	68	M	nkno	0	1	1	1	0	0	0	3	Moderately	No	No	No	Unknown	WT	0	155	832.00	Partial	Yes	Yes	No	2	Unknown	Unknown	Unknown	Unknown	Unknown
LCR 119	None Detected	Not applicable	WT	No	64	M	nkno	1	1	0	1	0	0	0	2	Moderately	No	Unknown	Unknown	Unknown	WT	0	189	306.00	SD	No	No	No	9000	Unknown	Unknown	Unknown	Unknown	Unknown
LCR 121	KRAS p.G13D	500	KRAS p.G13D	No	31	F	nkno	0	1	0	0	0	0	0	1	Moderately	No	No	No	MSS	WT	0	305	356.00	Partial	Yes	No	No	7	11600	18	0.5	3.6	0.5
LCR 122	KRAS p.G12D	500	KRAS p.G12D	No	82	M	nkno	0	1	0	0	0	0	0	1	Moderately	No	No	No	Unknown	WT	0	271	777.00	SD	Yes	No	No	28	6800	14	0.8	3.8	0.5

Table S4. Clinical characteristics of patients with discordant tissue and plasma *KRAS* mutation data

Univariable Analysis			
	False Negative N = 10	True Negative N = 127	P-Value
Age			
Mean (SD)	55.2 (11.08)	59.02 (12.83)	0.293
Median (Range)	58.5 (32, 69)	60 (18, 85)	
No. Missing (%)	0 (0)	2 (2)	
Gender, N (%)			
Female	3 (30)	51 (41)	0.737
Male	7 (70)	74 (59)	
Missing	0	2	
Race, N (%)			
A	1 (11)	3 (3)	0.447
B	0 (0)	10 (9)	
M	0 (0)	1 (1)	
O	0 (0)	0 (0)	
W	8 (89)	97 (87)	
Missing	1	16	
ECOG, N (%)			
0	10 (100)	96 (80)	0.653
1	0 (0)	15 (12)	
2	0 (0)	6 (5)	
3	0 (0)	2 (2)	
4	0 (0)	1 (1)	
Missing	0	7	
Met Site - Liver			
No	5 (50)	25 (20)	0.072
Yes	5 (50)	100 (80)	

	False Negative N = 10	True Negative N = 127	P-Value
Missing	0	2	
Met Site - Lungs			
No	7 (70)	88 (70)	>0.99
Yes	3 (30)	37 (30)	
Missing	0	2	
Met Site - Peritoneum			
No	8 (80)	89 (71)	0.818
Yes	2 (20)	36 (29)	
Missing	0	2	
Met Site - Pelvis			
No	10 (100)	121 (97)	>0.99
Yes	0 (0)	4 (3)	
Missing	0	2	
Met Site - Bone			
No	9 (90)	122 (98)	0.693
Yes	1 (10)	3 (2)	
Missing	0	2	
Met Site - Brain			
No	9 (90)	125 (100)	0.103
Yes	1 (10)	0 (0)	
Missing	0	2	
# of Met Sites			
0	0 (0)	3 (2)	0.604
1	8 (80)	77 (62)	
2	2 (20)	31 (25)	
3	0 (0)	13 (10)	
4	0 (0)	0 (0)	
Missing	0	3	

	False Negative N = 10	True Negative N = 127	P-Value
Histological Subtype			
Moderately	7 (70)	80 (73)	0.023
Moderately to Poorly	0 (0)	1 (1)	
Poorly	1 (10)	17 (15)	
Well	1 (10)	0 (0)	
Well to Moderately	1 (10)	12 (11)	
Missing	0	17	
Mucinous			
No	3 (30)	87 (85)	< 0.001*
Yes	7 (70)	15 (15)	
Missing	0	25	
Smoking Status			
Distant	3 (30)	19 (16)	0.446
No	6 (60)	93 (78)	
Yes	1 (10)	8 (7)	
Missing	0	7	
Family History			
No	6 (60)	83 (70)	0.776
Yes	4 (40)	36 (30)	
Missing	0	8	
MSI Status			
MSI	1 (50)	1 (8)	0.254
MSS	1 (50)	11 (85)	
Stable	0 (0)	1 (8)	
Missing	8	114	
BRAF Status			
Mutation Found	0 (0)	9 (12)	0.95
WT	5 (100)	69 (88)	

	False Negative N = 10	True Negative N = 127	P-Value
Missing	5	49	
Prior Surgery	1	6	
No	2 (20)	35 (28)	0.859
Yes	8 (80)	90 (72)	
Missing	0	2	
Prior Chemotherapy			
No	4 (40)	44 (35)	>0.99
Yes	6 (60)	81 (65)	
Missing	0	2	
Prior Radiation			
No	9 (90)	105 (85)	>0.99
Yes	1 (10)	19 (15)	
Missing	0	3	
CEA (+/- 8 weeks)			
Mean (SD)	2.14 (0.9)	380.82 (1663.89)	< 0.001*
Median (Range)	2 (1, 3)	17 (1, 13864)	
No. Missing (%)	3 (30)	24 (19)	
WBC			
Mean (SD)	5645.56 (2367.93)	7710.57 (3831.43)	0.056
Median (Range)	4600 (3480, 11070)	7300 (7.2, 20350)	
No. Missing (%)	1 (10)	5 (4)	
ALT			
Mean (SD)	21.67 (9.06)	77.56 (165.37)	0.299
Median (Range)	18 (9, 34)	24 (7, 922)	
No. Missing (%)	1 (10)	10 (8)	
Bilirubin			

	False Negative N = 10	True Negative N = 127	P-Value
Mean (SD)	0.66 (0.18)	0.85 (0.91)	>0.99
Median (Range)	0.7 (0.4, 1)	0.6 (0.1, 8)	
No. Missing (%)	2 (20)	34 (27)	
Albumin			
Mean (SD)	4.54 (0.25)	4.22 (3.34)	0.002
Median (Range)	4.6 (4.1, 4.9)	4 (1.8, 39)	
No. Missing (%)	1 (10)	12 (9)	
Creatinine			
Mean (SD)	0.88 (0.15)	1.41 (5.92)	0.308
Median (Range)	0.8 (0.7, 1.1)	0.8 (0.4, 65)	
No. Missing (%)	1 (10)	9 (7)	

*When a Bonferroni correction was applied, mucinous pathology and CEA remained significant (P<0.0017)

Table S5. Clinical characteristics of patients with false negatives in plasma *KRAS* mutation compared with tissue samples

Multivariable Analysis

	Odds Ratio Estimate
Age	0.989
CEA (within 8 weeks of blood DNA measurement)	0.897
WBC	0.999
ALT	0.959

Table S6. Association between clinical characteristics and absolute levels of ctDNA

		Univariable Analysis	
		Estimate (95% CI)	P-Value
Age		-0.035 (-0.07, 0)	0.051
Gender			
	Female	ref	
	Male	-0.625 (-1.672, 0.421)	0.237
Race			
	White	ref	
	Non-White	-0.456 (-2.813, 1.9)	0.699
ECOG PS			
	0	ref	
	1, 2	1.025 (-0.208, 2.259)	0.102
	≥3	3.31 (0.306, 6.314)	0.031
Met Site - Liver		1.433 (0.113, 2.752)	0.034
Met Site - Lungs		0.399 (-0.686, 1.484)	0.465
Met Site - Peritoneum		0.252 (-0.846, 1.351)	0.648
Met Site - Pelvis		-0.857 (-3.383, 1.669)	0.5

Univariable Analysis

	Estimate (95% CI)	P-Value
Met Site - Bone	0.267 (-1.445, 1.978)	0.757
Met Site - Brain	4.155 (-0.046, 8.357)	0.053
# of Met Sites		
0-1	ref	
≥2	0.872 (-0.158, 1.903)	0.096
Histological Subtype		
Poor	ref	
Moderate	-1.309 (-2.974, 0.356)	0.121
Well	-1.868 (-5.334, 1.597)	0.285
Mucinous		
No	ref	
Yes	-0.761 (-2.077, 0.554)	0.251
Smoking Status		
Distant	ref	
No	-0.018 (-1.639, 1.603)	0.983
Yes	-0.449 (-3.339, 2.44)	0.757
Family History		
No	ref	
Yes	0.166 (-1.035, 1.368)	0.783
MSI Status		
MSI	ref	
MSS	2.246 (-3.837, 8.329)	0.412
Prior Surgery		
No	ref	

Univariable Analysis

	Estimate (95% CI)	P-Value
Yes	-1.58 (-2.694, -0.466)	0.006
Prior Chemotherapy		
No	ref	
Yes	0.378 (-0.693, 1.449)	0.484
Prior Radiation		
No	ref	
Yes	-0.492 (-1.9, 0.917)	0.488
Log CEA (within 8 weeks of blood DNA measurement)	0.439 (0.22, 0.658)	<.001
WBC	0 (0, 0)	0.01
Log ALT	-0.07 (-0.566, 0.427)	0.78
Log Bilirubin	0.267 (-0.581, 1.115)	0.531
Log Albumin	-0.211 (-1.479, 1.058)	0.741
Log Creatinine	-0.06 (-0.731, 0.61)	0.857

Supplemental Table 7. Association between clinical characteristics and ctDNA concentration (log scale) in metastatic colorectal cancer patients

Multivariable Analysis	
	Estimate
Age	-0.013
Prior Surgery	
No	ref
Yes	-0.0799
Log CEA (within 8 weeks of blood DNA measurement)	0.3045
WBC	0.0002

Table S8. Patient characteristics and plasma mutations detected after EGFR blockade

Sample	Age	Gender	Tumor	EGFR Blockade	Duration on anti-EGFR therapy (weeks)	Plasma Mutation (nucleotide)	Plasma Mutation (codon)	Mutant fragments per 5 mL
Patient #5	60	M	Colorectal Cancer	Panitumumab	7	KRAS c.35G>A	KRAS p.G12D	66
Patient #16	72	F	Colorectal Cancer	Panitumumab	23	EGFR c.2380C>T	EGFR p.P794S	168
						KRAS c.183C>T	KRAS p.Q61H	90
Patient #17	57	F	Colorectal Cancer	Panitumumab	31	None Detected	None Detected	NA
Patient #18	47	F	Colorectal Cancer	Panitumumab	28	KRAS c.35G>T	KRAS p.G12V	120
						NRAS c.34G>A	NRAS p.G12S	129
Patient #19	42	M	Colorectal Cancer	Panitumumab	15	NRAS c.183A>T	NRAS p.Q61H	40
Patient #21	57	M	Colorectal Cancer	Panitumumab	15	KRAS c.35G>T	KRAS p.G12V	80
						NRAS c.183A>T	NRAS p.Q61H	30
						NRAS c.182A>G	NRAS p.Q61R	82
Patient #22	59	F	Colorectal Cancer	Panitumumab	15	EGFR c.2142G>C	EGFR p.K714N	948
						KRAS c.35G>C	KRAS p.G12A	40
						KRAS c.34G>T	KRAS p.G12C	30
						KRAS c.34G>A	KRAS p.G12S	120
						KRAS c.35G>A	KRAS p.G12D	104
						KRAS c.35G>T	KRAS p.G12V	100
Patient #24	57	M	Colorectal Cancer	Panitumumab	52	KRAS c.35G>C	KRAS p.G12A	15
						NRAS c.181C>A	NRAS p.Q61K	28
Patient #26	59	F	Colorectal Cancer	Panitumumab	23	KRAS c.35G>T	KRAS p.G12V	40
	73	M	Colorectal Cancer	Panitumumab	23	KRAS c.35G>T	KRAS p.G12V	114
Patient #1	64	M	Colorectal Cancer	Panitumumab	32	KRAS c.35G>C	KRAS p.G12A	1590
						KRAS c.34G>T	KRAS p.G12C	2160
						KRAS c.34G>C	KRAS p.G12R	660
						KRAS c.35G>A	KRAS p.G12D	3900
						KRAS c.35G>T	KRAS p.G12V	4940
						KRAS c.183A>T	KRAS p.Q61H	710
						KRAS c.182A>T	KRAS p.Q61L	640
						KRAS c.182A>G	KRAS p.Q61R	688
						NRAS c.181C>A	NRAS p.Q61H	1340
						NRAS c.183A>C	NRAS p.Q61K	4100
						NRAS c.182A>T	NRAS p.Q61L	6760
						NRAS c.182A>G	NRAS p.Q61R	625
Patient #2	53	M	Colorectal Cancer	Panitumumab	22	KRAS c.35G>T	KRAS p.G12V	93

Sample	Age	Gender	Tumor	EGFR Blockade	Duration on anti-EGFR therapy (weeks)	Plasma Mutation (nucleotide)	Plasma Mutation (codon)	Mutant fragments per 5 mL
Patient #4	67	F	Colorectal Cancer	Panitumumab	11	KRAS c.34G>C	KRAS p.G12R	30
						KRAS c.183A>T	KRAS p.Q61H	220
Patient #7	55	M	Colorectal Cancer	Panitumumab	23	KRAS c.35G>C	KRAS p.G12A	135
						KRAS c.35G>T	KRAS p.G12V	133
						NRAS c.183A>T	NRAS p.Q61H	848
						NRAS c.181C>A	NRAS p.Q61K	98
						NRAS c.182A>T	NRAS p.Q61L	374
Patient #9	50	M	Colorectal Cancer	Panitumumab	31	KRAS c.35G>A	KRAS p.G12D	61
						NRAS c.181C>A	NRAS p.Q61K	25
Patient #10	67	M	Colorectal Cancer	Panitumumab	23	KRAS c.35G>T	KRAS p.G12V	244
						KRAS c.183A>T	KRAS p.Q61H	83
						KRAS c.183A>C	KRAS p.Q61H	57
Patient #12	49	M	Colorectal Cancer	Panitumumab	23	KRAS c.35G>C	KRAS p.G12A	400
						KRAS c.34G>T	KRAS p.G12C	100
						KRAS c.183A>C	KRAS p.Q61H	429
BARD 101 PLS	64	M	Colorectal Cancer	Cetuximab	72	KRAS c.34G>C	KRAS p.G12R	13
						KRAS c.183A>T	KRAS p.Q61H	394
						NRAS c.182A>G	NRAS p.Q61R	4
BARD 102 PLS	45	F	Colorectal Cancer	Panitumumab	52	KRAS c.34G>C	KRAS p.G12R	208
						KRAS c.34G>C	KRAS p.G12R	308
						KRAS c.181C>G	KRAS p.Q61E	139
						KRAS c.182_183AA>CC	KRAS p.Q61P	265
BARD 103 PLS	67	F	Colorectal Cancer	Panitumumab	24	KRAS c.35G>A	KRAS p.G12D	13
						KRAS c.35G>T	KRAS p.G12V	130
CRC 188 PLS	41	F	Colorectal Cancer	Cetuximab	9	KRAS c.34G>T	KRAS p.G12C	3
CRC 189 PLS	35	F	Colorectal Cancer	Cetuximab	14	KRAS c.183A>T	KRAS p.Q61H	28
						KRAS c.183A>C	KRAS p.Q61H	13
CRC 190 PLS	54	M	Colorectal Cancer	Cetuximab	14	BRAF c.1799T>A	BRAF p.V600E	45
						KRAS c.35G>C	KRAS p.G12A	131
						KRAS c.183A>C	KRAS p.Q61H	10
						KRAS c.182A>G	KRAS p.Q61R	11
						NRAS c.182A>T	NRAS p.Q61L	2
						NRAS c.182A>G	NRAS p.Q61R	12
						KRAS c.35G>A	KRAS p.G12D	173

Sample	Age	Gender	Tumor	EGFR Blockade	Duration on anti-EGFR therapy (weeks)	Plasma Mutation (nucleotide)	Plasma Mutation (codon)	Mutant fragments per 5 mL
CRC 191 PLS	56	F	Colorectal Cancer	Panitumumab	7	KRAS c.34G>C	KRAS p.G12R	31
						KRAS c.35G>T	KRAS p.G12V	58
						KRAS c.183A>C	KRAS p.Q61H	250