CIRCULATING TUMOR DNA AS A BIOMARKER FOR CANCER

by

Jillian Phallen

A dissertation submitted to Johns Hopkins University

in conformity with the requirements for the degree of Doctor of Philosophy.

Baltimore, Maryland

October, 2017

ABSTRACT

Detection of circulating tumor DNA (ctDNA) has potential as a noninvasive, specific, broadly applicable biomarker for cancer. To evaluate the utility of ctDNA as a biomarker for pancreatic ductal adenocarcinoma (PDAC) we performed targeted analysis of tumors from 51 preoperative, stage II patients and examined tumor-specific mutations in the ctDNA. We detected ctDNA in 43% of preoperative patients, and found that detection post-resection predicted clinical relapse and poor outcome, with evidence of recurrence by ctDNA detected 6.5 months earlier than CT imaging. To further explore early detection of cancer using ctDNA we developed an approach called targeted error correction sequencing (TEC-Seq) that allows ultrasensitive direct evaluation of sequence changes in circulating cell-free DNA (cfDNA) using massively parallel sequencing. We used this approach to examine 58 cancer-related genes encompassing 81 kb. Analysis of plasma from 44 healthy individuals identified genomic changes related to clonal hematopoiesis in 16% of asymptomatic individuals but no alterations in driver genes related to solid cancers. Evaluation of 200 patients with colorectal, breast, lung, or ovarian cancer detected somatic mutations in the plasma of 71, 59, 59, and 68%, respectively, of patients with stage I or II disease. Analyses of mutations in the circulation revealed high concordance with alterations in the tumors of these patients. In patients with resectable colorectal cancers, higher amounts of preoperative circulating tumor DNA were associated with disease recurrence and decreased overall survival. We further applied the TEC-Seq approach to detect response to targeted tyrosine kinase inhibitors in lung cancer patients and found that patients with radiographic response to therapy had a drop in ctDNA from an average mutant allele fraction of 3.59% to 0.13% within 6-22 days, while non-responders had limited changes in ctDNA. Patients with ctDNA based molecular responses had improved progression-free survival (12.0 vs 1.5 months, P = 0.001), which was detected on average 38 days earlier and was more predictive than CT imaging. These observations provide a

broadly applicable approach for noninvasive detection of cancer and highlight the utility of ctDNA as a biomarker for early detection, identification of windows of opportunity for therapeutic intervention, and prediction of outcome.

Thesis Advisor: Dr. Victor E. Velculescu, M.D., Ph.D.

Thesis Readers: Dr. Robert B. Scharpf, Ph.D. and Dr. Valsamo Anagnostou, M.D., Ph.D.

ACKNOWLEDGEMENTS

I would like to thank my mentor, Victor Velculescu, for his guidance and support throughout my graduate training, for his impeccable attention to detail, for always having time to discuss data, for his unwavering kindness, and for his efforts towards making the lab a warm and exciting environment. I also want to thank Robert Scharpf and Valsamo Anagnostou for their mentorship and advice as members of my thesis committee and beyond, and all the members of the Cancer Genomics Lab for their comradery. My family also deserves many thanks especially my mom, Phyllis, who is a pillar of strength, and my husband, Dimitrios, whose motivation and clarity of mind are a constant inspiration to me. I greatly appreciate the contributions to research from patients with cancer as well as the support of Johns Hopkins University School of Medicine.

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CHAPTER 1:

INTRODUCTION

Cancer is a widespread and devastating disease of genetic origin. Several biomarkers have been developed for disease monitoring, however these are often unspecific, have limited clinical use, and are not applicable across multiple cancer stages and types. The development of noninvasive liquid biopsy methods based on the analysis of cfDNA provides the opportunity for a new generation of diagnostic approaches. Although cfDNA in the circulation was first described more than 50 years ago (1), abnormalities in cancer patients were observed only decades later (2, 3) and showed that such individuals have higher amounts of cfDNA. In patients with cancer, a fraction of cfDNA is tumor-derived and is termed circulating tumor DNA, or ctDNA. In principle, analysis of ctDNA has the advantage of identifying alterations that are extremely specific to the tumor. The application of next-generation sequencing (NGS), together with advanced computational methods, has recently allowed ctDNA-based tumor genotyping in a variety of cancer types (4-13). These approaches can be applied to monitor patients with late-stage cancers either through direct detection of ctDNA or through liquid biopsies guided by tumor tissue sequencing, and point to the possibility of noninvasive direct early-detection of cancer.

We first assessed the utility of liquid biopsy approaches to evaluate ctDNA for noninvasive detection of early-stage pancreatic cancer as well as for identifying recurrent or residual disease (6). Worldwide, over 250,000 patients develop PDAC every year and the vast majority die of their disease (14). PDAC comprises 85% of all pancreatic neoplasms, with 60–70% of cancers localized to the head of the pancreas, 20–25% in the body or tail and the remaining cases involving the entire organ (15). Currently, surgical resection of the tumor is the only potentially curative treatment. However, only a minority (15–20%) of patients are candidates for pancreatectomy at the time of diagnosis (16). This can largely be attributed to the fact that pancreatic cancer develops over decades as a result of the accumulation of genetic mutations and

other molecular abnormalities and clinical presentation often occurs very late in the history of the disease4. The 5-year survival rate for those diagnosed with pancreatic cancer remains <10% (14).

To identify genetic alterations that may be related to patient outcome and other clinical characteristics, we performed genomic analyses of pancreatic adenocarcinomas and used droplet digital PCR analyses of liquid biopsies to evaluate ctDNA for non-invasive detection of early-stage pancreatic cancer as well as for identifying recurrent or residual disease. These analyses provide predictors of clinical outcome in pancreatic cancer, have implications for personalized therapeutic intervention in these patients, and show the potential for early detection of cancer.

Through our analyses of liquid biopsies from pancreatic cancer patients we observed that detection of ctDNA is possible in patients with localized disease and we further explored the concept of direct detection of cancer at early stage. More than 14 million individuals are newly diagnosed with cancer worldwide each year, with the majority having invasive or metastatic disease (17). It is well established that much of the morbidity and mortality in human cancer is related to the late diagnosis, where surgical and pharmacologic therapies are less effective (18). While early detection of stage II pancreatic cancer patients using ctDNA was promising, we relied on the evaluation of mutations in the tumor tissue in order to follow alterations in the blood. In a true screening setting, prior knowledge of tumor-derived mutations would not be possible and droplet digital PCR approaches to evaluate specific mutations would not broadly capture the repertoire of mutations across cancer types and stages. Further challenges to early detection include extremely low levels of alterations in ctDNA for patients with localized disease. Unfortunately, clinically proven biomarkers that can be used to broadly diagnose and guide patient management early in the course of disease are not available. Serum-based protein biomarkers such as cancer antigen 125, carcinoembryonic antigen (CEA), prostate-specific antigen, and cancer antigen 19-9 are commonly used for monitoring cancer patients, but because

these proteins are also found in the serum of individuals without cancer, they are typically not useful for disease diagnosis (19-23). Other approaches for early detection of cancer, such as stool-based molecular tests or colonoscopies, are limited to individual tumor types and have challenges in patient compliance (24, 25). Currently, no widely applicable biomarkers have been developed for broad detection of human cancer.

We have developed an ultrasensitive approach for direct analysis of sequence alterations in commonly altered cancer genes in cfDNA without prior knowledge of alterations in the tumor (26). The sensitivity and specificity of the methodology were evaluated in a clinically relevant cohort of healthy individuals and those with early-stage disease in four common cancers. We identified sequence alterations in cell proliferation genes in individuals without cancer, established the sensitivity of the approach for detecting tumor-specific alterations in the plasma of cancer patients, evaluated concordance between plasma and tumor samples from the same patients, and showed that the amounts of ctDNA can serve as a predictive marker of patient outcome.

To extend our liquid biopsy analyses from early detection of cancer to the setting of disease monitoring, we used the TEC-Seq approach to assess the clinical value of a comprehensive analysis of ctDNA alterations to evaluate tumor burden at timepoints rapidly following targeted therapy in stage IV lung cancer patients. The management of oncogene-addicted cancer has been improved by the development of targeted therapies that act against a variety of cancer dependencies (27, 28). However, therapeutic efficacy of targeted agents has been limited by incomplete pharmacological suppression of tumors or through the selection of resistance mutations in clonal populations of tumor cells. Disease monitoring using computed tomography (CT) imaging is the current clinical practice for assessing response to targeted therapy, yet this approach does not fully represent the molecular and pathologic changes occurring in tumors

during therapy. Repeat tissue biopsies of accessible cancer lesions have been used to provide insights into therapeutic decision-making but rarely capture the complexity of intra- and inter-tumoral heterogeneity and are invasive procedures with potential complications. Theoretically, the ability to non-invasively track specific clonal populations of tumor cells through time has the potential to inform therapy sequence and combinatorial strategies. However, there are currently no approved or clinically recognized non-invasive molecularly defined strategies to assess early drug responsiveness or adaptive resistance in cancer patients before radiographic progression. A variety of studies have focused on changes in ctDNA during the course of therapy, but have largely focused on the analysis of specific or limited number of alterations that may only represent specific subclones of the tumor (5, 29, 30). More recent studies have used panels of commonly mutated driver genes to allow detection of multiple driver clones, typically at the time of diagnosis (7, 10, 26, 31). However, no study has yet assessed the utility of direct detection of ctDNA at early, clinically relevant timepoints during targeted therapy.

We hypothesized that kinetic changes in the amount of DNA released from tumor cells may occur within hours to days of treatment administration. We used TEC-Seq to evaluate patients previously diagnosed with late-stage non-small cell lung cancer who had response or progression on tyrosine kinase inhibitors, including afatinib, a second generation inhibitor of the epidermal growth factor receptor (EGFR) and erb-b2 receptor tyrosine kinase 2 (ERBB2), and osimertinib, a third-generation tyrosine kinase inhibitor targeting EGFR with activating and T790M resistance mutations (32, 33). These analyses investigated whether rapid changes and the overall levels in the amounts of ctDNA can serve as real-time and predictive biomarkers of patient outcome to a targeted cancer therapy.

Overall, we examined the utility of ctDNA as a biomarker for cancer using droplet digital PCR approaches to detect tumor-derived alterations in the blood of stage II pancreatic cancer patients

as well developed a novel next-generation sequencing approach for direct detection of ctDNA and applied it for detection of cancer at early stage and during the course of targeted therapy. These analyses, taken together, highlight the feasibility of noninvasive, sensitive and specific detection of cancer.

CHAPTER 2:

CLINICAL IMPLICATIONS OF GENOMIC ALTERATIONS IN THE TUMOR AND CIRCULATION OF PANCREATIC CANCER PATIENTS

METHODS

Samples obtained for sequencing analyses

Pancreatic ductal adenocarcinoma tumor specimens and matched germline specimens (from peripheral blood) from 51 patients were used for targeted genomic analyses (Tables 2.1 and 2.2). Plasma samples were obtained at the time of diagnosis from 44 of these patients as well as seven additional patients (Table 2.2). Informed consent for research use was obtained from all patients at the enrolling institution (University of Pennsylvania, Washington University in St. Louis, Herlev Hospital at the University of Copenhagen) before tissue banking and study approval was obtained. Primary tumor samples for genomic analyses were selected from patients with resectable stage II disease, verified to have >10% viable tumor cell content by histopathological assessment and demonstrated to be wild-type for the DAXX/ATRX loci, which have been shown to be associated with improved outcome in patients with pancreatic neuroendocrine tumors (34). For a subset of cases, plasma samples were obtained at multiple time points after surgery.

Sample preparation and next-generation sequencing

Sample preparation, library construction, exome and targeted capture, next-generation sequencing and bioinformatics analyses of tumor and normal samples were performed as previously described (35). In brief, DNA was extracted from frozen or formalin-fixed paraffin-embedded tissue, along with matched blood or saliva samples using the Qiagen DNA formalin-fixed paraffin-embedded tissue kit or Qiagen DNA blood mini kit (Qiagen, CA). Genomic DNA from tumor and normal samples were fragmented and used for Illumina TruSeq library construction (Illumina, San Diego, CA) according to the manufacturer's instructions or as previously described

(36). In brief, 50 ng–3mg of genomic DNA in 100 ml of TE was fragmented in a Covaris sonicator (Covaris, Woburn, MA) to a size of 150–450 bp. To remove fragments smaller than 150 bp, DNA was purified using Agencourt AMPure XP beads (Beckman Coulter, IN) in a ratio of 1.0 to 0.9 of PCR product to beads twice and washed using 70% ethanol as per the manufacturer's instructions. Purified, fragmented DNA was mixed with 36 ml of H2O, 10 ml of End-Repair Reaction Buffer, 5 ml of End-Repair Enzyme Mix (cat# E6050, NEB, Ipswich, MA). The 100-ml end-repair mixture was incubated at 20 C for 30 min, and purified using Agencourt AMPure XP beads (Beckman Coulter, IN) in a ratio of 1.0 to 1.25 of PCR product to beads and washed using 70% ethanol as per the manufacturer's instructions. To A-tail, 42 ml of end-repaired DNA was mixed with 5 ml of 10dA Tailing Reaction Buffer and 3 ml of Klenow (cat# E6053, NEB, Ipswich, MA). The 50-ml mixture was incubated at 37 C for 30 min and purified using Agencourt AMPure XP beads (Beckman Coulter, IN) in a ratio of 1.0 to 1.0 of PCR product to beads and washed using 70% ethanol as per the manufacturer's instructions. For adaptor ligation, 25 ml of A-tailed DNA was mixed with 6.7 ml of H2O, 3.3 ml of PE-adaptor (Illumina), 10 ml of 5Ligation buffer and 5 ml of Quick T4 DNA ligase (cat# E6056, NEB, Ipswich, MA). The ligation mixture was incubated at 20 C for 15 min and purified using Agencourt AMPure XP beads (Beckman Coulter, IN) in a ratio of 1.0 to 0.95 and 1.0 of PCR product to beads twice and washed using 70% ethanol as per the manufacturer's instructions. To obtain an amplified library, twelve PCRs of 25 ml each were set up, each including 15.5 ml of H2O, 5 ml of 5Phusion HF buffer, 0.5 ml of a dNTP mix containing 10mM of each dNTP, 1.25 ml of dimethylsulphoxide, 0.25 ml of Illumina PE primer #1, 0.25 ml of Illumina PE primer #2, 0.25 ml of Hotstart Phusion polymerase and 2 ml of the DNA. The PCR program used was: 98 C for 2min; 12 cycles of 98 C for 15 s, 65 C for 30 s, 72 C for 30 s; and 72 C for 5min. DNA was purified using Agencourt AMPure XP beads (Beckman Coulter, IN) in a ratio of 1.0 to 1.0 of PCR product to beads and washed using 70% ethanol as per the manufacturer's instructions.

Targeted regions were captured in solution using the Agilent SureSelect v.4 kit or a custom targeted panel for the 111 genes of interest according to the manufacturer's instructions (Agilent, Santa Clara, CA). The captured library was then purified with a Qiagen MinElute column purification kit and eluted in 17 ml of 70C EB to obtain 15 ml of captured DNA library. The captured DNA library was amplified in the following way: Eight 30 ml PCR reactions each containing 19 ml of H2O, 6 ml of 5Phusion HF buffer, 0.6 ml of 10mM dNTP, 1.5 ml of dimethylsulphoxide, 0.30 ml of Illumina PE primer #1, 0.30 ml of Illumina PE primer #2, 0.30 ml of Hotstart Phusion polymerase and 2 ml of captured exome library were set up. The PCR program used was: 98 C for 30 s; 14 cycles (exome) or 16 cycles (targeted) of 98 C for 10 s, 65 C for 30 s, 72 C for 30 s; and 72 C for 5min. To purify PCR products, a NucleoSpin Extract II purification kit (Macherey-Nagel, PA) was used following the manufacturer's instructions. Paired-end sequencing, resulting in 150 bases from each end of the fragment for targeted libraries, was performed using Illumina HiSeq 2000/2500 and Illumina MiSeq instrumentation (Illumina, San Diego, CA).

Analysis of next-generation sequencing data

Somatic mutations were identified using VariantDx (37) custom software for identifying mutations in matched tumor and normal samples. Before mutation calling, primary processing of sequence data for both tumor and normal samples were performed using Illumina CASAVA software (v1.8), including masking of adapter sequences. Sequence reads were aligned against the human reference genome (version hg18) using ELAND with additional realignment of select regions using the Needleman–Wunsch method25. Candidate somatic mutations, consisting of point mutations, insertions and deletions were then identified using VariantDx across the either the whole exome or regions of interest. VariantDx examines sequence alignments of tumor

samples against a matched normal while applying filters to exclude alignment and sequencing artifacts. In brief, an alignment filter was applied to exclude quality failed reads, unpaired reads and poorly mapped reads in the tumor. A base quality filter was applied to limit inclusion of bases with reported phred quality score 430 for the tumor and 420 for the normal. A mutation in the tumor was identified as a candidate somatic mutation only when (i) distinct paired reads contained the mutation in the tumor; (ii) the number of distinct paired reads containing a particular mutation in the tumor was at least 2% of the total distinct read pairs for targeted analyses and 10% of read pairs for exome and (iii) the mismatched base was not present in 41% of the reads in the matched normal sample as well as not present in a custom database of common germline variants derived from dbSNP and (iv) the position was covered in both the tumor and normal. Mutations arising from misplaced genome alignments, including paralogous sequences, were identified and excluded by searching the reference genome. Candidate somatic mutations were further filtered based on gene annotation to identify those occurring in protein coding regions. Functional consequences were predicted using snpEff and a custom database of CCDS, RefSeq and Ensembl annotations using the latest transcript versions available on hg18 from UCSC (https://genome.ucsc.edu/). Predictions were ordered to prefer transcripts with canonical start and stop codons and CCDS or Refseq transcripts over Ensembl when available. Finally, mutations were filtered to exclude intronic and silent changes, while retaining mutations resulting in missense mutations, nonsense mutations, frameshifts or splice-site alterations. A manual visual inspection step was used to further remove artefactual changes. In addition, all sequence data from tumors that harbored single-base substitutions in MLL, MLL2, MLL3 and ARID1A genes were independently analyzed using MuTect algorithm10 to confirm the presence of these alterations.

Analysis without a matched normal sample

For the identification of putative somatic mutations without a matched normal, additional filters were applied. First, mutations present in an unmatched normal sample, sequenced to a similar coverage and on the same platform as the matched normal, were removed. Second, alterations reported in the 1000 Genomes project, present in 41% of the population or listed as Common in dbSNP138 were filtered.

Statistical analyses of clinical and genetic data

Curves for overall survival and progression-free survival (calculated as the time from diagnosis to disease progression) were constructed using the Kaplan–Meier method and compared between groups using the log-rank test. Cox proportional hazards regression analysis was used to determine which independent factors jointly had a significant impact on overall survival. All P values were based on two-sided testing and differences were considered significant at $P<0.05$. Statistical analyses of clinical and genetic features were performed with SPSS version 22 for windows.

Digital PCR analyses

KRAS, BRAF or PIK3CA somatic point mutations were identified through sequencing analysis of tumor tissues. In cases with matched plasma samples, point mutations were detected in the plasma using droplet digital PCR (ddPCR) using the BioRad QX200 Droplet Digital PCR System (Hercules, CA). In brief, specific ddPCR assays for each point mutation were obtained from

BioRad (Hercules, CA) and applied to assess the mutant allele fraction (mutant genomic equivalents/total genomic equivalents). Before analysis of each point mutation in the patient plasma sample, a panel of at least 160 normal control analyses was used to confirm the mutation specificity of the assay. In addition, control samples of wild-type DNA were included in each analysis.

RESULTS

Next-generation sequencing analyses of pancreatic cancer

We used next-generation sequencing to examine targeted regions in 51 patient tumors. These approaches allowed us to identify sequence changes, including single base and small insertion or deletion mutations in 116 specific genes in the targeted analyses (Figure 2.1 and Table 2.1). The pancreatic cancers analyzed were stage II tumors in patients who underwent potentially curative resections (Table 2.2). Given the low neoplastic cellularity of pancreatic cancers5, we enriched for neoplastic cells by macrodissecting primary tumors, and performed high-coverage sequencing of these enriched samples. We obtained a per-base sequencing coverage of 754-fold for each tumor analyzed by targeted cancer gene sequencing (Table 2.3).

Non-invasive detection of early-stage pancreatic cancer

In parallel to the sequencing analyses of neoplastic tissues, we evaluated the utility of using somatic mutations in ctDNA to identify patients likely to recur after surgical intervention. Through sequencing analyses of tumor samples, we identified somatic mutations that could be used to detect ctDNA in 51 patients from whom plasma was available, largely focusing on alterations in the KRAS gene. Using digital PCR (dPCR) approaches, we were able to demonstrate that these alterations were detectable in the plasma of 22 patients (43%) at the time of diagnosis, with a specificity of 499.9% (Table 2.4). Consistent with recent reports (4, 10),

these results suggest that a significant fraction of early-stage pancreatic cancers could be diagnosed non-invasively using approaches that focus on a few specific genetic alterations.

dPCR analyses were performed using plasma samples obtained at various time points after surgical resection (Table 2.5). These analyses revealed that patients with detectable ctDNA in their plasma were more likely to relapse than those with undetectable alterations ($P<0.02$, log-rank test, Figure 2.2). Disease progression using ctDNA was detected at an average of 3.1 months after surgery compared with 9.6 months using standard computed tomography imaging ($P<0.0004$, paired t-test, Figure 2.3). The presence of ctDNA at the time of diagnosis also provided a predictor of disease recurrence ($P<0.015$, log-rank test, Figure 2.4). These analyses suggest that tests to detect sequence alterations in cell-free DNA may provide a highly specific approach for early detection of residual or recurrent disease after surgical resection.

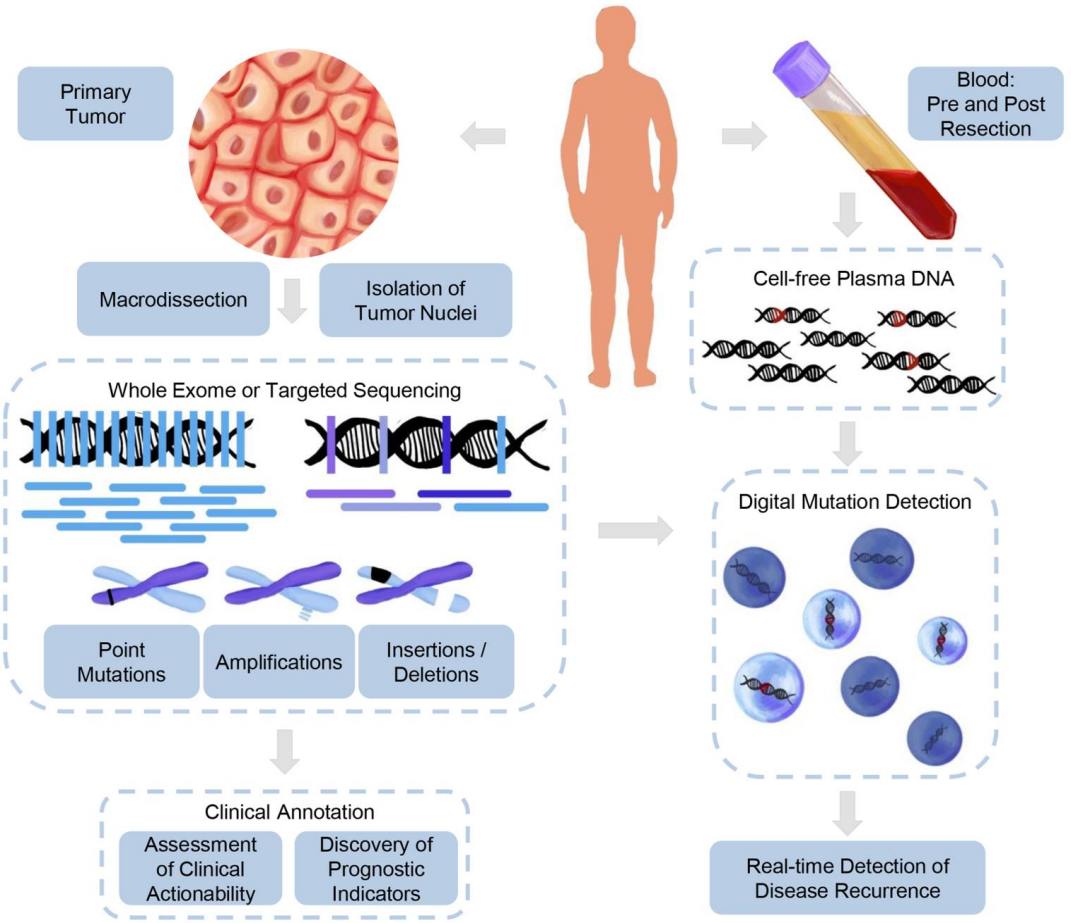

Figure 2.1. Schematic of next-generation sequencing and ctDNA analyses. Next-generation sequencing analyses were performed for tumor specimens obtained from pancreatic ductal adenocarcinoma patients using either whole-exome or targeted analyses focused on 116 genes. Genomic alterations were evaluated for potential clinical utility or as prognostic indicators. Somatic mutations identified through genomic analyses were used to evaluate patient plasma for detection of ctDNA at the time of diagnosis or after surgical intervention.

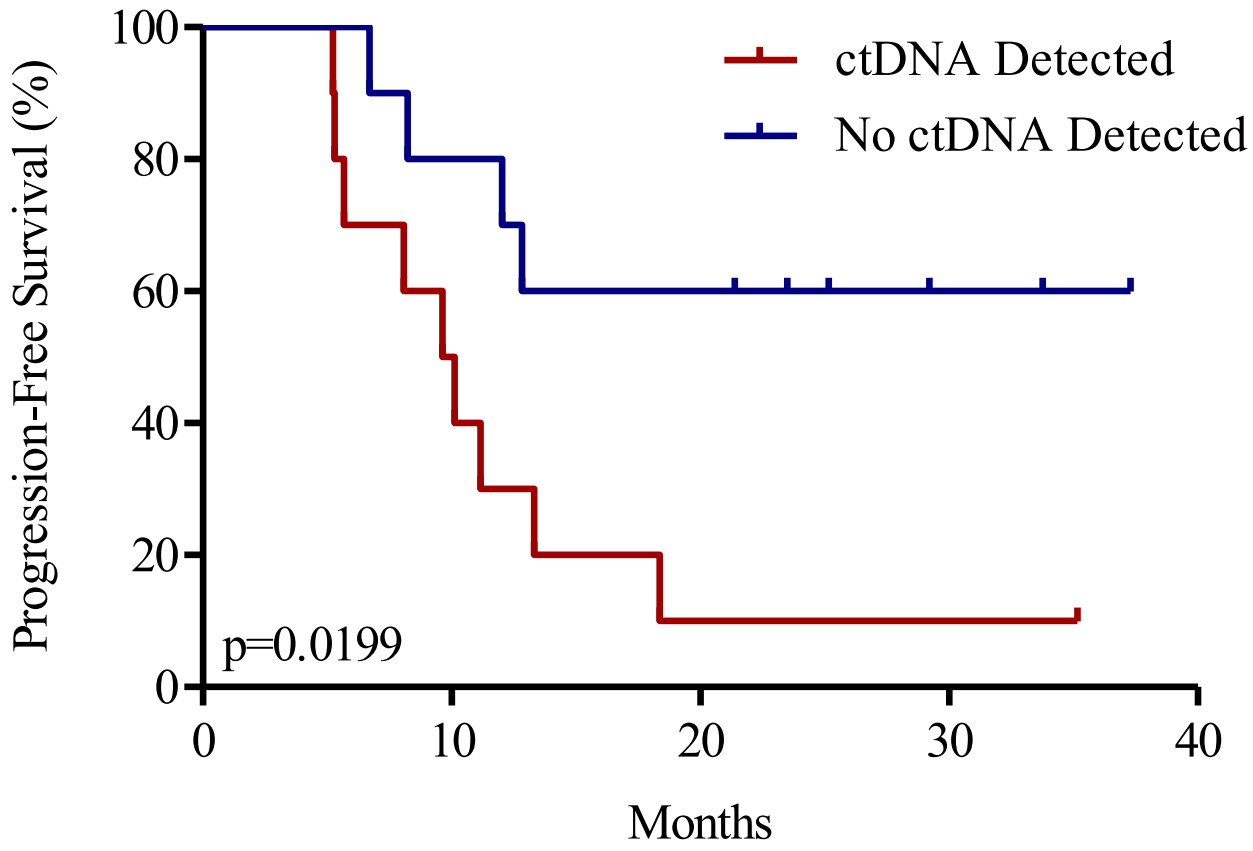

Figure 2.2. Detection of residual disease using ctDNA analyses. Patients with detectable ctDNA after surgical resection (n=10) were more likely to relapse and die from disease compared with those with undetectable ctDNA (n=10). The median time to recurrence as determined by CT imaging was 9.9 months for individuals with detectable ctDNA and was not reached for those without detectable ctDNA (P=0.0199, log-rank).

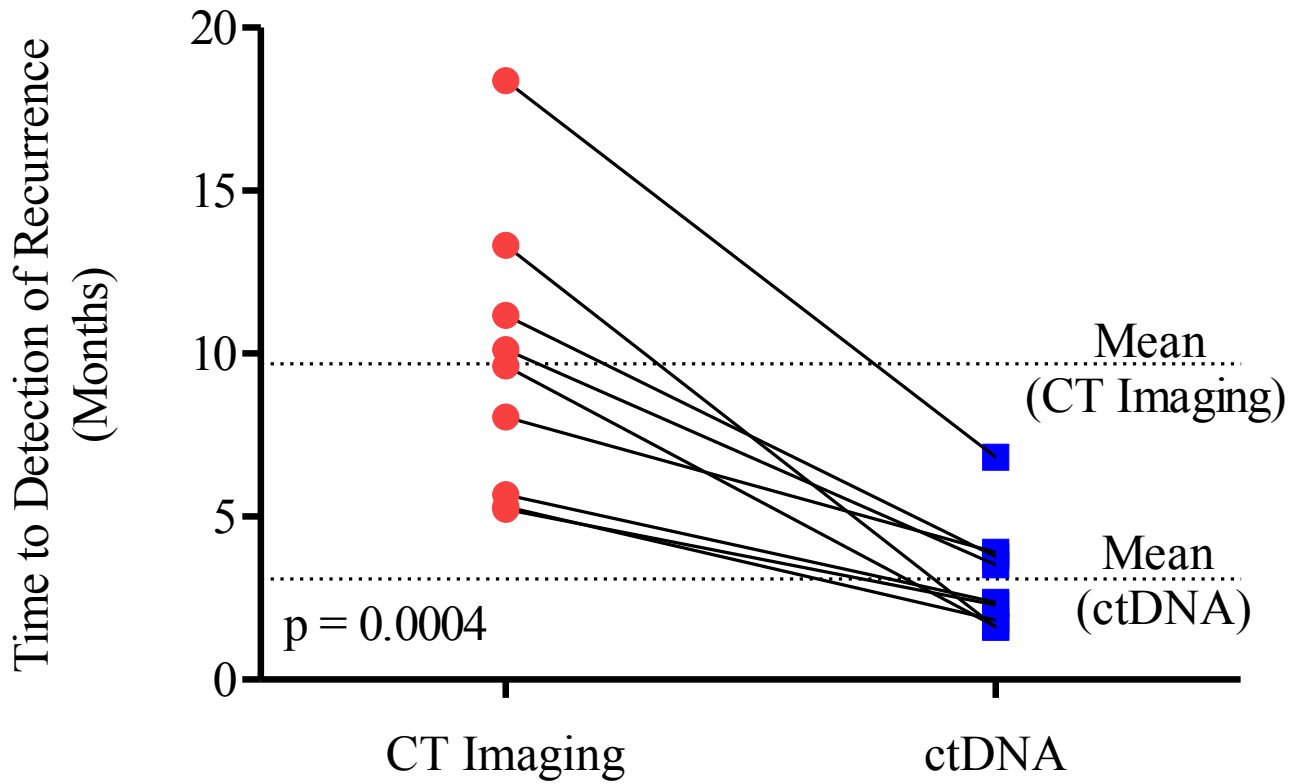

Figure 2.3. Detection of residual disease using CT imaging and ctDNA analyses. Comparison between the time to detection of recurrence using ctDNA and standard-of-care CT imaging revealed that the average time to recurrence was 3.1 months for individuals with detectable ctDNA and 9.6 months for those patients with positive imaging results (n=9, P=0.0004, paired t-test).

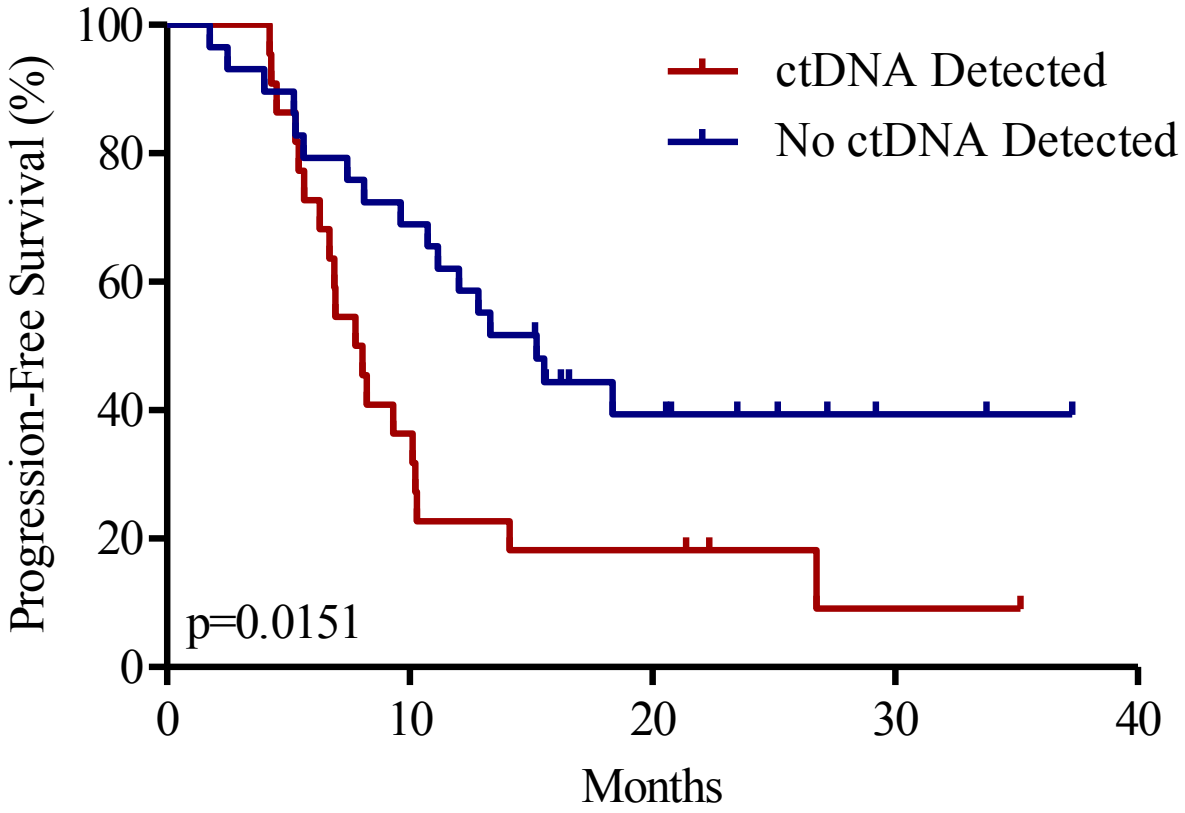

Figure 2.4. Prediction of Recurrence using ctDNA Detected at Baseline. The hazard ratio for tumor recurrence among individuals with detectable circulating tumor DNA (ctDNA) compared to those without detectable ctDNA was 2.39 (95% CI = 1.18–4.81, log rank). The median time to recurrence was 15.2 months for individuals without detectable ctDNA (n=29) and 7.9 months for those with detectable ctDNA (n=22).

Table 2.1. Summary of genes analyzed using targeted cancer gene sequencing.

ABL1	CREBBP	IDH1	MSH6	PTPN11
AKT1	CTNNB1	IDH2	MYC	RB1
AKT2	DAXX	IGF1R	MYCN	RET
ALK	DNMT3A	IGF2R	MYD88	RNF43
APC	EGFR	IKZF1	NF1	ROS1
AR	ERBB2	JAK1	NF2	RUNX1
ARID1A	ERBB3	JAK2	NOTCH1	SF3B1
ARID1B	ERBB4	JAK3	NOTCH2	SMAD2
ASXL1	EZH2	KDR	NOTCH3	SMAD3
ATM	FBXW7	KIT	NOTCH4	SMAD4
ATRX	FGFR1	KRAS	NPM1	SMARCB1
BAP1	FGFR2	MAML1	NRAS	SMO
BRAF	FGFR3	MDM2	PALB2	STAG2
BRCA1	FGFR4	MDM4	PAX5	STK11
BRCA2	FLT3	MED12	PBRM1	TET2
CBL	FOXL2	MEN1	PDGFRA	TGFBR2
CCND1	GATA1	MET	PDGFRB	TNFAIP3
CCNE1	GATA2	MLH1	PIK3CA	TP53
CDH1	GNA11	MLL	PIK3R1	TPMT
CDK4	GNAQ	MLL2	PMS2	TSC1
CDK6	GNAS	MLL3	PRSS1	TSC2
CDKN2A	HNF1A	MPL	PTCH1	TSHR
CEBPA	HRAS	MSH2	PTEN	VHL
				WT1

Table 2.2. Summary of Pancreatic Ductal Adenocarcinoma Cases Analyzed.

Case ID	Age at Surgery	Histology	Gender	Differentiation	Tumor Size (cm)	Tumor Stage	Neoadjuvant	Adjuvant	Progression Free Survival (Months)	Progression Free Survival	Overall Survival (Months)	Overall Survival
CGPLPA14	68	Adenocarcinoma	Male	Poor	3.5	IIa	No	Yes	6.7	1	13.1	1
CGPLPA15	70	Adenocarcinoma	Female	Well	3.5	IIb	No	Yes	6.9	1	12.1	1
CGPLPA16	56	Adenocarcinoma	Female	Moderate	3.2	IIb	No	Yes	5.7	1	18.5	1
CGPLPA17	65	Adenocarcinoma	Male	Well	2.5	IIb	No	Yes	10.2	1	11.4	1
CGPLPA19	73	Adenocarcinoma	Male	Poor	4.0	IIa	No	No	6.9	1	6.9	1
CGPLPA20	58	Adenocarcinoma	Female	Poor	1.5	IIb	No	Yes	10.1	1	41.8	0
CGPLPA23	58	Adenocarcinoma	Female	Moderate	3.4	IIb	No	Yes	4.2	1	5.6	1
CGPLPA24	59	Adenocarcinoma	Male	Moderate	Not Available	IIb	No	Yes	12.0	1	13.5	1
CGPLPA25	69	Adenocarcinoma	Female	Poor	1.0	IIb	No	Yes	4.5	1	5.0	1
CGPLPA26	64	Adenocarcinoma	Male	Well	4.0	IIb	No	Yes	1.8	1	5.5	1
CGPLPA27	59	Adenocarcinoma	Male	Moderate	2.0	IIb	No	Yes	2.5	1	2.5	1
CGPLPA28	79	Adenocarcinoma	Female	Well	3.0	IIb	No	Yes	15.5	1	34.6	1
CGPLPA31	37	Adenocarcinoma	Male	Moderate	4.0	IIb	No	Yes	18.4	1	37.1	0
CGPLPA33	67	Adenocarcinoma	Female	Well	4.0	IIb	No	Yes	5.2	1	5.9	1
CGPLPA34	73	Adenocarcinoma	Male	Well	8.0	IIb	No	Yes	5.3	1	11.6	1
CGPLPA35	77	Adenocarcinoma	Female	Moderate	3.0	IIb	No	Yes	8.1	1	9.8	1
CGPLPA36	65	Adenocarcinoma	Male	Poor	3.0	IIb	No	Yes	35.2	0	35.2	0
CGPLPA37	67	Adenocarcinoma	Female	Not Available	3.5	IIb	No	Yes	4.3	1	16.7	1
CGPLPA39	67	Adenocarcinoma	Female	Well	3.5	IIb	No	Yes	33.6	0	33.6	0
CGPLPA40	64	Adenocarcinoma	Male	Well	2.5	IIb	No	Yes	14.1	1	15.0	1
CGPLPA41	69	Adenocarcinoma	Male	Well	4.5	IIb	No	Yes	7.4	1	15.0	1
CGPLPA42	73	Adenocarcinoma	Male	Moderate	4.5	IIb	No	Yes	10.7	1	20.9	1
CGPLPA43	69	Adenocarcinoma	Male	Well	2.0	IIa	No	No	26.8	1	32.2	0
CGPLPA45	61	Adenocarcinoma	Female	Well	2.5	IIb	No	Yes	27.2	0	27.2	0
CGPLPA50	77	Adenocarcinoma	Female	Well	2.2	IIb	No	Yes	10.3	1	17.5	1
CGPLPA51	49	Adenocarcinoma	Female	Not Available	3.0	IIb	No	Yes	29.2	0	29.2	0
CGPLPA55	67	Adenocarcinoma	Male	Poor	3.0	IIb	No	Yes	7.8	1	9.7	1
CGPLPA56	62	Adenocarcinoma	Male	Well	2.0	IIb	No	Yes	15.2	1	25.2	0
CGPLPA57	73	Adenocarcinoma	Male	Well	3.0	IIb	No	Yes	25.2	0	25.2	0
CGPLPA60	53	Adenocarcinoma	Male	Moderate	3.0	IIb	No	Yes	11.2	1	24.3	0
CGPLPA63	59	Adenocarcinoma	Male	Moderate	3.5	IIb	No	Yes	13.3	1	23.7	0
CGPLPA65	66	Adenocarcinoma	Female	Moderate	12.5	IIb	No	Yes	8.1	1	23.3	0
CGPLPA73	66	Adenocarcinoma	Male	Poor	4.0	IIb	No	Yes	5.3	1	6.7	1

CGPLPA75	75	Adenocarcinoma	Female	Poor	7.0	IIb	No	Yes	5.4	1	6.7	1
CGPLPA77	69	Adenocarcinoma	Male	Moderate	2.0	IIb	No	Yes	21.4	0	21.4	0
CGPLPA78	70	Adenocarcinoma	Male	Not Available	1.5	IIb	No	Yes	20.8	0	20.8	0
CGPLPA80	70	Adenocarcinoma	Male	Well	2.0	IIb	No	Yes	20.6	0	20.6	0
CGPLPA81	62	Adenocarcinoma	Male	Moderate	5.0	IIb	No	Yes	8.2	1	8.5	1
CGPLPA82	63	Adenocarcinoma	Male	Poor	2.7	IIb	No	Yes	4.0	1	4.1	1
CGPLPA83	72	Adenocarcinoma	Male	Poor	2.0	IIb	No	Yes	6.3	1	6.8	1
CGPLPA84	68	Adenocarcinoma	Female	Well	3.5	IIb	No	Yes	5.6	1	7.7	1
CGPLPA88	69	Adenocarcinoma	Female	Well	3.0	IIa	No	Yes	16.2	0	16.2	0
CGPLPA89	62	Adenocarcinoma	Female	Moderate	2.7	IIb	No	Yes	15.6	0	15.6	0
CGPLPA90	68	Adenocarcinoma	Male	Moderate	2.4	IIb	No	Yes	15.2	0	15.2	0
CGPLPA13	47	Adenocarcinoma	Male	Well	3.5	III	No	Yes	9.3	1	12.5	1
CGPLPA29	68	Adenocarcinoma	Female	Not Available	2.3	Ib	No	Yes	37.3	0	37.3	0
CGPLPA30	65	Adenocarcinoma	Male	Not Available	2	III	No	Yes	9.6	1	12.9	1
CGPLPA64	74	Adenocarcinoma	Female	Moderate	4	III	No	Yes	23.5	0	23.5	0
CGPLPA66	77	Adenocarcinoma	Female	Moderate	1.6	III	No	Yes	12.8	1	22.8	0
CGPLPA72	72	Adenocarcinoma	Male	Not Available	3	IIb	No	Yes	22.3	0	22.3	0
CGPLPA87	65	Adenocarcinoma	Male	Well	Not Available	Ia	No	Yes	16.6	0	16.6	0

Table 2.3. Summary of next-generation sequencing analyses performed.

Case ID	Sample Type	Read Length	Bases Sequenced	Bases Mapped to Genome	Percent Mapped to Genome	Bases Mapped to Target	Percent Mapped to Target	Targeted bases with at least 10 reads	Targeted bases with at least 10 reads (%)	Average High Quality Total Coverage	Average High Quality Distinct Coverage
CGPLPA14	Tumor	150	1,422,206,700	1,233,948,600	87%	589,739,532	48%	617,026	97%	895.6	785.0
CGPLPA14	Normal	150	1,097,615,400	973,933,050	89%	480,874,737	49%	618,000	97%	734.4	686.3
CGPLPA15	Tumor	150	1,432,918,800	1,138,913,400	79%	633,520,028	56%	613,867	96%	961.4	686.3
CGPLPA15	Normal	150	1,282,901,400	1,158,667,350	90%	591,188,745	51%	619,785	97%	890.3	831.6
CGPLPA16	Tumor	150	1,325,756,700	1,137,578,550	86%	620,235,011	55%	617,943	97%	949.8	173.1
CGPLPA16	Normal	150	1,369,431,000	1,198,075,800	87%	598,680,103	50%	618,967	97%	915.8	794.0
CGPLPA17	Tumor	150	1,273,804,200	1,096,218,600	86%	545,386,390	50%	615,865	96%	829.6	719.8
CGPLPA17	Normal	150	1,156,002,900	1,040,958,300	90%	548,090,254	53%	618,586	97%	830.3	773.2
CGPLPA19	Tumor	150	1,450,699,200	1,294,802,100	89%	720,184,514	56%	620,257	97%	1091.2	213.3
CGPLPA19	Normal	150	923,131,200	818,532,600	89%	419,071,315	51%	618,661	97%	643.2	561.2
CGPLPA20	Tumor	150	1,388,595,600	1,273,161,750	92%	621,750,824	49%	620,316	97%	944.6	892.4
CGPLPA20	Normal	150	1,141,947,900	1,022,130,750	90%	539,339,890	53%	617,010	97%	820.4	771.2
CGPLPA23	Tumor	150	1,276,030,800	1,004,910,000	79%	538,518,453	54%	613,081	96%	820.5	434.5
CGPLPA23	Normal	150	1,168,223,400	1,044,452,250	89%	527,833,659	51%	616,857	97%	805.1	748.4
CGPLPA24	Tumor	150	1,368,000,000	1,250,000,000	91%	600,317,082	48%	620,384	97%	907.4	687.1
CGPLPA25	Tumor	150	1,325,500,800	1,144,549,650	86%	619,895,244	54%	616,688	97%	944.2	842.4
CGPLPA25	Normal	150	1,102,142,100	1,005,024,000	91%	514,688,345	51%	618,797	97%	786.7	723.9
CGPLPA26	Tumor	150	763,197,900	660,287,550	87%	339,718,956	51%	612,229	96%	493.2	456.5
CGPLPA26	Normal	150	779,267,700	697,112,550	89%	360,672,012	52%	614,646	96%	524.5	488.2
CGPLPA27	Tumor	150	846,427,500	741,015,150	88%	375,084,381	51%	613,466	96%	549.8	512.7
CGPLPA27	Normal	150	780,752,400	703,056,450	90%	365,704,778	52%	616,224	97%	525.9	487.0
CGPLPA28	Tumor	150	977,580,300	878,913,000	90%	466,375,081	53%	616,159	97%	716.6	666.7
CGPLPA28	Normal	150	963,573,000	876,802,800	91%	448,269,780	51%	617,579	97%	688.3	638.6
CGPLPA31	Tumor	150	1,442,430,000	1,231,364,250	85%	636,015,051	52%	616,795	97%	953.0	850.1
CGPLPA31	Normal	150	1,673,482,800	1,311,890,400	78%	710,364,927	54%	616,529	97%	1084.8	890.7
CGPLPA33	Tumor	150	1,733,820,600	1,293,419,550	75%	755,071,067	58%	611,022	96%	1133.3	683.7
CGPLPA33	Normal	150	1,388,404,500	1,122,970,800	81%	580,438,755	52%	618,657	97%	871.2	739.5
CGPLPA34	Tumor	150	1,252,716,900	1,166,142,900	93%	577,954,111	50%	621,622	97%	869.8	781.0
CGPLPA35	Tumor	150	1,448,322,600	1,209,805,800	84%	614,782,917	51%	616,478	97%	924.0	828.0
CGPLPA35	Normal	150	1,523,119,500	1,155,712,500	76%	619,509,476	54%	614,486	96%	931.7	714.1
CGPLPA36	Tumor	150	1,595,898,600	1,396,011,150	87%	767,902,754	55%	618,417	97%	1177.3	1013.7

CGPLPA36	Normal	150	1,368,051,000	1,073,128,650	78%	572,338,961	53%	614,464	96%	864.0	722.0
CGPLPA37	Tumor	150	1,389,140,100	1,192,749,600	86%	637,329,732	53%	617,222	97%	964.5	840.8
CGPLPA37	Normal	150	1,403,295,300	1,073,562,150	77%	586,179,382	55%	613,935	96%	884.8	727.5
CGPLPA39	Tumor	150	1,130,873,700	1,031,655,750	91%	521,195,716	51%	619,367	97%	789.1	718.5
CGPLPA39	Normal	150	1,353,234,300	1,103,716,200	82%	596,625,068	54%	618,965	97%	904.6	756.4
CGPLPA40	Tumor	150	1,486,219,500	1,320,968,850	89%	713,934,786	54%	618,452	97%	1075.4	954.4
CGPLPA40	Normal	150	1,274,490,300	981,103,350	77%	530,900,815	54%	614,055	96%	802.3	712.5
CGPLPA41	Tumor	150	1,420,233,000	1,195,861,200	84%	687,813,722	58%	616,947	97%	1039.9	873.1
CGPLPA41	Normal	150	1,485,501,600	1,153,747,650	78%	634,839,180	55%	615,599	96%	964.9	809.8
CGPLPA42	Tumor	150	1,381,733,100	1,160,990,100	84%	659,950,398	57%	614,881	96%	1002.3	754.5
CGPLPA42	Normal	150	1,455,513,000	1,065,667,950	73%	576,250,237	54%	614,823	96%	876.2	761.7
CGPLPA43	Tumor	150	1,305,021,900	1,163,152,950	89%	612,966,256	53%	617,406	97%	930.0	844.1
CGPLPA43	Normal	150	1,348,954,500	1,007,244,000	75%	548,707,683	54%	615,198	96%	833.2	724.1
CGPLPA45	Tumor	150	1,207,611,000	1,065,541,950	88%	566,123,421	53%	618,594	97%	862.2	680.9
CGPLPA45	Normal	150	1,133,718,900	1,042,314,150	92%	502,563,596	48%	622,745	98%	763.3	667.7
CGPLPA50	Tumor	150	892,917,600	774,671,400	87%	405,099,468	52%	611,192	96%	621.8	443.2
CGPLPA50	Normal	150	1,114,662,000	1,022,400,300	92%	516,589,764	51%	619,687	97%	785.5	698.6
CGPLPA51	Tumor	150	1,310,000,000	1,120,000,000	86%	587,000,000	52%	617,018	97%	890.8	663.3
CGPLPA51	Normal	150	1,460,000,000	1,330,000,000	91%	711,000,000	53%	619,485	97%	1080.8	899.4
CGPLPA55	Tumor	150	1,258,117,500	1,090,245,750	87%	571,086,668	52%	616,173	97%	876.4	706.6
CGPLPA55	Normal	150	1,080,838,200	963,629,250	89%	498,882,825	52%	618,398	97%	754.6	592.9
CGPLPA56	Tumor	150	1,139,238,600	1,027,636,650	90%	546,588,989	53%	617,006	97%	832.9	681.0
CGPLPA56	Normal	150	1,163,296,800	1,055,723,850	91%	577,632,226	55%	619,354	97%	886.8	821.1
CGPLPA57	Tumor	150	1,421,929,500	1,279,022,100	90%	671,418,966	52%	617,267	97%	1023.7	769.9
CGPLPA57	Normal	150	1,490,680,200	1,335,699,600	90%	686,759,055	51%	618,341	97%	1048.1	726.3
CGPLPA60	Tumor	150	1,064,281,500	936,046,050	88%	497,472,976	53%	619,076	97%	763.5	600.7
CGPLPA60	Normal	150	961,133,100	890,114,250	93%	467,206,901	52%	620,430	97%	716.2	637.3
CGPLPA63	Tumor	150	1,440,349,800	1,254,282,000	87%	685,755,803	55%	616,687	97%	1037.0	526.4
CGPLPA63	Normal	150	1,053,303,000	975,994,500	93%	511,180,718	52%	620,515	97%	783.6	733.5
CGPLPA65	Tumor	150	998,749,200	890,638,950	89%	457,905,966	51%	615,244	96%	698.4	575.7
CGPLPA65	Normal	150	1,444,104,900	1,270,918,950	88%	636,112,669	50%	618,303	97%	958.5	725.9
CGPLPA73	Tumor	150	1,482,431,100	1,216,092,300	82%	724,672,761	60%	614,615	96%	1101.7	597.7
CGPLPA73	Normal	150	1,122,234,600	1,040,857,200	93%	539,267,848	52%	621,805	97%	817.7	750.3
CGPLPA75	Tumor	150	1,361,733,000	1,180,700,850	87%	638,268,594	54%	616,074	96%	968.0	754.7
CGPLPA75	Normal	150	1,247,388,300	1,159,974,300	93%	655,366,609	56%	619,155	97%	995.9	903.8
CGPLPA77	Tumor	150	1,293,679,200	1,144,525,200	88%	604,144,412	53%	617,003	97%	920.6	773.1
CGPLPA77	Normal	150	1,135,085,100	1,068,456,000	94%	596,796,267	56%	619,086	97%	907.8	826.4
CGPLPA78	Tumor	150	1,414,849,200	1,197,070,800	85%	654,034,434	55%	616,298	97%	995.4	683.9

CGPLPA78	Normal	150	1,039,368,600	973,212,750	94%	527,655,278	54%	620,350	97%	803.4	746.2
CGPLPA80	Tumor	150	1,249,176,000	976,683,900	78%	545,997,777	56%	611,837	96%	833.0	611.8
CGPLPA80	Normal	150	1,245,922,200	1,152,128,700	92%	584,937,511	51%	619,538	97%	891.4	789.1
CGPLPA81	Tumor	150	1,094,793,600	983,800,800	90%	497,918,684	51%	619,055	97%	759.3	675.8
CGPLPA81	Normal	150	1,148,752,800	1,063,345,050	93%	548,237,313	52%	618,682	97%	838.0	755.5
CGPLPA82	Tumor	150	1,030,927,800	841,387,800	82%	466,002,645	55%	613,502	96%	713.0	532.9
CGPLPA82	Normal	150	1,085,075,700	996,816,600	92%	510,624,495	51%	618,557	97%	780.9	708.3
CGPLPA83	Tumor	150	1,185,284,700	1,010,383,650	85%	549,141,680	54%	615,386	96%	839.5	586.9
CGPLPA83	Normal	150	1,100,280,600	1,023,636,450	93%	517,833,703	51%	618,434	97%	791.8	714.4
CGPLPA84	Tumor	150	1,476,325,800	1,336,581,750	91%	664,798,233	50%	619,904	97%	1013.2	887.2
CGPLPA84	Normal	150	1,283,497,500	1,126,293,600	88%	394,197,633	35%	615,972	96%	603.1	541.5
CGPLPA88	Tumor	150	1,167,415,500	1,066,878,300	91%	554,605,027	52%	620,645	97%	843.2	739.0
CGPLPA88	Normal	150	1,864,864,200	1,635,930,750	88%	567,045,390	35%	619,465	97%	865.7	754.5
CGPLPA89	Tumor	150	1,162,636,800	1,028,794,050	88%	541,294,076	53%	617,290	97%	822.5	722.2
CGPLPA89	Normal	150	1,439,229,900	1,342,554,750	93%	697,132,331	52%	621,249	97%	1054.7	916.8
CGPLPA90	Tumor	150	1,197,470,100	1,049,859,000	88%	568,299,409	54%	616,559	97%	860.9	758.2
CGPLPA90	Normal	150	1,113,039,600	1,037,476,050	93%	565,283,810	54%	620,102	97%	856.7	757.5

Table 2.4. Summary of pancreatic ductal adenoma cases analyzed with digital PCR pre-surgery

Case Data		Pre-Surgery						CT Imaging		
Case ID	Mutation Analyzed	Date of Blood Draw	Volume of Plasma (ml)	Total Genomic Equivalents Analyzed	Wild Type Genomic Equivalents	Mutant Genomic Equivalents	Mutant Allele Fraction (%)	Date of Scan	CT Scan (Months from Surgery)	Result of Scan (1 - Positive, 0 - Negative)
CGPLPA13	KRAS G12V	1/21/2011	4.0	2,322	2,320	2	0.07%	10/28/2011	9.3	1
CGPLPA14	KRAS G12V	1/27/2011	4.0	3,202	3,200	2	0.05%	8/16/2011	6.7	1
CGPLPA15	KRAS G12D	3/2/2011	4.0	3,370	3,360	10	0.28%	9/25/2011	6.9	1
CGPLPA16	KRAS G12D	3/22/2011	4.0	58,177	58,160	17	0.03%	9/8/2011	5.7	1
CGPLPA17	KRAS G12I	5/4/2011	4.0	19,825	19,820	5	0.02%	3/6/2012	10.2	1
CGPLPA19*	KRAS G12R	5/31/2011	4.0	2,544	2,542	2	0.08%	12/25/2011	6.9	1
CGPLPA20	KRAS G12R	6/27/2011	4.0	2,344	2,340	4	0.16%	4/26/2012	10.1	1
CGPLPA23	KRAS G12D	8/30/2011	4.0	30,032	30,020	12	0.04%	1/4/2012	4.2	1
CGPLPA24	KRAS G12D	9/7/2011	4.0	5,486	5,486	Not Detected	N/A	9/2/2012	12.0	1
CGPLPA25	KRAS G12D	9/15/2011	4.0	9,627	9,620	7	0.08%	1/29/2012	4.5	1
CGPLPA26	KRAS G12R	9/28/2011	4.0	7,580	7,580	Not Detected	N/A	11/20/2011	1.8	1
CGPLPA27	KRAS G12D	10/6/2011	4.0	30,802	30,800	2	0.02%	12/20/2011	2.5	1
CGPLPA28	KRAS G12V	10/27/2011	4.0	15,584	15,580	4	0.00%	2/4/2013	15.5	1
CGPLPA29	KRAS G12V	11/9/2011	4.0	4,816	4,816	Not Detected	N/A	12/2/2014	37.3	0
CGPLPA30	KRAS G12D	11/11/2011	4.0	14,560	14,560	Not Detected	N/A	8/26/2012	9.6	1
CGPLPA31	KRAS G12D	11/15/2011	4.0	2,604	2,604	Not Detected	N/A	5/19/2013	18.4	1
CGPLPA33	KRAS G12R	12/8/2011	4.0	2,120	2,120	Not Detected	N/A	5/13/2012	5.2	1
CGPLPA34	KRAS G12C	12/29/2011	4.0	2,882	2,880	2	0.07%	6/5/2012	5.3	1
CGPLPA35	KRAS G12D	1/2/2012	4.0	9,360	9,360	Not Detected	N/A	9/2/2012	8.1	1
CGPLPA36	KRAS G12D	1/12/2012	4.0	10,564	10,560	4	0.07%	12/2/2014	35.2	0
CGPLPA37	KRAS G12D	2/6/2012	4.0	6,266	6,260	6	0.09%	6/14/2012	4.3	1
CGPLPA39	KRAS G12S	2/23/2012	4.0	8,022	8,020	2	0.00%	12/2/2014	33.8	0
CGPLPA40	KRAS G12V	2/29/2012	4.0	4,311	4,300	11	0.26%	4/28/2013	14.1	1
CGPLPA41	KRAS G12D	3/21/2012	4.0	10,840	10,840	Not Detected	N/A	10/30/2012	7.4	1
CGPLPA42	KRAS G13D	4/2/2012	4.0	2,840	2,840	Not Detected	N/A	2/18/2013	10.7	1
CGPLPA43	KRAS G12R	4/11/2012	4.0	1,522	1,520	2	0.13%	6/23/2014	26.8	1
CGPLPA45	KRAS G12D	6/6/2012	4.0	20,423	20,420	Not Detected	N/A	8/31/2014	27.2	0
CGPLPA50	KRAS G12V	7/6/2012	3.0	10,465	10,458	7	0.07%	5/11/2013	10.3	1
CGPLPA51	KRAS G12V	7/9/2012	4.0	10,962	10,960	Not Detected	N/A	12/2/2014	29.2	0
CGPLPA55	KRAS G12V	10/22/2012	4.0	7,875	7,866	9	0.11%	6/12/2013	7.8	1

CGPLPA56	KRAS G12R	11/5/2012	4.0	6,434	6,434	Not Detected	N/A	2/5/2014	15.2	1
CGPLPA57	KRAS G12V	11/7/2012	4.0	15,384	15,380	4	0.00%	12/2/2014	25.2	0
CGPLPA60	KRAS G12V	12/3/2012	2.2	1,252	1,252	Not Detected	N/A	11/3/2013	11.2	1
CGPLPA63	KRAS G12R	12/21/2012	3.0	2,920	2,920	Not Detected	N/A	1/25/2014	13.3	1
CGPLPA64	KRAS G13D	12/27/2012	2.5	1,096	1,096	Not Detected	N/A	12/2/2014	23.5	0
CGPLPA65	KRAS G12R	1/2/2013	2.5	8,764	8,748	16	0.18%	9/1/2013	8.1	1
CGPLPA66	KRAS G12D	1/17/2013	2.5	7,516	7,516	Not Detected	N/A	2/6/2014	12.8	1
CGPLPA72	KRAS G12D	1/31/2013	3.0	3,930	3,920	10	0.25%	12/2/2014	22.3	0
CGPLPA73	KRAS G12D	2/5/2013	2.5	4,982	4,980	2	0.00%	7/14/2013	5.3	1
CGPLPA75	KRAS G12D	2/19/2013	3.0	3,543	3,540	3	0.10%	8/1/2013	5.4	1
CGPLPA77	KRAS G12R	2/28/2013	2.2	1,946	1,944	2	0.08%	12/2/2014	21.4	0
CGPLPA78	KRAS G12V	3/19/2013	3.2	8,020	8,020	Not Detected	N/A	12/2/2014	20.8	0
CGPLPA80	PIK3CA E542K	3/25/2013	2.7	1,636	1,636	Not Detected	N/A	12/2/2014	20.6	0
CGPLPA81	KRAS G12D	4/2/2013	3.0	4,260	4,238	22	0.52%	12/5/2013	8.2	1
CGPLPA82	KRAS G12D	4/23/2013	3.0	2,182	2,182	Not Detected	N/A	8/21/2013	4.0	1
CGPLPA83	KRAS G12D	5/14/2013	2.5	2,847	2,840	7	0.25%	11/19/2013	6.3	1
CGPLPA84	KRAS G12V	6/3/2013	3.0	4,110	4,110	Not Detected	N/A	11/19/2013	5.6	1
CGPLPA87	KRAS G13D	7/23/2013	2.5	1,980	1,980	Not Detected	N/A	12/2/2014	16.6	0
CGPLPA88	BRAF V600E	8/2/2013	2.5	1,128	1,128	Not Detected	N/A	12/2/2014	16.2	0
CGPLPA89	KRAS G12V	8/21/2013	3.2	24,062	24,060	2	0.00%	12/2/2014	15.6	0
CGPLPA90	KRAS G12R	9/3/2013	2.5	5,260	5,260	Not Detected	N/A	12/2/2014	15.2	0

*CT imaging of this patient did not identify tumor recurrence, although this patient later died of disease and is indicated as having a recurrence at the time of death

Table 2.5. Summary of pancreatic ductal adenoma cases analyzed with digital PCR post-surgery.

Case Data		Post-Surgery						
Case ID	**Mutation Analyzed**	**Date of Blood Draw**	**Blood Draw (Months from Surgery)**	**Volume of Plasma (ml)**	**Total Genomic Equivalents Analyzed**	**Wild Type Genomic Equivalents**	**Mutant Genomic Equivalents**	**Mutant Allele Fraction (%)**
CGPLPA14	KRAS G12V	4/6/2011	2.3	4	6,776	6,776	Not Detected	N/A
CGPLPA16	KRAS G12D	6/1/2011	2.4	4	6,805	6,800	5	0.07%
CGPLPA20	KRAS G12R	10/11/2011	3.5	3.5	9,990	9,988	2	0.02%
CGPLPA24	KRAS G12D	10/19/2011	1.4	1.5	810	810	Not Detected	N/A
CGPLPA29	KRAS G12V	1/30/2012	2.7	1.5	1,648	1,648	Not Detected	N/A
CGPLPA30	KRAS G12D	12/30/2011	1.6	3.7	6,193	6,190	3	0.05%
CGPLPA31	KRAS G12D	6/7/2012	6.8	4	10,880	10,874	6	0.05%
CGPLPA33	KRAS G12R	2/15/2012	2.3	4	3,925	3,922	3	0.08%
CGPLPA34	KRAS G12C	2/22/2012	1.8	4	6,786	6,784	2	0.03%
CGPLPA36	KRAS G12D	2/10/2012	1.0	3.5	6,065	6,060	5	0.09%
CGPLPA39	KRAS G12S	4/20/2012	1.9	3.5	1,984	1,984	Not Detected	N/A
CGPLPA51	KRAS G12V	11/13/2012	4.2	4	16,722	16,720	2	0.01%
CGPLPA57	KRAS G12V	4/3/2013	4.9	4	5,744	5,742	2	0.03%
CGPLPA60	KRAS G12V	3/26/2013	3.8	3	4,125	4,122	3	0.06%
CGPLPA63	KRAS G12R	2/7/2013	1.6	4	4,618	4,616	2	0.04%
CGPLPA64	KRAS G13D	4/12/2013	3.5	4	9,202	9,200	2	0.03%
CGPLPA65	KRAS G12R	4/29/2013	3.9	3.5	6,198	6,196	2	0.03%
CGPLPA66	KRAS G12D	4/12/2013	2.8	4	3,922	3,920	Not Detected	N/A
CGPLPA77	KRAS G12R	5/3/2013	2.1	3	9,638	9,638	Not Detected	N/A
CGPLPA81	KRAS G12D	5/28/2013	1.9	3.5	4,914	4,912	Not Detected	N/A

CHAPTER 3:

DIRECT DETECTION OF EARLY-STAGE CANCERS USING CIRCULATING TUMOR DNA

METHODS

Study design

This study presents a retrospective analysis of cf DNA using an ultrasensitive sequencing and analysis platform to detect somatic sequence alterations in early-stage cancers. We analyzed 250 plasma samples from 244 individuals, including 44 healthy individuals and 200 patients with colorectal (n = 42), lung (n = 71), ovarian (n = 42), or breast (n =45) cancer over a range of stages, with most patients exhibiting localized disease. We estimated that analysis of at least 42 patients for each tumor type would provide a 96% power to detect 50% of cases with a 95% CI of 35 to 65%. We evaluated the sensitivity and specificity of the TEC-Seq method to detect ctDNA in early-stage patients without previous knowledge of alterations in their tumors. We detected sequence alterations in hematopoietic expansion genes in healthy individuals, established the sensitivity of the approach for detecting tumor-specific alterations in the blood of cancer patients, evaluated concordance be- tween alterations identified in cf DNA and tumor samples from the same patients, and assessed whether preoperative ctDNA can serve as a marker of patient outcome.

Patient and sample characteristics

Plasma samples from healthy individuals and plasma and tissue samples from patients with breast, lung, ovarian, and colorectal cancers were obtained from ILSBio/Bioreclamation, Aarhus University, the Academic Medical Center of the University of Amsterdam, and University of

California, San Diego. All samples were obtained under Institutional Review Board–approved protocols with informed consent for research use at participating institutions.

Plasma samples from healthy individuals were obtained at the time of routine screening, including for colonoscopies or Pap smears. Individuals were considered healthy if they had no previous history of cancer and negative screening results. Plasma samples from individuals with colorectal, lung, ovarian, or breast cancer were obtained at the time of diagnosis, before tumor resection. Serially collected plasma samples from lung cancer patients were collected over a course of treatment during which the patients experienced stable or progressive disease. Matched formalin-fixed, paraffin-embedded (FFPE) or frozen tumor tissue and buffy coat (as a source of germline DNA) were obtained from patients whenever available. Tumor specimens were obtained from primary resection, with the exception of stage IV colorectal cancer patients with liver-only metastases, for whom the samples were obtained from the liver metastases. All tumor samples had ≥10% viable tumor cell content by histopathologic assessment. Clinical data for all patients included and sample data for the tissue types assayed in this study are listed in Tables 3.1 and 3.2.

Sample preparation and NGS of cfDNA

Whole blood was collected in EDTA tubes and processed immediately or within 2 hours after storage at 4°C to separate plasma and cellular components by centrifugation at 800g for 10 min at 4°C. Plasma was centrifuged a second time at 18,000g at room temperature to remove any remaining cellular debris and stored at −80°C until the time of DNA extraction. DNA was isolated from plasma using the Qiagen Circulating Nucleic Acids Kit (Qiagen GmbH) and eluted

in LoBind tubes (Eppendorf AG). Concentration and quality of cf DNA were assessed using the Bioanalyzer 2100 (Agilent Technologies).

TEC-Seq NGS cf DNA libraries were prepared from 5 to 250 ng of cf DNA. Genomic libraries were prepared using the NEBNext DNA Library Prep Kit for Illumina [New England Biolabs (NEB)] with four main modifications to the manufacturer's guidelines: (i) The library purification steps used the on-bead AMPure XP approach to minimize sample loss during elution and tube transfer steps (38); (ii) NEBNext End Repair, A-tailing, and adapter ligation enzyme and buffer volumes were adjusted as appropriate to accommodate the on-bead AMPure XP purification strategy; (iii) a pool of eight unique Illumina dual index adapters with 8–base pair (bp) barcodes was used in the ligation reaction instead of the standard Illumina single or dual index adapters with 6- or 8-bp barcodes, respectively; and (iv) cf DNA libraries were amplified with Phusion Hot Start Polymerase. Incorporation of these modifications improved conversion efficiency from 13.4% before modifications to 34.1% in validation analyses of 38 cases incorporating these changes. Analysis of plasma samples from healthy individuals and cancer patients revealed a conversion efficiency of 40%, with a significant correlation between input DNA amount and the number of distinct molecules analyzed (Pearson correlation $r = 0.55$; 95% CI, 0.46 to 0.64; $P < 0.0001$; Figure 3.1).

Briefly, cf DNA was combined with End Repair Reaction Buffer (NEB) and End Repair Enzyme Mix (NEB) and incubated for 30 min at 20°C. The end-repair reaction was purified with Agencourt AMPure XP Beads (Beckman Coulter). A-tailing was performed by adding 6 ml of dA-Tailing Reaction Buffer (NEB) and 3.6 ml of Klenow (NEB) to the end-repaired cf DNA and incubating for 30 min at 37°C. A-tailed cf DNA was purified using Agencourt AMPure XP Buffer (Beckman Coulter). Adaptor oligonucleotides containing the TEC-Seq dual index pools and Quick T4 DNA Ligase (NEB) were mixed with A-tailed, on-bead cf DNA and incubated for

15 min at 20°C. Ligated cf DNA was purified with two rounds of Agencourt AMPure XP Buffer. The cf DNA library was amplified using Phusion Hot Start DNA polymerase (Thermo Fisher Scientific) and PCR primers published for the Nextera DNA Library Prep Kit: 5′-AATGATACGGCGACCACCGA-3′ and 5′-CAAGCAGAAGACGGCATACGA-3′ (Illumina Inc.). For each genomic library, PCRs contained 2 ml of cf DNA library, 15.5 ml of H2O,

1.25 ml of dimethyl sulfoxide, 5.0 ml of 5X Phusion HF Buffer, 0.5 ml of deoxynucleoside triphosphate (dNTP) mix containing 10 mM each dNTP (Life Technologies), 0.5 ml of each primer, and 0.25 ml of Phusion Hot Start Polymerase. The following PCR conditions were used: 98°C for 30 s; 12 cycles of 98°C for 10 s, 60°C for 30 s, and 72°C for 30 s; and 72°C for 5 min. Purification of the amplified cf DNA library was per- formed using Agencourt AMPure XP Beads. Concentration and quality of cf DNA libraries were assessed using the Bioanalyzer 2100 (Agilent Technologies).

Targeted capture was performed using the Agilent SureSelect reagents and a custom set of hybridization probes targeting 58 genes (Table 3.3) per the manufacturer's guidelines. The captured library was amplified with Phusion Hot Start Polymerase (NEB). The concentration and quality of captured cf DNA libraries were assessed on the Bioanalyzer 2100 using the DNA 1000 Kit (Agilent Technologies). TEC-Seq libraries were sequenced using 100-bp paired-end runs on the Illumina HiSeq 2000/2500 (Illumina).

Sample preparation and NGS of tumor-normal pairs

Sample preparation, library construction, targeted capture, NGS, and bioinformatic analyses of tumor and normal samples were performed as previously described (36, 37). Briefly, DNA was extracted from matched FFPE or frozen tumor tissue and buffy coat samples using the Qiagen DNA FFPE Tissue Kit or Qiagen DNA Blood Mini Kit (Qiagen GmbH). Genomic DNA from tumor and normal samples was fragmented and used for Illumina TruSeq library construction (Illumina) as previously described (36, 37). Targeted regions of interest were captured using Agilent SureSelect in-solution capture reagents and a custom-targeted panel for genes of interest according to the manufacturer's instructions (Agilent). Paired-end sequencing, resulting in 150 bases from each end of the fragment for targeted libraries, was performed using Illumina MiSeq (Illumina).

Analyses of NGS data from cfDNA

Primary processing of NGS data for cf DNA samples was performed using Illumina CASAVA (Consensus Assessment of Sequence and Variation) software (version 1.8), including demultiplexing and masking of dual-index adapter sequences. Sequence reads were aligned against the human reference genome (version hg18 or hg19) using NovoAlign with additional realignment of select regions using the Needleman- Wunsch method (37). The positions of the alterations we have identified have not been affected by the different genome builds.

Next, candidate somatic mutations, consisting of point mutations, small insertions, and deletions, were identified using VariantDx (37) across the targeted regions of interest. VariantDx examined

sequence alignments of cf DNA plasma samples while applying filters to exclude alignment and sequencing artifacts. Specifically, an alignment filter was applied to exclude quality-failed reads, unpaired reads, and poorly mapped reads in the plasma. A base quality filter was applied to only include bases with a reported Phred quality score >30.

A mutation identified in cf DNA was considered a candidate somatic mutation only when (i) three distinct paired reads contained the mutation in the plasma (each redundantly sequenced at least three times) with a distribution of start and cycle positions when compared to the reference genome, and the number of distinct paired reads containing a particular mutation in the plasma was at least 0.1% of the total distinct read pairs; or (ii) four distinct paired reads contained the mutation in the plasma (each redundantly sequenced at least four times) with a distribution of start and cycle positions when compared to the reference genome, and the number of distinct paired reads containing a particular mutation in the plasma was at least 0.05% and less than 0.1% of the total distinct read pairs; and (iii) the mismatched base was not present in >1% of the reads in a panel of unmatched normal samples and not present in a custom database of common germline variants derived from dbSNP (The Single Nucleotide Polymorphism Database).

Mutations arising from misplaced genome alignments, including paralogous sequences, were identified and excluded by searching the reference genome. Candidate somatic mutations were further filtered on the basis of gene annotation to identify those occurring in protein- coding regions. Functional consequences were predicted using snpEff and a custom database of CCDS (Consensus Coding Sequence), RefSeq, and Ensembl annotations using the latest transcript versions available on hg18 and hg19 from the University of California, Santa Cruz (https://genome.ucsc.edu/). Predictions were ordered to prefer transcripts with canonical start and stop codons and CCDS or RefSeq transcripts over Ensembl when available. Finally, mutations

were filtered to exclude intronic and silent changes, while retaining mutations resulting in missense mutations, nonsense mutations, frameshifts, or splice site alterations.

Candidate alterations were defined as somatic hotspots if the nucleotide change and amino acid change were identical to an alteration observed in ≥20 cancer cases reported in the COSMIC database. Alterations that were not hotspots were retained only if either (i) seven or more distinct paired reads contained the mutation in the plasma, and the number of distinct paired reads containing a particular mutation in the plasma was at least 0.1% and less than 0.2% of the total distinct read pairs, or (ii) six or more distinct paired reads contained the mutation in the plasma, and the number of distinct paired reads containing a particular mutation in the plasma was at least 0.2% of the total distinct read pairs.

Candidate mutations were further limited through identification and removal of common germline variants present in ≥25% of reads or <25% of reads if the variant was recurrent and most of the alterations at that position had a mutant allele fraction of ≥25%. Variants known to be at a somatic hotspot position or producing a truncating mutation in a tumor suppressor gene were not excluded as germline changes. Because of the high frequency of mutations in specific genes and the possible confounding between somatic and germline changes, we limited analyses in the APC gene to frameshift or nonsense mutations and in KRAS, HRAS, and NRAS to positions 12, 13, 61, and 146. Finally, we excluded hematopoietic expansion-related variants that have been previously described, including those in DNMT3A, IDH1, and IDH2 and specific alterations within ATM (residue 3008), GNAS (residue 202), or JAK2 (residue 617) (39-41).

To evaluate the sensitivity of the TEC-Seq approach using dilutions of cell lines with known mutations, we used a mixture of cell lines obtained from American Type Culture Collection and combined in ratios to reflect the mutant allele frequency. The cell lines in the mutant pool

included CCL-237, CRL-2158, CRL-2547, CRL-7585, CRL-9068, CRL-2177, CCL-231, CRL-2871, CRL-5908, CRL-5908, CCL-224, and CRL-5894. To evaluate sensitivity and specificity, we used dilutions of a cell line (CGBR4C, CRL-2338), which had been previously sequenced to examine both mutant and wild-type bases in the 58 genes in our panel (42). For analyses at all dilutions, we considered those alterations where the mutant allele fraction was expected to be at 0.1% or higher. To calculate the per-base error rate for conventional sequencing in samples from healthy individuals, we summed the number of false-positive calls at each genomic position and divided this by the total coverage at that base for the 44 healthy individuals. The upper limit of the per-base error rate of TEC-Seq was determined by assuming one alteration per base if no error was identified and dividing by the total coverage at each base for the 44 healthy individuals analyzed.

To compare the TEC-Seq bioinformatic approach to iDES-enhanced CAPP-Seq (cancer personalized profiling by deep sequencing), we used the bioinformatic components of iDES combined with the requirement of multiple distinct read families based on endogenous and exogenous barcodes (10, 11) (https://cappseq.stanford.edu/ides/).

Analyses of NGS data from tumor-normal pairs

Primary processing of NGS data from tumor-normal pairs and identification of putative somatic mutations were completed using Illumina CASAVA software (version 1.8) and VariantDx custom software, respectively, as previously described (37).

Statistical analyses

We used a variety of methods for determining significance. To test the linear association between expected and observed mutant allele fractions (Figure 3.2), we used Pearson's product moment correlation coefficient. To quantify the difference in mean error rate by genomic position for conventional sequencing and TEC-Seq, we used a paired (by genomic position) t test assuming equal variances. Differences in means of unpaired (independent) samples were tested using a two-sample t test assuming equal variances (such as for comparisons involving the concentration of cf DNA in plasma between healthy and cancer populations). To assess whether high mutant allele fractions are associated with patient outcomes, we defined patients with high mutant allele fractions as those with values >3 median absolute deviations from the median mutant allele fraction observed in 31 colorectal cancer patients analyzed. We used a median absolute deviation rather than an SD because the mutant allele fractions were skewed, and the median absolute deviation provides a more robust-to-outlier measure of the SD. We compared PFS and OS between patients with low and high mutant allele fraction using the log-rank test in univariate analyses and the Cox proportional hazards in multivariate analyses (43, 44).

RESULTS

Targeted error correction sequencing

We developed a methodology for comprehensive analysis of sequence alterations in driver genes that are commonly mutated in colorectal, lung, ovarian, breast, and other cancers. Similar to targeted analyses of cancer tissues (37), we first selected genes that were frequently mutated in these tumors and focused our analyses on either the entire coding regions or the most highly mutated exons of these genes. An analysis of the frequency of these alterations in the COSMIC database of somatic mutations in cancer (45) revealed that more than three quarters of patients would be expected to have at least one mutation in 55 genes among the intended cancers as well as other common tumor types (Tables 3.1 and 3.4). We hypothesized that a larger panel of genes would increase the probability of detecting at least one gene alteration in the plasma from any given cancer patient. Because alterations in the blood have previously been reported in healthy individuals, we examined three additional genes as well as specific sequence positions in three genes of the 55-gene panel (Table 3.3) that were known to be somatically altered in clonal hematopoietic expansion, myelodysplasia, or other hematological malignancies (39-41).

Detection of sequence alterations using conventional NGS is limited to a relatively high fraction of mutant to wild-type DNA (>1%) and, as such, is typically not useful for analyses of ctDNA, which may be present in minute amounts in the blood. Although methods have been developed for analysis of ctDNA in late-stage cancer patients (4-12), no method has been systematically applied for analysis of early-stage disease. We developed a custom capture and sequencing approach called targeted error correction sequencing (TEC-Seq) to allow sensitive and specific

detection of low-abundance sequence alterations using NGS (Figure 3.3). This methodology is based on targeted capture of multiple regions of the genome and deep sequencing (~30,000×) of DNA fragments. The 58 genes analyzed in this study comprised 80,930 aptured bases. Specific steps were performed for analysis of rare tumor-specific alterations in DNA molecules and for elimination of potential amplification, sequencing, and contamination errors as well as other sources of alterations in the blood. These included (i) optimizing library generation and capture for conversion of cf DNA for subsequent analyses; (ii) maximizing representation of unique cf DNA molecules analyzed using mapping positions and a small number of prespecified barcodes; (iii) redundant sequencing, where multiple identical DNA molecules are generated and sequenced and any sequence changes are reconciled; (iv) filtering of mapping and sequencing artifacts; and (v) identifying and removing germline and hematopoietic cell proliferation alterations.

Conceptually, the number of genome equivalents analyzed provides a lower limit of detection for any genomic analysis. A high sensitivity approach would aim to maximize the number of unique molecules assessed while allowing for a broad and facile analysis in a range that is above the actual number of fragments present in a biologic sample. We optimized methodologies for extraction and conversion of cf DNA to genomic libraries. Initially, we considered using the start and end genome mapping positions of paired-end sequenced fragments as “endogenous barcodes” to distinguish between individual molecules. However, Monte Carlo simulations suggested that the tight size distribution of cf DNA molecules observed in the plasma would result in a smaller number of possible end mapping combinations and therefore underestimate the true complexity of cf DNA in the circulation (Figure 3.4). To extend the complexity of endogenous barcodes, we introduced a limited set of sequence indices as “exogenous barcodes” in the initial steps of library generation. Kinde et al. (46) reported the use of a large number of random exogenous barcodes as unique identifiers for analysis of rare mutations in DNA populations. However, simulations with

a relatively small number of long prespecified exogenous barcodes (n=4-16) suggested that these, in combination with endogenous barcodes, would be sufficient to distinguish among different cf DNA molecules in the plasma from a typical blood draw (Figure 3.4). Extending the number of barcodes substantially beyond this number has the theoretical disadvantage of misassignment among barcodes through sequencing errors and of primer dimers that can form during library formation.

We first evaluated the characteristics of the TEC-Seq approach for detecting known tumor-specific alterations from a mixture of DNA from tumor cell lines at different dilutions (ranging from 100 to 0.1%) with unrelated wild-type DNA. Libraries with eight exogenous barcodes were sequenced with an average of ~32,224 sequence reads at each position among the 58 genes analyzed (Tables 3.5 and 3.6). We designed thresholds that were expected to identify >99% of alterations with a mutant allele fraction of 0.5% at the anticipated sequencing depth. Alterations were considered if they were present in all copies of multiple sequences of each DNA molecule with identical endogenous and exogenous barcodes and were not removed by additional error filtering steps. Hotspot alterations at positions previously observed to be frequently altered in cancer patients were evaluated with more sensitive thresholds because the a priori probability that these alterations were tumor-derived is higher than that of other alterations. Alterations present in common germline variant databases or in 25% or greater of reads were considered germline and removed from further analysis, unless the mutations were identical to known hotspot alterations or represented truncating mutations in common tumor suppressor genes. Analysis of the altered positions in the dilution samples revealed high concordance to the expected fraction of mutant molecules ($r = 0.93$; $P < 0.0001$, Pearson correlation; Figure 3.2 and Tables 3.5 and 3.6), as well as high sensitivity and specificity. The analytical sensitivity was 97.4% overall and 100 and 89% for detecting mutations present at 0.2 and 0.1%, respectively, using minimum thresholds of

0.05% in hotspot positions and 0.1% at all other locations. No false positives were detected over the 80,930 bases analyzed in 38 dilution analyses, resulting in less than one error in 3 million bases sequenced (error rate of $<3.3 \times 10^{-7}$ false-positive mutation calls per base; specificity, >99.9999%; Tables 3.5 and 3.6).

Evaluation of plasma from healthy individuals

We used TEC-Seq to examine plasma specimens from 44 healthy individuals (Tables 3.1, 3.2 and 3.7). These individuals were not known to have cancer and provided their blood samples as part of a routine cancer screening visit (colonoscopy or Papanicolaou test). Samples were processed within 2 hours from collection and centrifuged twice at high speed to ensure that cells and cellular debris were removed and that only cf DNA was analyzed. From the ~4 ml of plasma obtained from each individual, we generated TEC-Seq libraries and sequenced these to ~30,000-fold coverage. Through these analyses, no mutations were observed in the cancer driver genes analyzed in our panel, consistent with the estimated specificity observed in our dilution analyses. Although conventional sequencing of these samples would have resulted in thousands of putative alterations among the regions analyzed, the TEC-Seq analyses significantly reduced the sequencing error rate to fewer than one false positive per 3 million bases sequenced ($<3 \times 10^{-7}$ false-positive mutation calls per base; $P < 0.0001$, paired t test; Figure 3.5). We compared the TEC-Seq error rate to those obtained through other liquid biopsy analyses. Reanalysis of our sequence data from 15 healthy individuals using the recently developed integrated digital error suppression (iDES) method (10, 11) resulted in multiple false-positive alterations in the healthy cases, consistent with the reported error rate of this approach (11).

Analysis of six genes related to hematopoietic proliferation identified six individuals with a single mutation in their plasma samples, and a seventh individual had two detectable alterations (16% of patients analyzed; Table 3.8). All of the alterations were identified in DNA methyltransferase 3a (DNMT3A), a gene that is clonally altered under preleukemic conditions and myelodysplasia (39-41). Three of the mutations were predicted to result in the R882C change previously observed in clonal hematopoiesis, but other alterations have not been previously reported. These mutations were identified at mutant allele fractions of 0.16 to 5.3%, substantially lower than previous observations in blood cells of healthy individuals (39-41). Our analyses suggest that a higher fraction of asymptomatic individuals may harbor such somatic alterations than had been previously reported through cellular analyses of these genes in the blood.

Analysis of plasma from patients with cancer

We next analyzed plasma samples from 194 patients with breast cancer (n = 45), colorectal cancer (n = 42), lung cancer (n = 65), and ovarian cancer (n = 42). The cohort consisted of untreated patients who had localized or metastatic dis- ease, with most of the patients diagnosed at stages I and II (Tables 3.5 and 3.6). We found that the concentration of cf DNA in plasma from cancer patients was ~29 ng/ml, significantly higher than that observed in healthy individuals (average of 7 ng/ml; P = 0.001, unpaired t test; Figure 3.6 A). In the colorectal cancer cohort, where a larger number of later-stage patients were analyzed, we found that samples from patients with metastatic disease had higher concentration of cf DNA than those from patients with earlier stages of disease (average of 66 ng/ml for stage IV patients versus 21 ng/ml for stages I to III; P = 0.006, unpaired t test; Figure 3.6 B).

We examined the cf DNA from these patients using the TEC-Seq approach. Of the 194 patients analyzed, more than three quarters of colorectal cancer patients, two-thirds of ovarian cancer patients, and most of the lung and breast cancer patients had detectable alterations in driver genes (Table 3.9). These detection rates were higher in some cases than the theoretical estimates for these cancer types (Tables 3.4 and 3.9). More than three quarters of patients with advanced disease (stages III and IV) and 62% of patients with localized disease (stages I and II) were detected among all tumor types (Table 3.9). The amounts of ctDNA varied among cancer types, with breast cancer having the lowest mutant allele fraction ($P = 0.028$, unpaired t test; Figure 3.6 C). Similar to observations of cf DNA, the amounts of ctDNA were higher in metastatic disease compared to earlier-stage disease among all cancer types ($P < 0.0001$, unpaired t test; Figure 3.6 D and 3.7). Eighty of 128 detected cases had at least one alteration in a gene hotspot position (Figure 3.7). The affected genes and distribution of alterations for each tumor type were similar to common driver gene alterations that have previously been reported in these cancers (Figure 3.8). On average, 2.1 alterations, including 0.9 changes at hotspot positions, were observed in each patient with detectable ctDNA, with lung and colorectal cancers having a higher number of alterations per case (Figure 3.7). By limiting analysis only to a specific set of hotspot variants as others have reported (11), the fraction of cases detected was reduced to 56% of those identified by TEC-Seq. These observations highlight the benefit of analyzing a broader panel of driver gene regions to increase the possibility of detecting tumor-specific alterations in the plasma.

Comparison of mutations in plasma with those in matched tumor and blood cells of the 194 patients in our study, 152 cases had matched tumor and normal tissues that we analyzed using an independent targeted NGS approach (Tables 3.5, 3.6 and 3.7). We examined these cases to determine whether the mutations identified in the plasma were tumor-specific or may have originated during blood cell expansion. The plasma analyses performed using TEC-Seq were

performed separately and did not rely on any knowledge of alterations identified through these parallel tissue analyses.

We detected 87 changes in the circulation of 194 patients at allele fractions >25% and considered these to be likely germline variants. Analysis of 63 of these variants in the available corresponding blood cells identified all these changes to be germline (Table 3.10). These observations suggested that cf DNA can be used to accurately identify germline changes in the context of tumor-derived and blood cell proliferation alterations and, similarly, that this approach can be used to distinguish these changes from somatic alterations.

Similar to our observations in healthy individuals, we identified alterations in DNMT3A and five other genes involved in blood cell proliferation in the plasma of cancer patients (Table 3.8). The fraction of patients with detectable changes in these genes correlated with age, as previously observed ($P = 0.013$, unpaired t test) (39-41). Unlike tumor- specific alterations, the allele fractions of blood cell proliferation alterations in cf DNA were similar among healthy individuals and patients with cancer, regardless of stage. Analysis of matched white blood cells from individuals with alterations in these genes identified the corresponding mutation in most of the cases, consistent with the notion that the alterations in cf DNA originated from these cells (Table 3.8).

After accounting for blood cell proliferation and germline alterations, we identified 313 candidate tumor-specific changes in the plasma samples from 128 of the 194 patients analyzed. We further evaluated 216 of these alterations in 100 patients where matched tumor tissue and blood cells were available. We found that 155 of the 216 (72%) alterations were identical in both plasma and tumor samples (Figure 3.9). Among stage III and IV patients, 65 of 84 (77%) variants were concordant, whereas for early-stage patients, 90 of 132 (68%) alterations were concordant. In line

with these observations, we found that 70 of the 75 (93%) alterations with a mutant allele fraction >1% in the plasma were detected in the tumor tissue of the same individual. Overall, 82 of the 100 (82%) patients had at least one alteration observed in the circulation that was identical to that in the tumor specimen.

To evaluate reproducibility of the approach between separate blood draws in the same patients, we assessed six late-stage patients with lung cancer where blood was obtained early during the course of treatment. These patients were undergoing treatment but were observed to have progressive or stable disease. Despite the difference in time between the blood draws, we found that 90% of the alterations observed in the second blood draw were present at the time of the first blood draw (17 of 19 alterations), with one patient having no alterations at both time points (Figure 3.10). All alterations present with a mutant allele fraction ≥1% were observed at both time points.

In a subset of colorectal cancer patients, we evaluated whether the observations we detected in the plasma could be independently confirmed using droplet digital polymerase chain reaction (ddPCR), a method that is highly sensitive for detection of single-base substitutions (47). We examined six driver alterations detected in the plasma: two that were also detected in matched tumors and four that were absent. Five of the six driver alterations were detected in the plasma by ddPCR at levels similar to those observed by TEC-Seq (Figure 3.11 A). Those not detected in tumors by targeted sequencing were similarly not identified through ddPCR approaches. We also evaluated 10 mutations that corresponded to the most common changes in KRAS, PIK3CA, and BRAF that we detected in these tumors but were not present in the plasma of these patients. Although we confirmed that these alterations were in the tumors of these patients, we found that those not detected by TEC-Seq analyses remained undetected by ddPCR in the plasma,

presumably because the amounts of ctDNA corresponding to these alterations were extremely low in these patients (Figure 3.11 B).

To assess the possibility that tumor heterogeneity may be responsible for the apparent lack of concordance between specific alterations in the plasma and those in the tumor, we analyzed multiple tumor sites from colorectal cancer patient CGCRC307 using ddPCR. We characterized 10 different regions of the tumor as well as a subsequent metastatic site for an R201C alteration in the GNAS gene that we detected in the plasma but not in the tumor of this patient. Although we found a BRAF V600E alteration in all samples analyzed, the GNAS R201C substitution was not detected in the original tumor biopsy but was detected as a subclonal change in only a portion of the primary tumor, suggesting that it developed later in tumorigenesis (Figure 3.12). The GNAS R201C change identified had been previously reported in colorectal cancers (42) and has been shown to promote intestinal tumorigenesis through activation of both Wnt and ERK pathways (48). Consistent with this notion, we found the GNAS alteration to be clonal in the metastatic lesion that was identified 2 years after the primary tumor in this patient (Figure 3.12). These results suggest that plasma alterations not detected in the matched tumor specimens may represent bona fide somatic mutations in ctDNA derived from heterogeneous primary or occult lesions.

ctDNA and disease progression

Tumor-specific markers may be useful for evaluating disease progression. In colorectal cancer, CEA is commonly used to monitor patients after therapy to determine recurrence or progressive disease (23, 49). Of the 29 colorectal cancer patients for whom CEA values were available, all 10

cases with CEA concentrations >5 ng/ml had detectable ctDNA (Tables 3.5, 3.6 and 3.10). However, among the 19 patients with negative or borderline CEA results, 13 had detectable ctDNA, including patients of all stages (Tables 3.11 and 3.12). There was no significant correlation be- tween ctDNA and CEA concentrations (Pearson correlation coefficient = −0.017; P = 0.93).

We next examined whether preoperative ctDNA analyses may be related to disease recurrence and survival after surgical resection. We hypothesized that elevated amounts of ctDNA were more likely to be associated with large primary lesions that were incompletely resected or with occult metastases. A total of 31 colorectal cancer patients had potentially curative resections, including 8 stage I, 9 stage II, 10 stage III, and 4 stage IV patients with liver-only metastases. For these patients, the median mutant allele fraction was 0.21%. However, several patients had mutant allele fractions >3 median absolute deviations from the median mutant allele fraction, or >2%. As predicted, we found that high amounts of ctDNA correlated with poor prognosis (Figure 3.13). Patients with increased ctDNA had a shorter progression-free survival (PFS) and overall survival (OS) compared to those with lower ctDNA amounts (P < 0.0001 for PFS and OS, log-rank test; Figure 3.14 A and B). The prognostic value for PFS was statistically significant in multivariate models, adjusted for stage as a categorical covariate (hazard ratio, 36.3; 95% confidence interval (CI), 2.8 to 471.1; P = 0.006, Cox proportional hazards model). These same predictions were observed in patients with resectable stage I to III disease (P = 0.0006 for PFS and P < 0.0001 for OS, log-rank test; Figure 3.14 C and D). We also evaluated other thresholds of increased amounts of ctDNA and found that these were statistically significantly associated with worse outcome (P = 0.008 for 0.5% mutant allele fraction and P = 0.0001 for 1% mutant allele fraction, log-rank test). In addition, we found that considering ctDNA amounts as a continuous variable correlated with outcome (hazard ratio, 1.13; 95% CI, 1.03 to 1.24; P =0.01 for PFS and

OS, Cox univariate test). Together, these results indicate that liquid biopsy analyses offer both a quantitative and qualitative assessment of disease progression. Although previous analyses have found a limited association between preoperative CEA concentrations and OS (23, 49), CEA concentrations among our patients were not associated with disease outcome (P = 0.75 for PFS and P = 0.73 for OS, log- rank test; Figure 3.15). These analyses from a limited and heterogeneous cohort of patients suggest that preoperative ctDNA amounts may provide a useful marker of disease outcome in operable colorectal cancer.

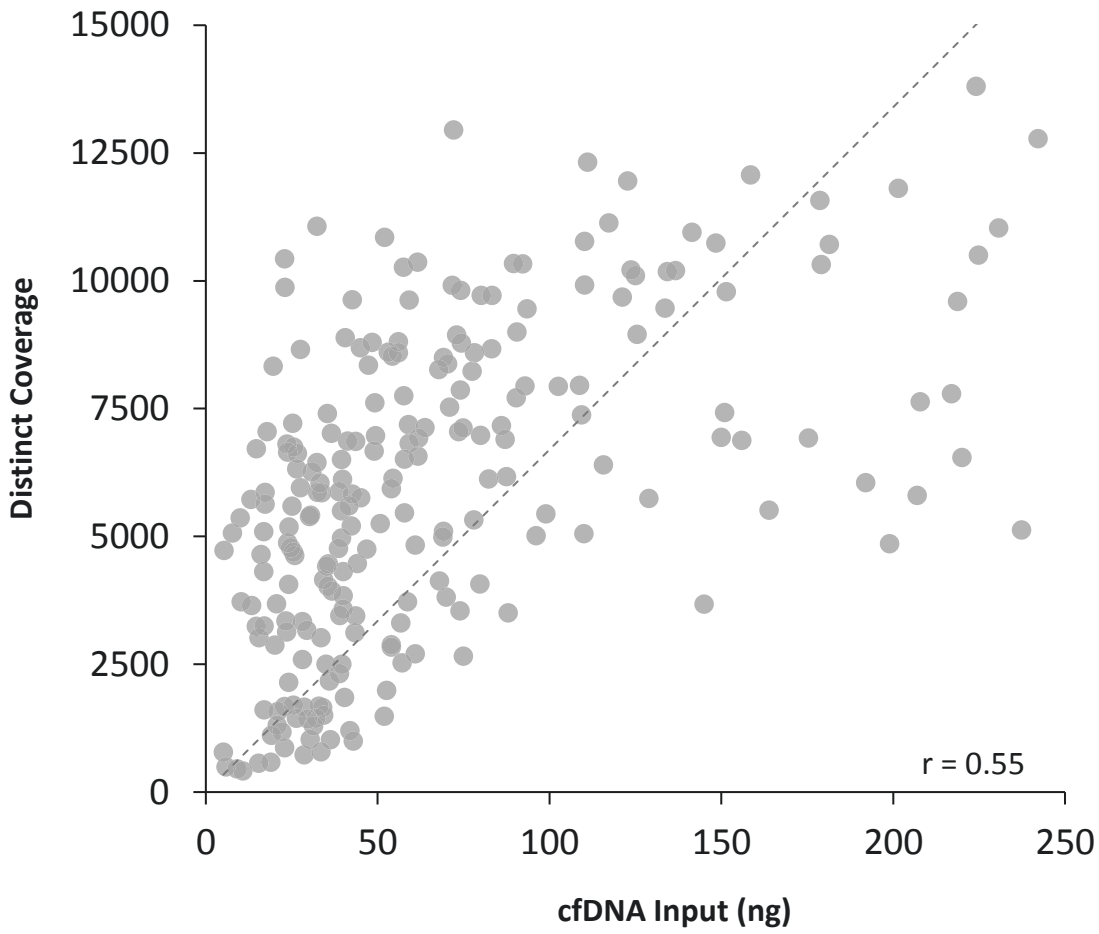

Figure 3.1. Conversion efficiency of cfDNA. Correlation of library input cfDNA with distinct sequencing coverage in cases with cfDNA <250 ng (n=230) (Pearson correlation: r=0.55, 95% CI=0.46-0.64, p<0.0001).

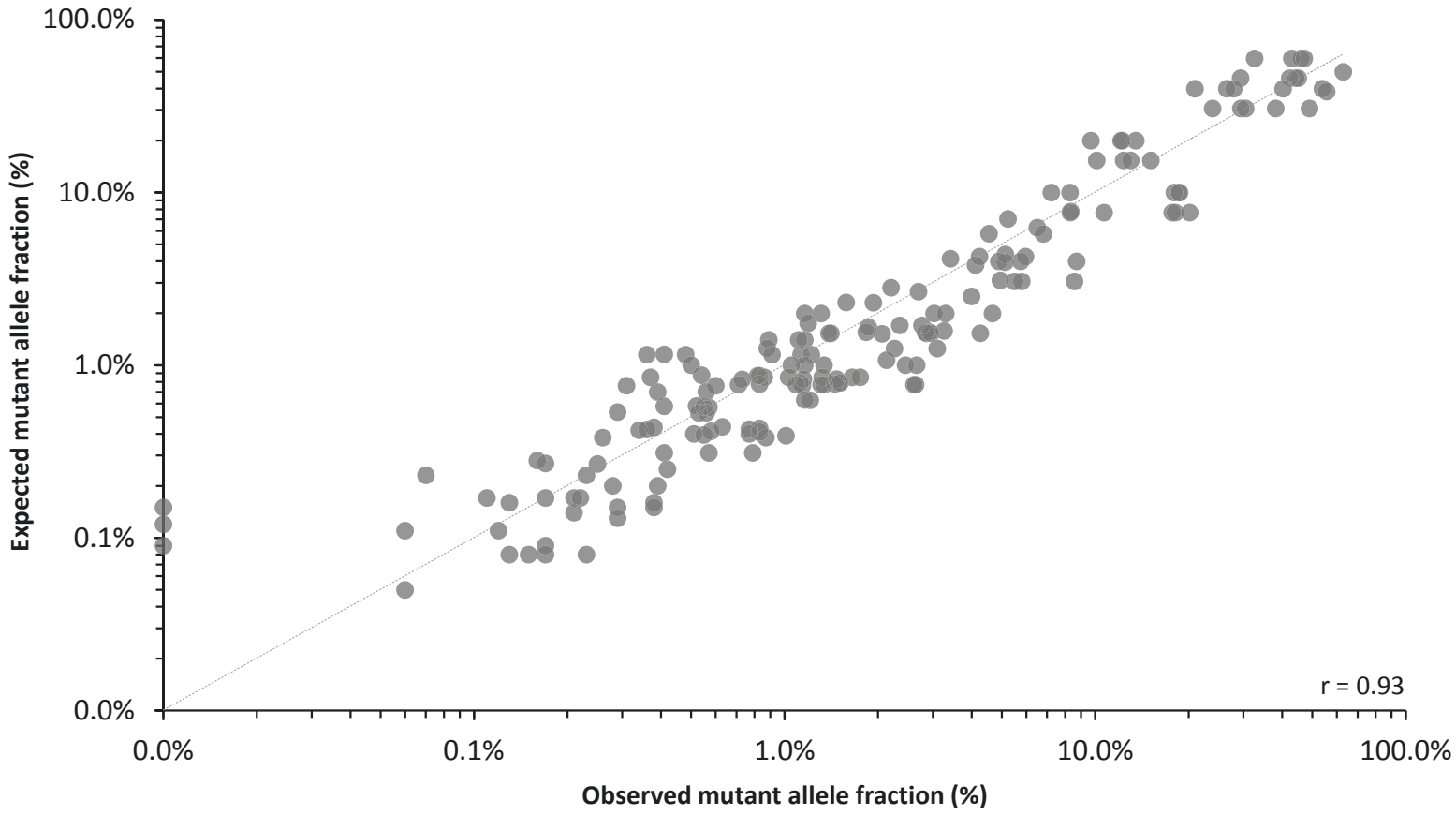

Figure 3.2. Validation of the TEC-Seq approach. Correlation between observed and expected mutant allele fractions from mutant pools of tumor cell line DNA mixed with varying dilutions of genomic DNA (Pearson correlation: r=0.93, 95% CI=0.91, p=0.0001, r^2=0.87).

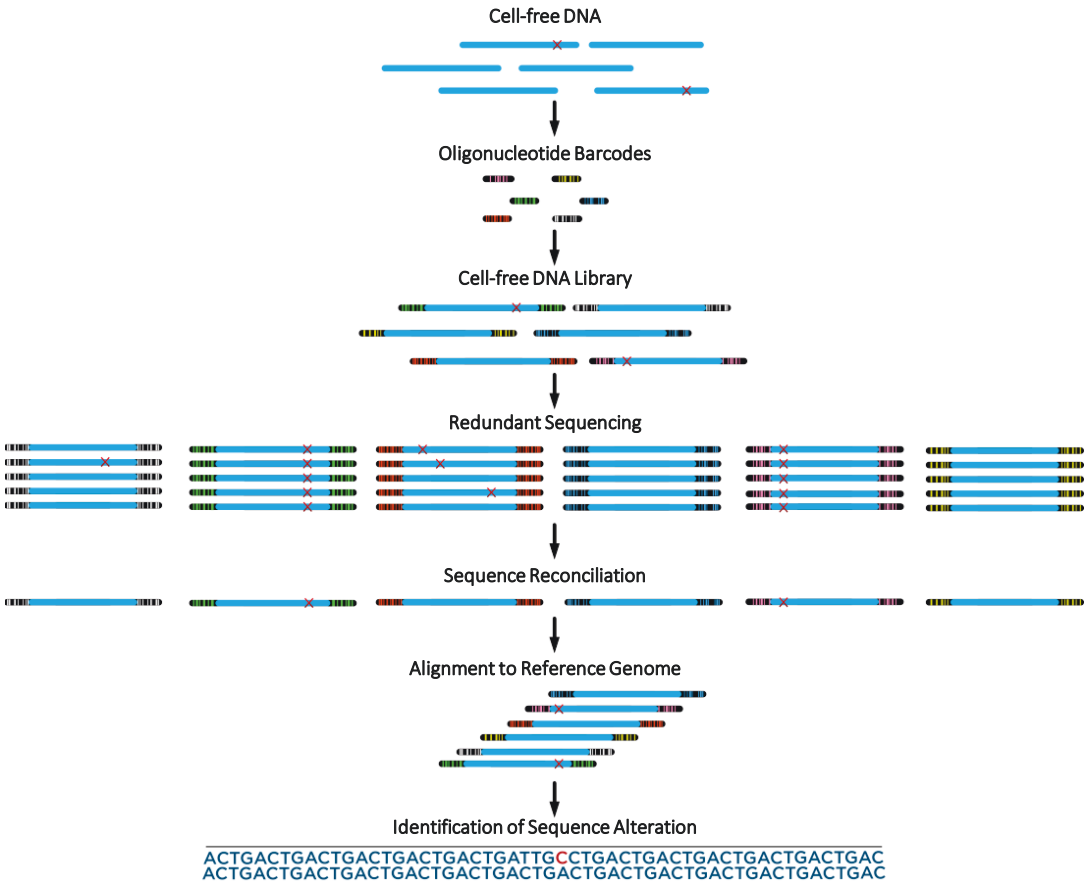

Figure 3.3. Schematic of the TEC-Seq method. cfDNA is extracted from the blood and converted to a genomic library through ligation of a pool containing a small number of dual-index barcode adapters. The resulting cfDNA library is captured and redundantly sequenced to produce multiple duplicates of each DNA fragment. Sequence reconciliation among duplicate fragments identifies alterations present in identical DNA molecules with the same start and end position and exogenous barcodes. Alignment to the reference genome of multiple distinct molecules containing identical redundant changes is used to identify bona fide alterations.

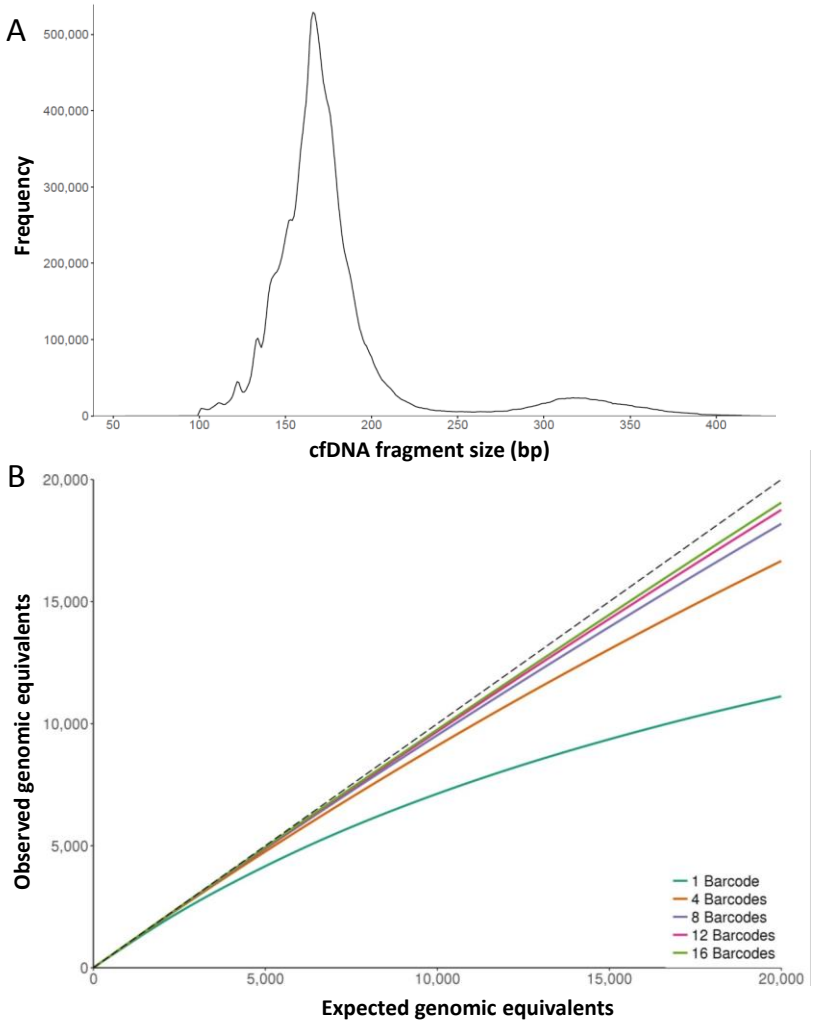

Figure 3.4. Simulations using limited exogenous barcodes. Monte Carlo simulations were performed to evaluate the effect of varying the quantity of exogenous barcodes on the number of genomic equivalents that can be distinguished through next-generation sequencing as compared to the expected number of genomic equivalents. (A) Representative distribution of cfDNA fragment sizes observed from sequencing data of ten colorectal cancer patients. (B) Using a sliding number of expected genomic equivalents (F), we sampled F fragment lengths from the distribution in (A) with replacement. Sample fragments were then randomly assigned start and end positions relative to an arbitrary base (x). Exogenous barcodes were randomly assigned to each fragment for simulation of 1, 4, 8, 12, and 16 barcodes. The number of observed genomic equivalents was then calculated from the unique combinations of endogenous (start and end position) and exogenous barcodes. These analyses indicate that a limited number of exogenous barcodes with endogenous barcodes improves the number of genome equivalents that can be analyzed at the sequencing depths typically utilized.

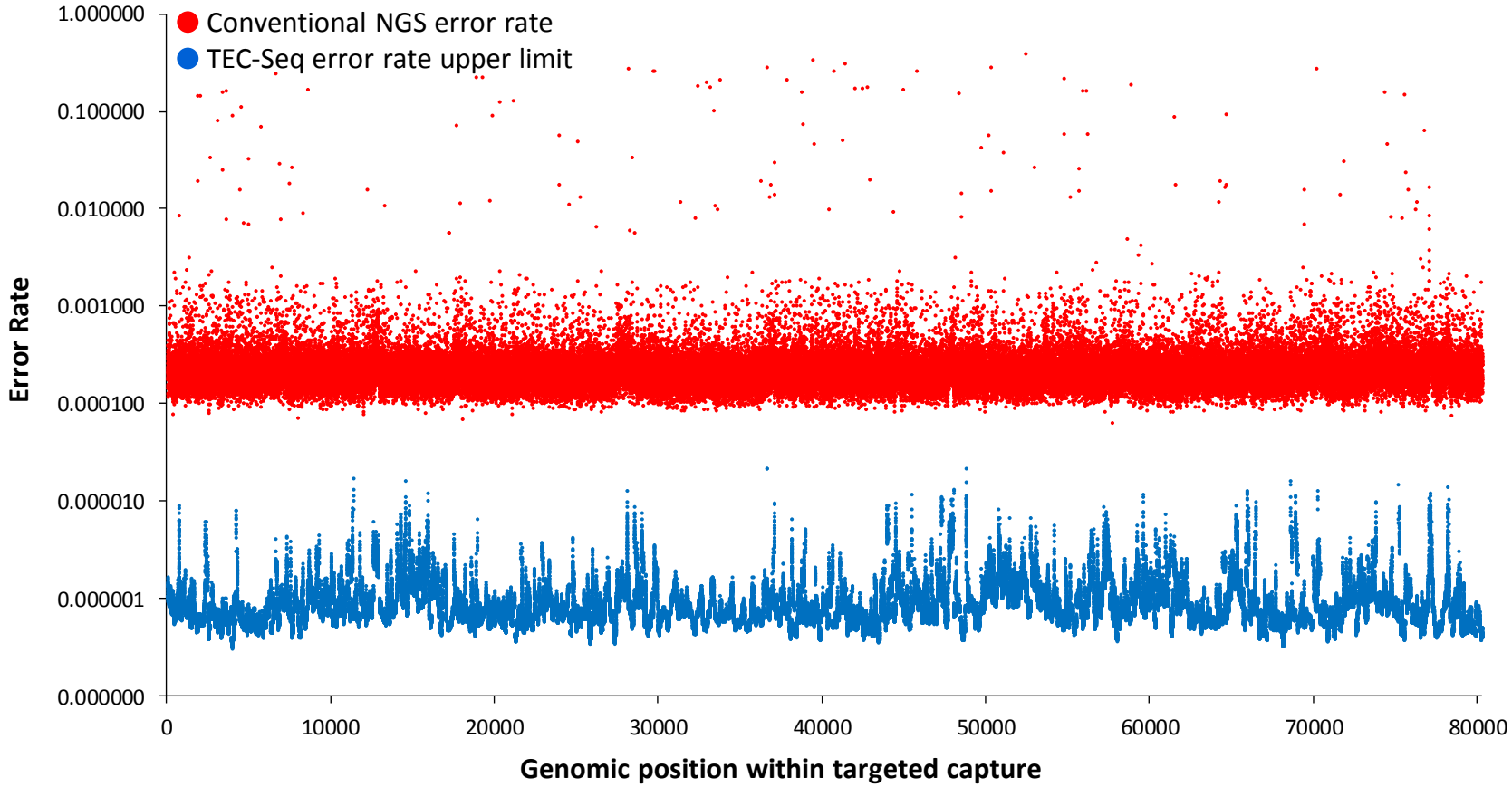

Figure 3.5. TEC-Seq error correction. Sequencing error rates of conventional NGS and theoretical upper limit for TEC-Seq are indicated at each base in the captured regions of interest ($P < 0.0001$, paired t test). Error rates are determined by identifying the number of alterations at each base (or assuming one alteration per base if no error was identified) divided by the total coverage at each base among the 44 healthy individuals analyzed.

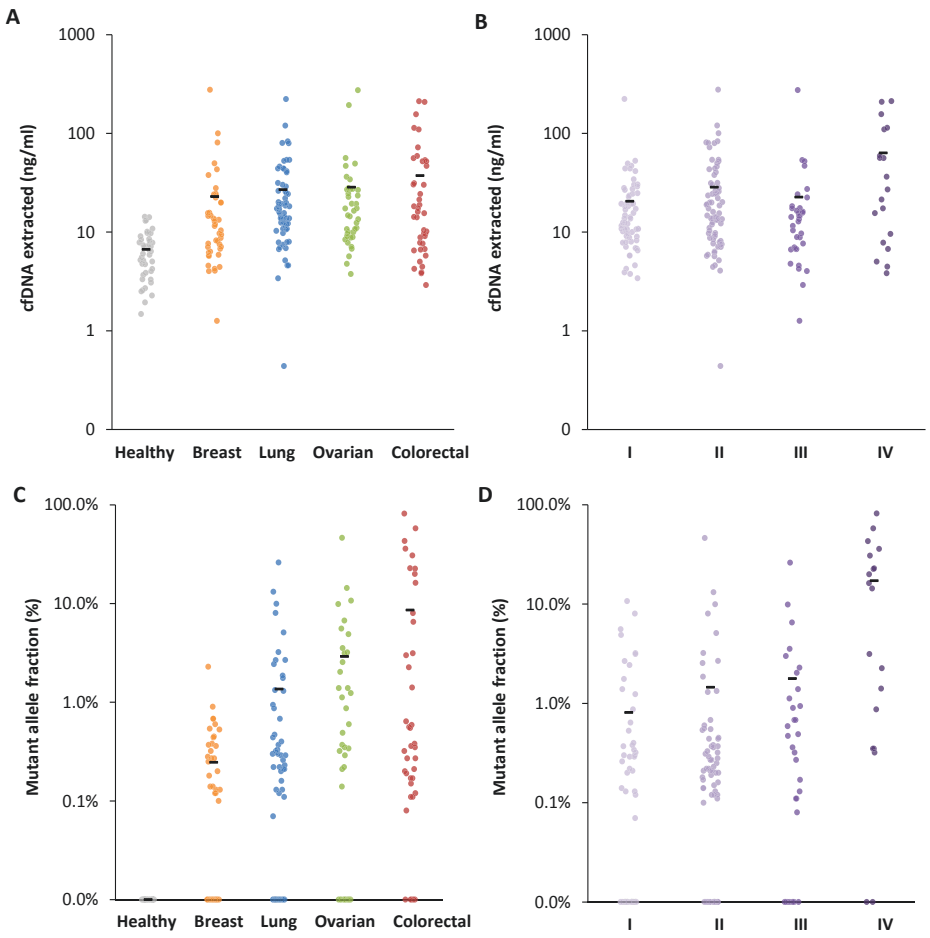

Figure 3.6. cfDNA and ctDNA in healthy individuals and patients with cancer. Amount of cfDNA extracted from all healthy individuals and patients with different cancer types (A) and from cancer patients of different stages (B). Mutant allele fraction of ctDNA detected in healthy individuals and patients with different cancer types (C) and in cancer patients of different stages (D). Means for each group are represented by the black bars in the columns analyzed. In patients for whom multiple alterations were detected, the highest value is indicated.

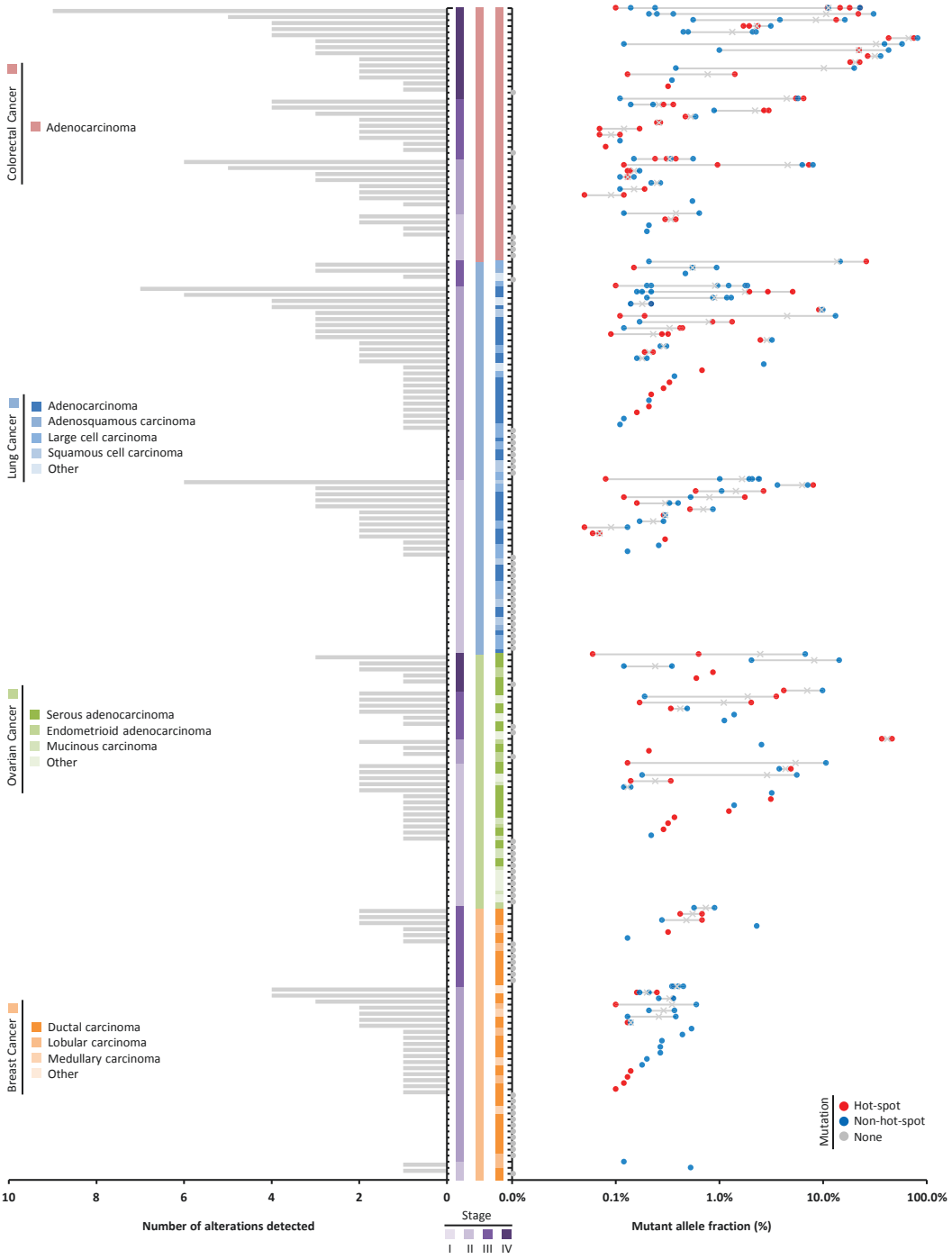

Figure 3.7. ctDNA in patients with breast, colorectal, lung, and ovarian cancer. Patients (n = 194) are each represented by a tick mark. (Left) Bar chart shows the number of alterations detected for each case. (Middle) Stage, cancer type, and histopathological subtype are represented by colored vertical bars. (Right) Mutant allele fractions for each alteration detected per patient are indicated with an "x" at the mean. Alterations are colored on the basis of hotspot status and whether any alterations were detected in the case.

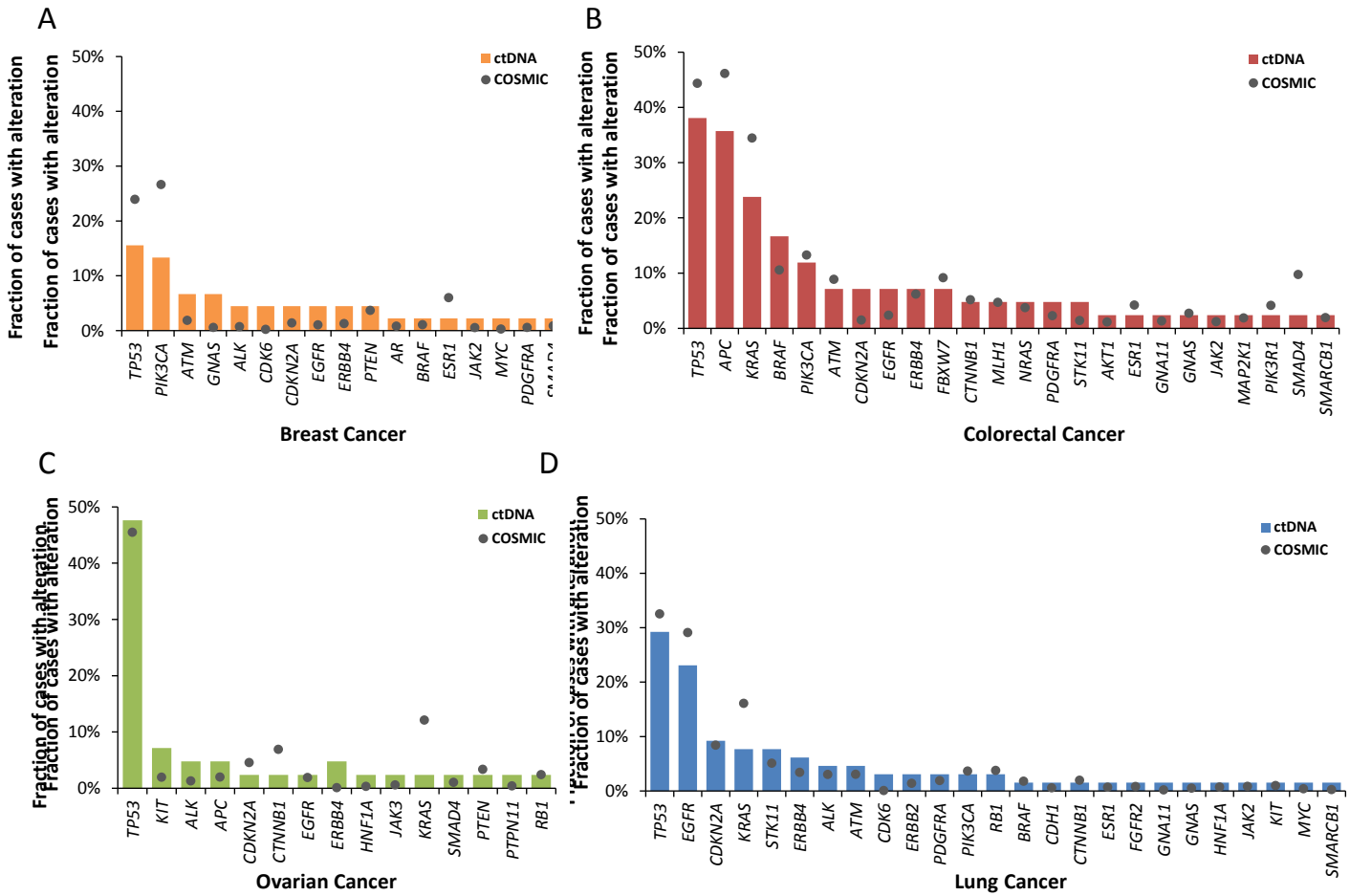

Figure 3.8. Mutation frequencies in cancer genes. Bar charts depict the fraction of patients with an alteration in a cancer driver gene observed in the plasma using TEC-Seq for breast (A), colorectal (B), ovarian (C), and lung (D) cancer cohorts. The fraction of cancer cases reported in the COSMIC database with an alteration in the same genes is shown in the overlaid dot plot. The fraction of patients in our study and in the COSMIC database with an alteration in the genes of interest was similar for 75 out of 81 genes analyzed ($P>0.05$ for 75 of 81 genes, Fisher's exact test).

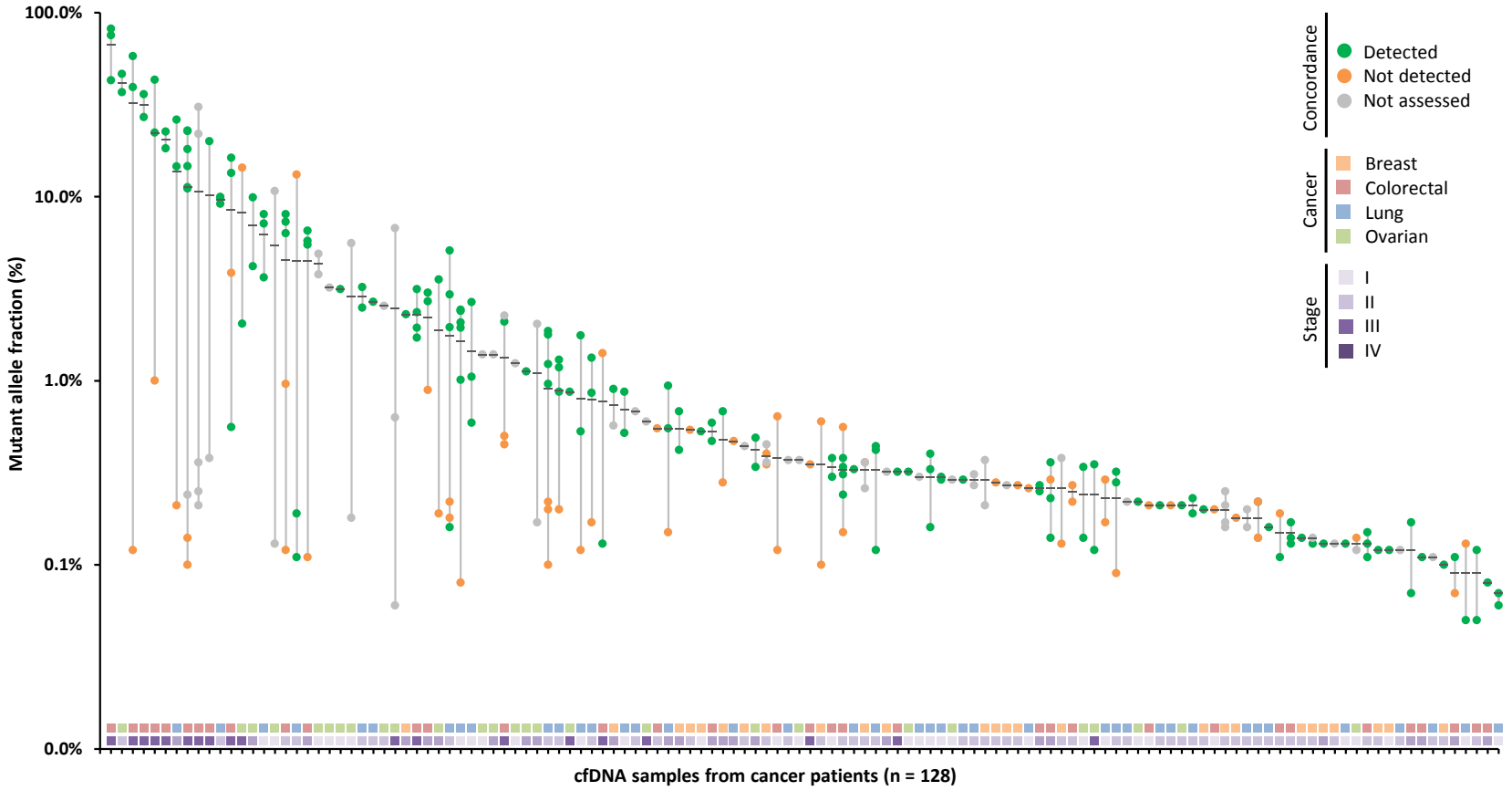

Figure 3.9. Concordance between alterations in plasma and tissue. Mutant allele fractions observed in the plasma are indicated for each alteration identified with a black bar at the mean. The presence of alterations in matched tumor specimens is indicated with green dots, whereas nonconcordant alterations are indicated in orange, and those that are not assessed are indicated in gray. Stage and cancer type for each patient are plotted in the two horizontal tracks at the bottom of the figure.

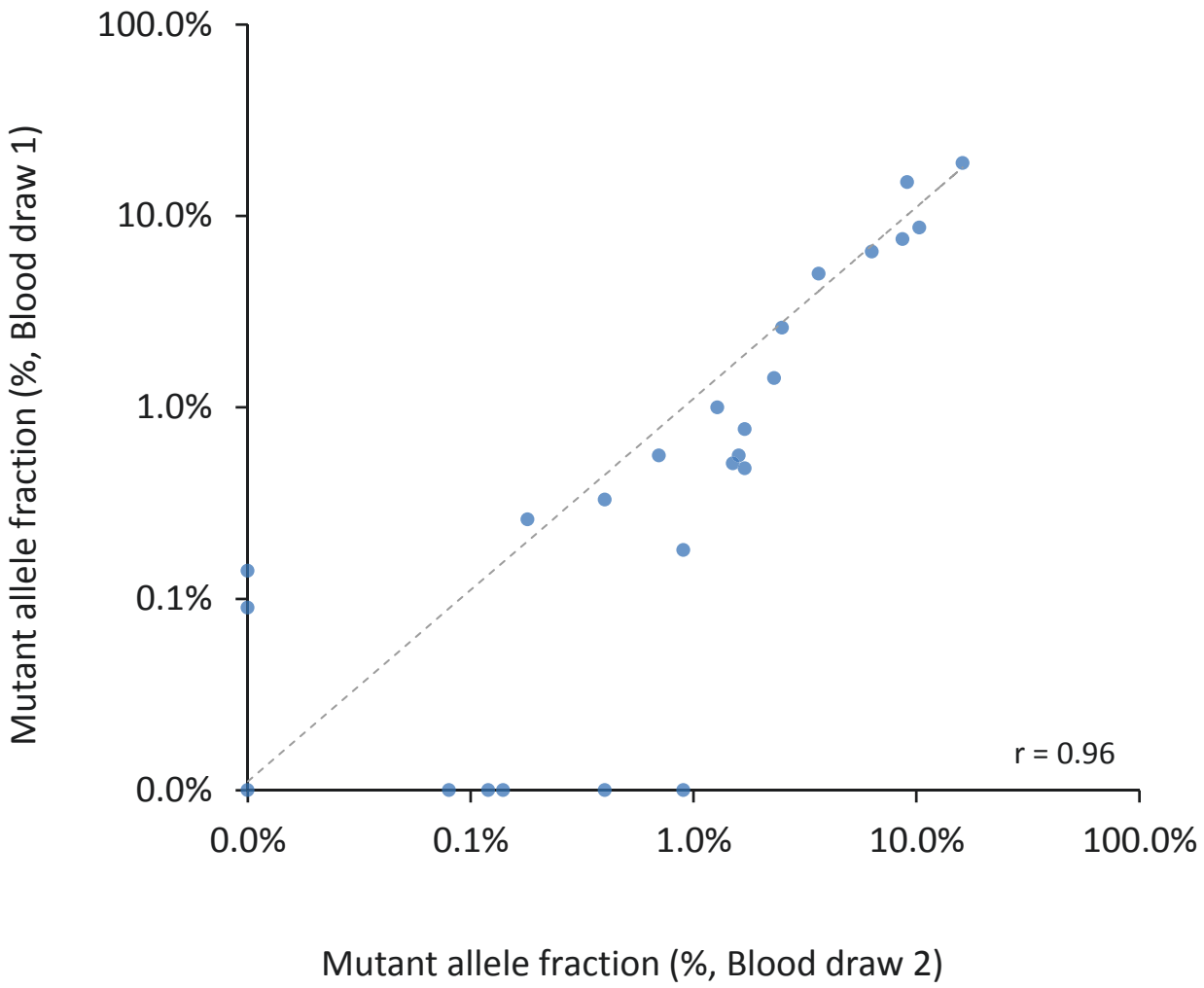

Figure 3.10. ctDNA mutant allele fractions in serial blood draws. Mutant allele fractions for alterations identified in two serial blood draws from six patients are indicated for each time point (Pearson r=0.96, 95% CI=0.92 – 0.98, p<0.0001, r2=0.93).

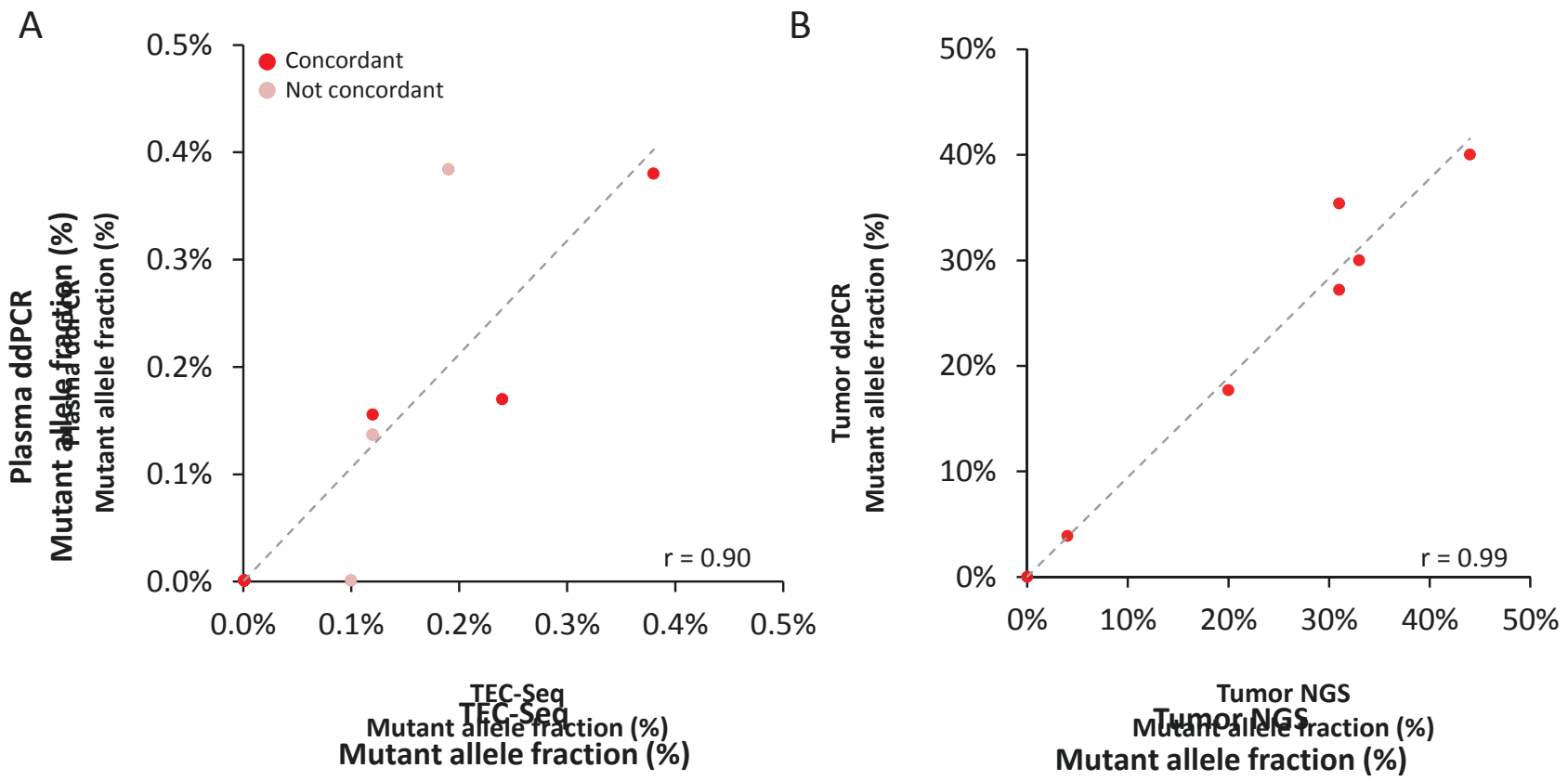

Figure 3.11. Comparison of ctDNA mutant allele fractions measured by TEC-Seq and ddPCR. Correlation of independent detection of alterations in cfDNA using ddPCR and TEC-Seq (A. Pearson r=0.90, 95% CI=0.72–0.96, p<0.0001, r2=0.81) and in tumor tissue using ddPCR and conventional NGS (B. Pearson r=0.99, 95% CI=0.95–1.00, p<0.0001, r2=0.98). Nine alterations in panel A were not detected by either plasma ddPCR or TEC-Seq. Alterations analyzed in the plasma (A) that were confirmed to be concordant with alterations in the matched tumor are indicated in bright red whereas alterations not concordant with those in the tumor appear in light red.

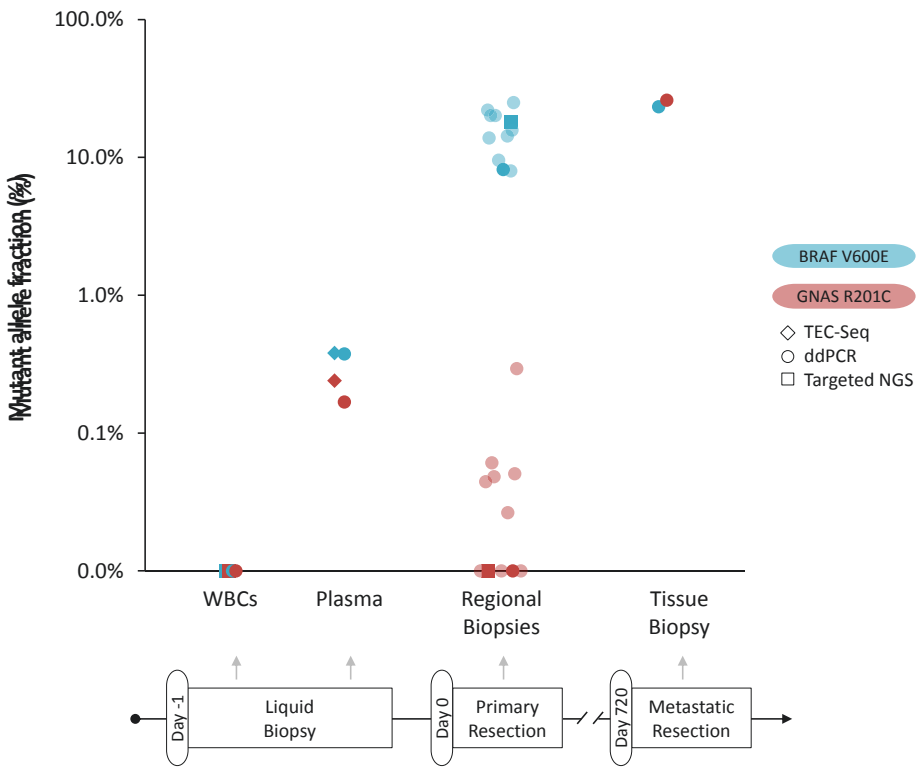

Figure 3.12. ctDNA and tumor heterogeneity. Analysis of two alterations, BRAF V600E (blue) and GNAS R201C (red), identified in a stage II CRC patient by three independent methods: TEC-Seq (diamonds), ddPCR (circles), and targeted NGS (squares). A liquid biopsy obtained one day before primary resection of the tumor yielded white blood cells and plasma for analysis of germline DNA and cfDNA, respectively. Both alterations were assessed in the white blood cells by targeted NGS and ddCPR, and in the plasma with TEC-Seq and ddPCR. Tissue from the primary resection was cored to obtain multiple biopsies, each analyzed separately by ddPCR for both alterations. Two analyses of one biopsy performed using targeted NGS and ddPCR are shown in darker shades compared to biopsies assessed by ddPCR alone. Tissue from a metastatic lesion was analyzed with ddPCR for both alterations. These analyses indicate that alterations identified in the plasma using TEC-Seq may represent heterogeneous changes that are present in only a portion of the primary tumor and/or occult lesions.

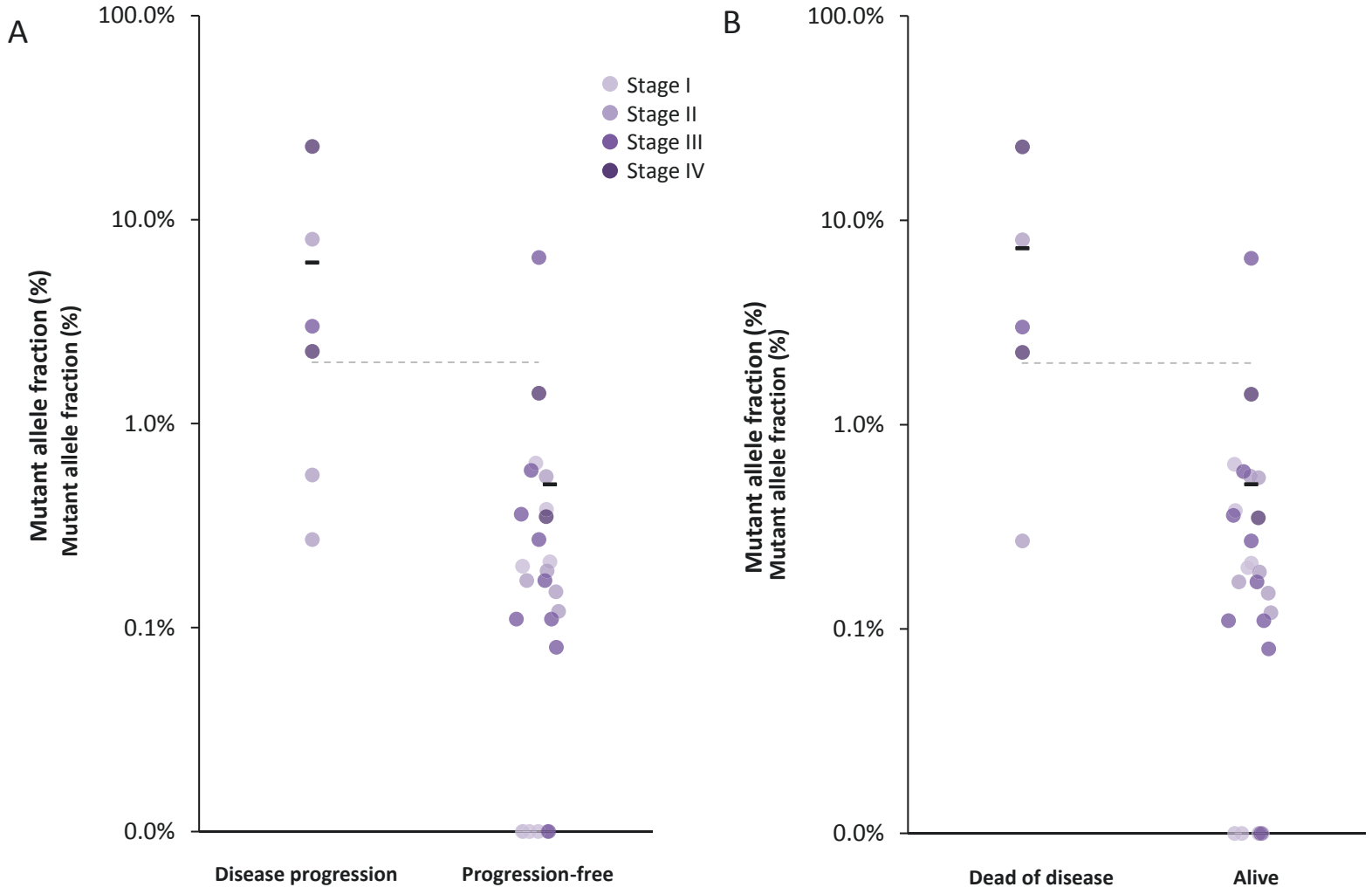

Figure 3.13. Pre-operative ctDNA mutant allele. Mutant allele fractions of 31 CRC patients with stage I – IV disease organized based on progression-free survival status (A, P=0.0026, unpaired t test), and overall survival status (B, P=0.0006, unpaired t test).

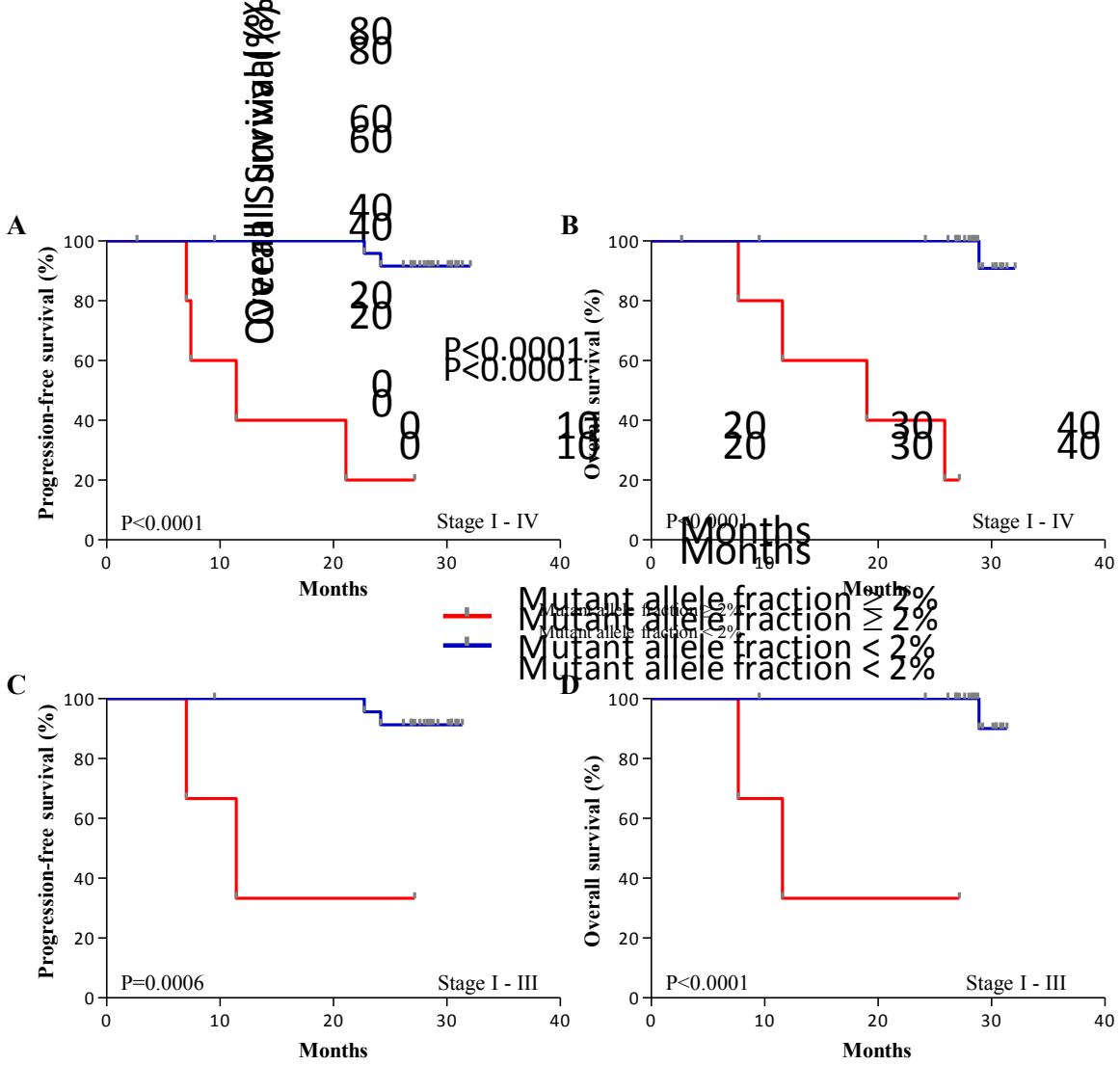

Figure 3.14. Preoperative ctDNA amounts and outcome in colorectal cancer patients. Kaplan-Meier curves depict PFS (A) ($P < 0.0001$, log-rank test) and OS (B) ($P < 0.0001$, log-rank test) of 31 colorectal cancer patients, stages I to IV, stratified on the basis of a ctDNA mutant allele fraction threshold of 2%. Kaplan-Meier analyses of the 27 patients with stage I to III disease for PFS (C), ($P = 0.0006$, log-rank test) and OS (D) ($P < 0.0001$, log-rank test) were performed using the same threshold to examine the association of ctDNA with outcome in patients without stage IV disease.

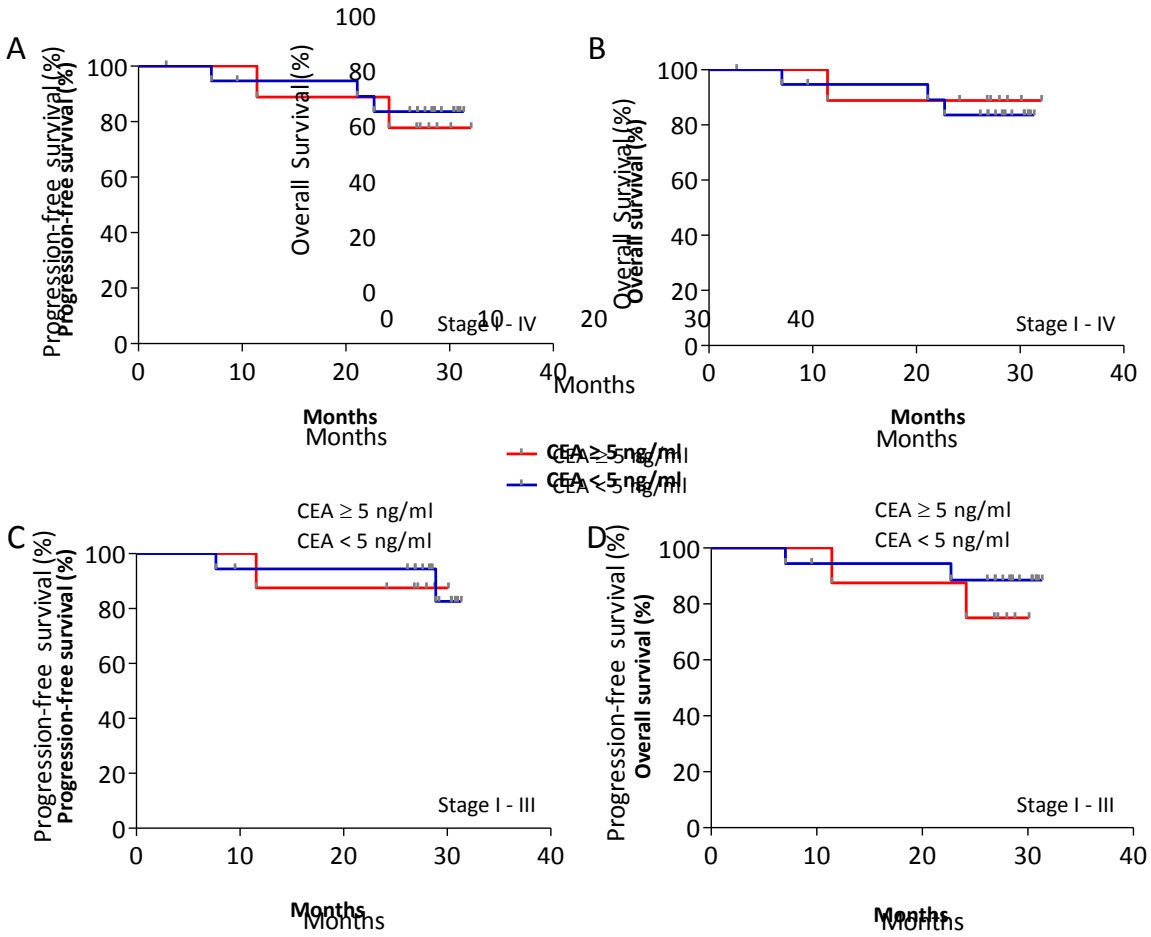

Figure 3.15. Pre-operative CEA in colorectal cancer patients. Kaplan-Meier curves depict progression-free survival (A, p = 0.7533, Log-rank test) and overall survival (B, p = 0.7329, Log-rank test) of 31 CRC patients, stage I – IV, stratified based on a CEA threshold of 5 ng/ml. Kaplan-Meier analyses of the 27 patients with stage I – III disease for progression-free survival (C, Log-rank test p = 0.4282) and overall survival (D, Log-rank test p = 7345) were performed using the same threshold in order to examine the association of CEA with outcome in patients without stage IV disease. Similar results were obtained using CEA thresholds of 2.5 ng/ml and 3 ng/ml.

Table 3.1. Summary of clinical data for patients analyzed.

Patient	Patient Type	Age at Diagnosis	Gender	Stage at Diagnosis	TNM Staging	Site of Primary Tumor	Resected Tumor Location	Histopathological Diagnosis	Degree of Differentiation	Location of Metastases at Diagnosis
CGPLBR53	Breast Cancer	57	F	III	T2N3M0	Right Breast	Right Breast	Infiltrating Ductal Carcinoma	Moderate	None
CGPLH80	Healthy	37	F	NA	NA	NA	NA	NA	NA	NA
CGPLH78	Healthy	34	F	NA	NA	NA	NA	NA	NA	NA
CGPLH79	Healthy	37	F	NA	NA	NA	NA	NA	NA	NA
CGPLBR49	Breast Cancer	37	F	II	T2N1M0	Left Breast	Left Breast	Infiltrating Ductal Carcinoma	Poor	None
CGPLH82	Healthy	38	F	NA	NA	NA	NA	NA	NA	NA
CGPLBR38	Breast Cancer	54	F	I	T1N0M0	Left Breast	Left Breast	Infiltrating Ductal Carcinoma	Moderate	None
CGPLBR55	Breast Cancer	53	F	III	T3N1M0	Right Breast	Right Breast	Infiltrating Ductal Carcinoma	Poor	None
CGPLBR69	Breast Cancer	43	F	II	T2N0M0	Breast	Breast	Infiltrating Ductal Carcinoma	Moderate	None
CGPLH85	Healthy	34	F	NA	NA	NA	NA	NA	NA	NA
CGPLBR39	Breast Cancer	33	F	II	T2N0M0	Left Breast	Left Breast	Infiltrating Ductal Carcinoma	Poor	None
CGPLBR57	Breast Cancer	54	F	III	T2N2M0	Left Breast	Left Breast	Infiltrating Ductal Carcinoma	NA	None

CGPLBR72	Breast Cancer	67	F	II	T2N0M0	Breast	Breast	Infiltrating Ductal Carcinoma	Well	None
CGPLBR48	Breast Cancer	47	F	II	T2N1M0	Left Breast	Left Breast	Infiltrating Ductal Carcinoma	Poor	None
CGPLH86	Healthy	50	F	NA	NA	NA	NA	NA	NA	NA
CGPLH76	Healthy	53	F	NA	NA	NA	NA	NA	NA	NA
CGPLBR71	Breast Cancer	65	F	II	T2N0M0	Breast	Breast	Infiltrating Ductal Carcinoma	Poor	None
CGPLBR42	Breast Cancer	82	F	II	T1N1M0	Left Breast	Left Breast	Infiltrating Medullary Carcinoma	Poor	None
CGPLOV15	Ovarian Cancer	54	F	III	T3N1M0	Ovary	Ovary	Adenocarcinoma	Poor	None
CGPLH84	Healthy	45	F	NA	NA	NA	NA	NA	NA	NA
CGPLBR103	Breast Cancer	48	F	II	T2N1M0	Left Breast	Left Breast	Infiltrating Ductal Carcinoma	Moderate	None
CGPLOV17	Ovarian Cancer	42	F	I	T1aN0M0	Ovary	Ovary	Endometrioid Adenoarcinoma	Moderate	None
CGCRC301	Colorectal Cancer	76	F	I	T2N0M0	Rectum	Rectum	Adenocarcinoma	Moderate	None
CGPLBR75	Breast Cancer	63	F	II	T2N1M0	Left Breast	Left Breast	Infiltrating Ductal Carcinoma	Moderate	None
CGPLBR76	Breast Cancer	53	F	II	T2N0M0	Right Breast	Right Breast	Infiltrating Ductal Carcinoma	Well	None
CGPLLU176	Lung Cancer	58	M	I	T2N0M0	Lung	Lung	Adenosquamous Carcinoma	Moderate	None

CGCRC332	Colorectal Cancer	NA	NA	IV	NA	Colorectal	Liver-only metastasis	Adenocarcinoma	NA	Liver
CGPLBR70	Breast Cancer	60	F	II	T2N1M0	Breast	Breast	Infiltrating Ductal Carcinoma	Moderate	None
CGCRC293	Colorectal Cancer	55	M	IV	T3N2M1	Rectum	Liver-only metastasis	Adenocarcinoma	Moderate	Synchronous Liver
CGPLOV18	Ovarian Cancer	57	F	I	T1cN0M0	Ovary	Ovary	Clear Cell Adenocarcinoma	Moderate	None
CGPLBR68	Breast Cancer	64	F	III	T4N1M0	Breast	Breast	Infiltrating Ductal Carcinoma	Poor	None
CGPLBR41	Breast Cancer	51	F	III	T3N1M0	Left Breast	Left Breast	Infiltrating Ductal Carcinoma	Moderate	None
CGPLOV20	Ovarian Cancer	52	F	II	T2aN0M0	Left Ovary	Left Ovary	Endometrioid Adenoarcinoma	Poor	None
CGPLLU145	Lung Cancer	53	M	II	T2aN0M0	Lung	Lung	Adenocarcinoma	Poor	None
CGPLLU178	Lung Cancer	58	F	I	T2aN0M0	Right Lung	Right Lung	Squamous Cell Carcinoma	Moderate	None
CGPLH75	Healthy	46	F	NA	NA	NA	NA	NA	NA	NA
CGPLOV24	Ovarian Cancer	14	F	I	T1aN0M0	Ovary	Ovary	Germ Cell Tumor	Poor	None
CGPLBR63	Breast Cancer	48	F	II	T2N1M0	Left Breast	Left Breast	Infiltrating Ductal Carcinoma	Moderate	None
CGPLBR74	Breast Cancer	46	F	II	T2N0M0	Left Breast	Left Breast	Infiltrating Medullary Carcinoma	Moderate	None
CGPLBR100	Breast Cancer	44	F	III	T2N2M0	Left Breast	Left Breast	Infiltrating Ductal Carcinoma	NA	None

CGPLLU147	Lung Cancer	60	M	III	T3N2M0	Lung	Lung	Adenosquamous Carcinoma	Poor	None
CGCRC311	Colorectal Cancer	59	M	I	T2N0M0	Sigmoideum	Sigmoideum	Adenocarcinoma	Moderate	None
CGPLBR73	Breast Cancer	60	F	II	T2N1M0	Breast	Breast	Infiltrating Ductal Carcinoma	Moderate	None
CGPLOV25	Ovarian Cancer	18	F	I	T1aN0M0	Ovary	Ovary	Germ Cell Tumor	Poor	None
CGPLBR40	Breast Cancer	66	F	III	T2N2M0	Left Breast	Left Breast	Infiltrating Ductal Carcinoma	Poor	None
CGCRC312	Colorectal Cancer	75	M	III	T3N1M0	Ascending	Ascending	Adenocarcinoma	Moderate	None
CGPLLU169	Lung Cancer	64	M	I	T1bN0M0	Lung	Lung	Squamous Cell Carcinoma	Moderate	None
CGPLLU197	Lung Cancer	55	M	I	T1N0M0	Left Lung	Left Lung	Adenocarcinoma	NA	None
CGPLH81	Healthy	54	F	NA	NA	NA	NA	NA	NA	NA
CGPLBR99	Breast Cancer	41	F	III	T3N3M0	Right Breast	Right Breast	Infiltrating Ductal Carcinoma	Poor	None
CGPLBR44	Breast Cancer	61	F	III	T2N2M0	Left Breast	Left Breast	Infiltrating Ductal Carcinoma	Poor	None
CGPLBR59	Breast Cancer	42	F	I	T1N0M0	Left Breast	Left Breast	Infiltrating Ductal Carcinoma	Moderate	None
CGPLOV10	Ovarian Cancer	48	F	I	T1aN0MX	Right Ovary	Right Ovary	Serous Adenocarcinoma	Poor	None
CGCRC291	Colorectal Cancer	69	F	IV	T3N2M1	Coecum	Liver-only metastasis	Adenocarcinoma	Moderate	Synchronous Liver

CGPLLU179	Lung Cancer	66	M	I	T2N0M0	Lung	Lung	Large Cell Carcinoma	Poor	None
CGCRC321	Colorectal Cancer	68	M	I	T2N0M0	Rectum	Rectum	Adenocarcinoma	Moderate	None
CGCRC298	Colorectal Cancer	64	M	II	T3N0M0	Rectum	Rectum	Adenocarcinoma	Moderate	None
CGCRC292	Colorectal Cancer	51	M	IV	T3N2M1	Sigmoideum	Liver-only metastasis	Adenocarcinoma	Moderate	Synchronous Liver and Lung
CGPLLU204	Lung Cancer	50	M	I	T1aN0M0	Left Lung	Left Lung	Squamous Cell Carcinoma	Moderate	None
CGPLLU170	Lung Cancer	46	M	II	T3N0M0	Lung	Lung	Squamous Cell Carcinoma	Poor	None
CGPLBR61	Breast Cancer	67	F	II	T2N1M0	Left Breast	Left Breast	Infiltrating Ductal Carcinoma	Moderate	None
CGPLBR102	Breast Cancer	47	F	II	T2N1M0	Right Breast	Right Breast	Infiltrating Ductal Carcinoma	Moderate	None
CGPLOV12	Ovarian Cancer	45	F	I	T1aN0MX	Ovary	Ovary	Endometrioid Adenoarcinoma	NA	None
CGPLBR43	Breast Cancer	49	F	II	T2N0M0	Left Breast	Left Breast	Infiltrating Ductal Carcinoma	Moderate	None
CGPLLU14	Lung Cancer	59	NA	IV	T1N1M0	Right Lower lobe	Right Lower lobe	Adenocarcinoma	Moderate	NA
CGCRC297	Colorectal Cancer	68	F	III	T3N1M0	Ascending	Ascending	Adenocarcinoma	Moderate	None
CGPLLU4	Lung Cancer	62	M	II	T2bN1M0	Lung	Lung	Adenocarcinoma	Moderate	None
CGPLH83	Healthy	60	F	NA	NA	NA	NA	NA	NA	NA

CGPLLU146	Lung Cancer	58	M	II	T3N0M0	Lung	Lung	Small Cell - Large Cell Adenocarcinoma	NA	None
CGPLOV2	Ovarian Cancer	19	F	I	NA	Ovary	Ovary	Mucinous Adenocarcinoma	NA	None
CGPLBR91	Breast Cancer	62	F	III	T2N2M0	Breast	Breast	Infiltrating Lobular Carcinoma	Poor	None
CGCRC295	Colorectal Cancer	76	F	IV	NA	Colorectal	Liver-only metastasis	Adenocarcinoma	NA	Metachronous Liver
CGCRC314	Colorectal Cancer	67	F	I	T2N0M0	Sigmoideum	Sigmoideum	Adenocarcinoma	Moderate	None
CGPLBR67	Breast Cancer	61	F	III	T4N1M0	Breast	Breast	Infiltrating Ductal Carcinoma	Well	None
CGPLBR80	Breast Cancer	54	F	II	T2N1M0	Breast	Breast	Infiltrating Lobular Carcinoma	Moderate	None
CGPLH77	Healthy	46	F	NA	NA	NA	NA	NA	NA	NA
CGPLLU205	Lung Cancer	65	M	II	T3N0M0	Left Lung	Left Lung	Adenocarcinoma	Poor	None
CGCRC300	Colorectal Cancer	65	M	I	T2N0M0	Rectum	Rectum	Adenocarcinoma	Moderate	None
CGPLOV44	Ovarian Cancer	69	F	I	T1aN0M0	Ovary	Ovary	Mucinous adenocarcinoma	NA	None
CGPLH90	Healthy	43	F	NA	NA	NA	NA	NA	NA	NA
CGPLLU214	Lung Cancer	54	M	II	T2N1M0	Lung	Lung	Squamous Cell Carcinoma	NA	None
CGPLOV6	Ovarian Cancer	56	F	II	T2aN0M0	Ovary	Ovary	Serous Adenocarcinoma	Moderate	None

CGPLLU207	Lung Cancer	60	F	II	T2N1M0	Lung	Lung	Adenocarcinoma	Well	None
CGPLBR64	Breast Cancer	60	F	II	T2N0M0	Left Breast	Left Breast	Infiltrating Ductal Carcinoma	Poor	None
CGPLLU208	Lung Cancer	56	F	II	T2N1M0	Lung	Lung	Adenocarcinoma	Moderate	None
CGPLBR77	Breast Cancer	56	F	III	T2N2M0	Right Breast	Right Breast	Infiltrating Lobular Carcinoma	Moderate	None
CGPLOV11	Ovarian Cancer	51	F	IV	T3cN0M1	Right Ovary	Right Ovary	Endometrioid Adenoarcinoma	Moderate	Omentum
CGPLOV22	Ovarian Cancer	64	F	III	T1cNXMX	Left Ovary	Left Ovary	Serous Adenocarcinoma	Well	None
CGPLOV28	Ovarian Cancer	63	F	I	T1aNxM0	Right Ovary	Right Ovary	Serous carcinoma	NA	NA
CGCRC303	Colorectal Cancer	64	M	III	T3N1M0	Ascending	Ascending	Adenocarcinoma	Moderate	None
CGPLH91	Healthy	36	F	NA	NA	NA	NA	NA	NA	NA
CGPLLU177	Lung Cancer	45	M	II	T3N0M0	Right Lung	Right Lung	Adenocarcinoma	NA	None
CGPLLU168	Lung Cancer	70	F	I	T2aN0M0	Lung	Lung	Adenocarcinoma	Poor	None
CGCRC299	Colorectal Cancer	71	M	I	T1N0M0	Rectum	Rectum	Adenocarcinoma	Moderate	None
CGPLLU198	Lung Cancer	49	F	I	T2N0M0	Left Lung	Left Lung	Adenocarcinoma	Moderate	None
CGPLLU5	Lung Cancer	73	M	II	T3N0M0	Lung	Lung	Squamous Cell Carcinoma	Poor	None

CGPLLU174	Lung Cancer	56	M	I	T2N0M0	Lung	Lung	Adenocarcinoma	Poor	None
CGCRC313	Colorectal Cancer	64	M	III	T4N1M0	Sigmoideum	Sigmoideum	Adenocarcinoma	Well	None
CGPLBR93	Breast Cancer	59	F	II	T1N0M0	Breast	Breast	Infiltrating Ductal Carcinoma	Moderate	None
CGPLLU173	Lung Cancer	72	F	I	T2N0M0	Right Lung	Right Lung	Adenocarcinoma	Moderate	None
CGPLLU206	Lung Cancer	55	M	III	T3N1M0	Right Lung	Right Lung	Squamous Cell Carcinoma	Poor	None
CGPLBR82	Breast Cancer	70	F	I	T1N0M0	Right Breast	Right Breast	Infiltrating Lobular Carcinoma	Moderate	None
CGPLLU115	Lung Cancer	59	M	I	T2aN0M0	Lung	Lung	Adenocarcinoma	Poor	None
CGCRC305	Colorectal Cancer	83	F	II	T3N0M0	Transversum	Transversum	Adenocarcinoma	Moderate	None
CGPLOV43	Ovarian Cancer	30	F	I	T1aN0M0	Ovary	Ovary	Mucinous cystadenocarcinoma	NA	None
CGPLH55	Healthy	46	F	NA	NA	NA	NA	NA	NA	NA
CGPLOV46	Ovarian Cancer	58	F	I	T1bN0M0	Ovary	Ovary	Serous carcinoma	NA	None
CGPLLU180	Lung Cancer	57	M	I	T2N0M0	Right Lung	Right Lung	Large Cell Carcinoma	Poor	None
CGPLLU175	Lung Cancer	47	M	I	T2N0M0	Lung	Lung	Squamous Cell Carcinoma	Moderate	None
CGPLOV27	Ovarian Cancer	25	F	I	T1aN0MX	Ovary	Ovary	Germ Cell Tumor	Moderate	None

CGPLLU30	Lung Cancer	55	M	III	T2bN0M0	Lung	Lung	Squamous Cell Carcinoma	Moderate	None
CGCRC315	Colorectal Cancer	74	M	III	T3N1M0	Sigmoideum	Sigmoideum	Adenocarcinoma	Moderate	None
CGPLLU6	Lung Cancer	75	M	I	T2N0M0	Left Lung	Left Lung	Large Cell Carcinoma	Poor	None
CGPLOV1	Ovarian Cancer	48	F	I	T1N0M0	Ovary	Ovary	Mucinous Adenocarcinoma	Poor	None
CGPLOV41	Ovarian Cancer	57	F	IV	T3N0M1	Ovary	Ovary	Serous carcinoma	NA	Omentum, Uterus, and Cervix
CGPLBR104	Breast Cancer	68	F	II	T2N0M0	Right Breast	Right Breast	Infiltrating Lobular Carcinoma	Moderate	None
CGPLLU165	Lung Cancer	68	F	II	T1N1M0	Right Lung	Right Lung	Adenocarcinoma	Well	None
CGPLLU144	Lung Cancer	52	M	II	T2aN1M0	Lung	Lung	Adenocarcinoma	Poor	None
CGPLOV16	Ovarian Cancer	40	F	III	T3aN0M0	Ovary	Ovary	Serous Adenocarcinoma	Moderate	None
CGPLOV50	Ovarian Cancer	30	F	III	T3cN0M0	Ovary	Ovary	Serous carcinoma	NA	None
CGPLBR86	Breast Cancer	51	F	II	T2N1M0	Left Breast	Left Breast	Infiltrating Ductal Carcinoma	Poor	None
CGPLLU9	Lung Cancer	72	NA	IV	NA	Left Upper Lobe of Lung	Left Upper Lobe of Lung	Squamous Cell Carcinoma	NA	NA
CGPLOV19	Ovarian Cancer	52	F	II	T2aN0M0	Ovary	Ovary	Endometrioid Adenoarcinoma	Moderate	None
CGPLOV31	Ovarian Cancer	45	F	III	T3aNxM0	Right Ovary	Right Ovary	Clear cell adenocarcinoma	NA	None

CGPLLU66	Lung Cancer	71	M	I	T2N0M0	Left Lung	Left Lung	Adenocarcinoma	Poor	None
CGPLH50	Healthy	55	F	NA	NA	NA	NA	NA	NA	NA
CGPLLU203	Lung Cancer	66	M	II	T3N0M0	Right Lung	Right Lung	Squamous Cell Carcinoma	Well	None
CGCRC340	Colorectal Cancer	NA	NA	IV	NA	Colorectal	Liver-only metastasis	Adenocarcinoma	NA	Liver
CGPLLU29	Lung Cancer	65	F	I	T1bN0M0	Lung	Lung	Adenocarcinoma	Poor	None
CGPLH59	Healthy	34	F	NA	NA	NA	NA	NA	NA	NA
CGPLLU54	Lung Cancer	55	M	II	T2N1M0	Right Lung	Right Lung	Adenocarcinoma	Poor	None
CGPLLU119	Lung Cancer	67	M	II	T2bN1M0	Lung	Lung	Squamous Cell Carcinoma	Moderate	None
CGPLBR96	Breast Cancer	78	F	II	T2N0M0	Left Breast	Left Breast	Infiltrating Lobular Carcinoma	Moderate	None
CGPLOV14	Ovarian Cancer	46	F	I	T1bN0MX	Ovary	Ovary	Serous Adenocarcinoma	Poor	None
CGPLLU116	Lung Cancer	64	M	I	T1bN0M0	Lung	Lung	Squamous Cell Carcinoma	NA	None
CGPLH60	Healthy	31	F	NA	NA	NA	NA	NA	NA	NA
CGCRC304	Colorectal Cancer	86	F	II	T3N0M0	Rectum	Rectum	Adenocarcinoma	Moderate	None
CGPLLU162	Lung Cancer	38	M	II	T1N1M0	Right Lung	Right Lung	Adenocarcinoma	Moderate	None

CGCRC307	Colorectal Cancer	78	F	II	T3N0M0	Ascending	Ascending	Adenocarcinoma	Moderate	None
CGPLLU65	Lung Cancer	54	F	II	T2N1M0	Lung	Lung	Adenocarcinoma	Well	None
CGPLOV47	Ovarian Cancer	41	F	I	T1aN0M0	Ovary	Ovary	Serous cystadenoma	NA	None
CGPLLU19	Lung Cancer	47	F	I	T1N0M0	Lung	Lung	Adenocarcinoma	Poor	None
CGCRC317	Colorectal Cancer	74	M	III	T3N2M0	Descending	Descending	Adenocarcinoma	Moderate	None
CGPLOV13	Ovarian Cancer	62	F	IV	T1bN0M1	Right Ovary	Right Ovary	Endometrioid Adenoarcinoma	Poor	Omentum
CGCRC294	Colorectal Cancer	67	F	II	T3N0M0	Sigmoideum	CRC	Adenocarcinoma	Moderate	None
CGCRC296	Colorectal Cancer	76	F	II	T4N0M0	Coecum	Coecum	Adenocarcinoma	Poor	None
CGPLLU31	Lung Cancer	59	M	II	T2aN1M0	Lung	Lung	Squamous Cell Carcinoma	Moderate	None
CGCRC320	Colorectal Cancer	73	F	I	T2N0M0	Ascending	Ascending	Adenocarcinoma	Moderate	None
CGPLOV38	Ovarian Cancer	46	F	I	T1cN0M0	Ovary	Ovary	Serous carcinoma	NA	None
CGPLOV49	Ovarian Cancer	68	F	III	T3bN0M0	Ovary	Ovary	Serous carcinoma	NA	None
CGPLLU202	Lung Cancer	68	M	I	T2aN0M0	Right Lung	Right Lung	Adenocarcinoma	NA	None
CGPLLU138	Lung Cancer	32	M	II	T2N1M0	Lung	Lung	Large Cell Carcinoma	Poor	None

CGCRC309	Colorectal Cancer	83	F	III	T3N2M0	Transversum	Transversum	Adenocarcinoma	Poor	None
CGPLLU135	Lung Cancer	54	F	II	T2N1M0	Lung	Lung	Adenocarcinoma	Well	None
CGPLH45	Healthy	58	F	NA	NA	NA	NA	NA	NA	NA
CGPLLU117	Lung Cancer	59	M	III	T3N1M0	Lung	Lung	Carcinoma	Moderate	None
CGPLOV26	Ovarian Cancer	35	F	I	T1aN0M0	Ovary	Ovary	Germ Cell Tumor	Poor	None
CGPLOV23	Ovarian Cancer	47	F	I	T1aN0M0	Ovary	Ovary	Serous Adenocarcinoma	Poor	None
CGPLOV32	Ovarian Cancer	53	F	I	T1aNxM0	Left Ovary	Left Ovary	Mucinous cystadenoma	NA	None
CGPLLU64	Lung Cancer	55	F	I	T2N0M0	Lung	Lung	Adenocarcinoma	Poor	None
CGCRC306	Colorectal Cancer	80	F	II	T4N0M0	Ascending	Ascending	Adenocarcinoma	Moderate	None
CGPLH48	Healthy	38	F	NA	NA	NA	NA	NA	NA	NA
CGCRC318	Colorectal Cancer	81	M	I	T2N0M0	Coecum	Coecum	Adenocarcinoma	Moderate	None
CGPLBR101	Breast Cancer	46	F	II	T2N1M0	Left Breast	Left Breast	Infiltrating Lobular Carcinoma	Moderate	None
CGPLLU9	Lung Cancer	72	NA	IV	NA	Left Upper Lobe of Lung	Left Upper Lobe of Lung	Squamous Cell Carcinoma	NA	NA
CGPLH52	Healthy	40	F	NA	NA	NA	NA	NA	NA	NA

CGPLH56	Healthy	42	F	NA	NA	NA	NA	NA	NA	NA
CGCRC302	Colorectal Cancer	73	M	II	T3N0M0	Transversum	Transversum	Adenocarcinoma	Moderate	None
CGPLLU20	Lung Cancer	61	F	II	T1N1M0	Lung	Lung	Adenocarcinoma	Moderate	None
CGPLLU59	Lung Cancer	NA	NA	IV	T1bN0	Lung	Lung	Adenocarcinoma	NA	NA
CGPLH37	Healthy	39	F	NA	NA	NA	NA	NA	NA	NA
CGPLBR97	Breast Cancer	44	F	II	T2N0M0	Left Breast	Left Breast	Infiltrating Ductal Carcinoma	Moderate	None
CGCRC308	Colorectal Cancer	72	F	III	T4N2M0	Ascending	Ascending	Adenocarcinoma	Moderate	None
CGPLH54	Healthy	47	F	NA	NA	NA	NA	NA	NA	NA
CGPLH53	Healthy	50	F	NA	NA	NA	NA	NA	NA	NA
CGPLH57	Healthy	39	F	NA	NA	NA	NA	NA	NA	NA
CGPLBR88	Breast Cancer	48	F	II	T1N1M0	Left Breast	Left Breast	Infiltrating Ductal Carcinoma	Poor	None
CGCRC316	Colorectal Cancer	80	M	III	T3N2M0	Transversum	Transversum	Adenocarcinoma	Moderate	None
CGPLBR66	Breast Cancer	57	F	III	T4N1M0	Breast	Breast	Infiltrating Ductal Carcinoma	Well	None
CGPLLU137	Lung Cancer	35	F	I	T2N0M0	Lung	Lung	Squamous Cell Carcinoma	NA	None

CGPLH49	Healthy	39	F	NA	NA	NA	NA	NA	NA	NA
CGCRC339	Colorectal Cancer	NA	NA	IV	NA	Colorectal	Liver-only metastasis	Adenocarcinoma	NA	Liver
CGPLBR92	Breast Cancer	58	F	II	T2N1M0	Breast	Breast	Infiltrating Medullary Carcinoma	Poor	None
CGPLLU118	Lung Cancer	60	M	II	T2bN0M0	Lung	Lung	Small Cell Carcinoma	Poor	None
CGPLH58	Healthy	45	F	NA	NA	NA	NA	NA	NA	NA
CGPLOV21	Ovarian Cancer	51	F	IV	TanyN1M1	Ovary	Ovary	Serous Adenocarcinoma	Poor	Omentum amd Appendix
CGPLOV7	Ovarian Cancer	28	F	I	T1aN0M0	Right Ovary	Right Ovary	Mucinous Adenocarcinoma	Moderate	None
CGCRC334	Colorectal Cancer	NA	NA	IV	NA	Colorectal	Liver-only metastasis	Adenocarcinoma	NA	Liver
CGPLOV48	Ovarian Cancer	52	F	I	T1bN0M0	Ovary	Ovary	Serous carcinoma	NA	None
CGPLH46	Healthy	35	F	NA	NA	NA	NA	NA	NA	NA
CGPLH62	Healthy	41	F	NA	NA	NA	NA	NA	NA	NA
CGPLLU28	Lung Cancer	38	M	I	T2aN0M0	Lung	Lung	Squamous Cell Carcinoma	Poor	None
CGPLLU161	Lung Cancer	41	F	II	T3N0M0	Lung	Lung	Adenocarcinoma	Well	None
CGPLH43	Healthy	49	F	NA	NA	NA	NA	NA	NA	NA

CGPLLU163	Lung Cancer	66	M	II	T1N1M0	Left Lung	Left Lung	Adenocarcinoma	Poor	None
CGPLH47	Healthy	50	F	NA	NA	NA	NA	NA	NA	NA
CGPLOV8	Ovarian Cancer	43	F	I	T1N0M0	Left Ovary	Left Ovary	Mucinous Adenocarcinoma	NA	None
CGPLLU166	Lung Cancer	52	F	II	T2N1M0	Right Lung	Right Lung	Adenocarcinoma	Poor	None
CGPLLU209	Lung Cancer	65	M	II	T2aN0M0	Lung	Lung	Large Cell Carcinoma	Poor	None
CGCRC319	Colorectal Cancer	80	F	III	T2N1M0	Descending	Descending	Adenocarcinoma	Moderate	None
CGPLOV37	Ovarian Cancer	40	F	I	T1cN0M0	Ovary	Ovary	Serous carcinoma	NA	None
CGPLLU139	Lung Cancer	57	M	II	T2N1M0	Lung	Lung	Large Cell Carcinoma	Poor	None
CGPLH63	Healthy	47	F	NA	NA	NA	NA	NA	NA	NA
CGPLH64	Healthy	55	F	NA	NA	NA	NA	NA	NA	NA
CGPLLU136	Lung Cancer	51	F	II	T2N1M0	Lung	Lung	Adenocarcinoma	Moderate	None
CGPLOV3	Ovarian Cancer	59	F	III	T3bN0M0	Ovary	Ovary	Serous Adenocarcinoma	Poor	None
CGPLH42	Healthy	54	F	NA	NA	NA	NA	NA	NA	NA
CGPLLU47	Lung Cancer	65	NA	IV	NA	Lung	Lung	Squamous Cell Carcinoma	NA	NA

CGCRC342	Colorectal Cancer	NA	NA	IV	NA	Colorectal	Liver-only metastasis	Adenocarcinoma	NA	Liver
CGPLLU57	Lung Cancer	NA	NA	III	NA	Lung	Lung	Squamous Cell Carcinoma	NA	None
CGPLH51	Healthy	48	F	NA	NA	NA	NA	NA	NA	NA
CGPLLU21	Lung Cancer	63	F	I	T2N0M0	Lung	Lung	Adenocarcinoma	Moderate	None
CGPLLU44	Lung Cancer	80	NA	IV	NA	Right Upper Lobe of Lung	Right Upper Lobe of Lung	Adenosquamous Carcinoma	NA	Liver / Bone
CGPLH40	Healthy	37	F	NA	NA	NA	NA	NA	NA	NA
CGPLH35	Healthy	48	F	NA	NA	NA	NA	NA	NA	NA
CGPLLU26	Lung Cancer	56	M	I	T2aN0M0	Lung	Lung	Adenocarcinoma	Moderate	None
CGPLOV42	Ovarian Cancer	52	F	I	T3aN0M0	Ovary	Ovary	Serous carcinoma	NA	None
CGPLH39	Healthy	41	F	NA	NA	NA	NA	NA	NA	NA
CGPLLU44	Lung Cancer	80	NA	IV	NA	Right Upper Lobe of Lung	Right Upper Lobe of Lung	Adenosquamous Carcinoma	NA	Liver / Bone
CGPLLU57	Lung Cancer	NA	NA	III	NA	Lung	Lung	Squamous Cell Carcinoma	NA	None
CGPLLU59	Lung Cancer	NA	NA	IV	T1bN0	Lung	Lung	Adenocarcinoma	NA	NA
CGPLOV5	Ovarian Cancer	38	F	IV	T3bN0M1	Ovary	Ovary	Serous Adenocarcinoma	Moderate	Peritoneum amd Appendix

CGCRC310	Colorectal Cancer	85	M	II	T4N0M0	Coecum	Coecum	Adenocarcinoma	Moderate	None
CGPLLU47	Lung Cancer	65	NA	IV	NA	Lung	Lung	Squamous Cell Carcinoma	NA	NA
CGPLOV40	Ovarian Cancer	53	F	IV	T3N0M1	Ovary	Ovary	Serous carcinoma	NA	Omentum, Uterus, and Appendix
CGPLH41	Healthy	49	F	NA	NA	NA	NA	NA	NA	NA
CGPLLU22	Lung Cancer	76	M	I	T2N0M0	Left Lung	Left Lung	Large Cell Carcinoma	Poor	None
CGPLOV9	Ovarian Cancer	47	F	II	T2cN0M0	Left Ovary	Left Ovary	Endometrioid Adenoarcinoma	Well	None
CGPLH36	Healthy	36	F	NA	NA	NA	NA	NA	NA	NA
CGPLH44	Healthy	30	F	NA	NA	NA	NA	NA	NA	NA
CGPLLU63	Lung Cancer	46	F	I	T1N0M0	Left Lung	Left Lung	Adenocarcinoma	Poor	None
CGPLLU164	Lung Cancer	66	M	II	T1N1M0	Left Lung	Left Lung	Adenocarcinoma	Poor	None
CGPLH61	Healthy	32	F	NA	NA	NA	NA	NA	NA	NA
CGPLLU23	Lung Cancer	56	M	I	T2N0M0	Left Lung	Left Lung	Adenosquamous Carcinoma	Moderate	None
CGPLLU2	Lung Cancer	72	M	II	T1bN0M0	Lung	Lung	Adenocarcinoma	Moderate	None
CGPLLU67	Lung Cancer	67	M	II	T2N1M0	Left Lung	Left Lung	Adenocarcinoma	Poor	None

CGPLH38	Healthy	47	F	NA	NA	NA	NA	NA	NA	NA
CGPLLU14	Lung Cancer	59	NA	IV	T1N1M0	Right Lower lobe	Right Lower lobe	Adenocarcinoma	Moderate	NA
CGCRC338	Colorectal Cancer	NA	NA	IV	NA	Colorectal	Liver-only metastasis	Adenocarcinoma	NA	Liver
CGCRC335	Colorectal Cancer	NA	NA	IV	NA	Colorectal	Liver-only metastasis	Adenocarcinoma	NA	Liver
CGPLLU3	Lung Cancer	55	M	I	T2aN0M0	Lung	Lung	Squamous Cell Carcinoma	Moderate	None
CGPLBR87	Breast Cancer	80	F	II	T2N1M0	Righ Breast	Righ Breast	Papillary Carcinoma	Well	None
CGCRC333	Colorectal Cancer	NA	NA	IV	NA	Colorectal	Liver-only metastasis	Adenocarcinoma	NA	Liver
CGCRC341	Colorectal Cancer	NA	NA	IV	NA	Colorectal	Liver-only metastasis	Adenocarcinoma	NA	Liver
CGPLLU68	Lung Cancer	61	M	II	T2N1M0	Lung	Lung	Adenocarcinoma	Moderate	None
CGPLBR83	Breast Cancer	53	F	II	T2N1M0	Right Breast	Right Breast	Infiltrating Ductal Carcinoma	Moderate	None
CGCRC337	Colorectal Cancer	NA	NA	IV	NA	Colorectal	Liver-only metastasis	Adenocarcinoma	NA	Liver
CGCRC336	Colorectal Cancer	NA	NA	IV	NA	Colorectal	Liver-only metastasis	Adenocarcinoma	NA	Liver
CGPLLU1	Lung Cancer	67	M	I	T1N0M0	Right Lung	Right Lung	Adenocarcinoma	NA	None
CGPLOV4	Ovarian Cancer	13	F	III	T3bN0M0	Ovary	Ovary	Granulosa	Poor	None

*NA denotes either not available or in the case of healthy samples not applicable. All samples were from patients that had not been previously treated with the exception of lung cancer cases CPPLLU14, CPPLLU44, CPPLLU47, CPPLLU57, CPPLLU59, and CPPLLU9. Multiple values for volume of plasma and cfDNA extracted are indicated for cases with serial blood draws.

Table 3.2. Summary of sample data for patients analyzed.

Patient	Analysis Type	Volume of Plasma (ml)	cfDNA Extracted (ng/ml)	cfDNA Input (ng/ml)
CGPLBR53	TEC-Seq and Targeted NGS	4.20	1.26	1.26
CGPLH80	TEC-Seq	4.00	1.94	1.94
CGPLH78	TEC-Seq	4.00	2.51	2.51
CGPLH79	TEC-Seq	4.00	3.68	3.68
CGPLBR49	TEC-Seq and Targeted NGS	4.00	5.74	5.74
CGPLH82	TEC-Seq	4.00	3.30	3.30
CGPLBR38	TEC-Seq and Targeted NGS	4.00	5.77	5.77
CGPLBR55	TEC-Seq and Targeted NGS	4.30	4.57	4.57
CGPLBR69	TEC-Seq and Targeted NGS	4.40	4.07	4.07
CGPLH85	TEC-Seq	4.00	2.58	2.58
CGPLBR39	TEC-Seq and Targeted NGS	4.30	7.53	7.53
CGPLBR57	TEC-Seq and Targeted NGS	4.30	4.02	4.02
CGPLBR72	TEC-Seq and Targeted NGS	3.90	4.43	4.43
CGPLBR48	TEC-Seq and Targeted NGS	3.90	7.07	7.07
CGPLH86	TEC-Seq	4.00	4.23	4.23
CGPLH76	TEC-Seq	4.00	4.03	4.03
CGPLBR71	TEC-Seq and Targeted NGS	3.10	7.64	7.64
CGPLBR42	TEC-Seq	4.00	6.34	6.34
CGPLOV15	TEC-Seq and Targeted NGS	5.00	4.77	4.77
CGPLH84	TEC-Seq	4.00	3.33	3.33
CGPLBR103	TEC-Seq	3.60	7.11	7.11
CGPLOV17	TEC-Seq and Targeted NGS	4.50	3.76	3.76
CGCRC301	TEC-Seq and Targeted NGS	4.10	6.51	6.51
CGPLBR75	TEC-Seq and Targeted NGS	4.50	5.89	5.89
CGPLBR76	TEC-Seq and Targeted NGS	4.90	8.71	8.71

CGPLLU176	TEC-Seq and Targeted NGS	3.20	7.86	7.86
CGCRC332	TEC-Seq and Targeted NGS	3.30	4.46	4.46
CGPLBR70	TEC-Seq and Targeted NGS	3.40	11.94	11.94
CGCRC293	TEC-Seq and Targeted NGS	7.20	3.83	3.83
CGPLOV18	TEC-Seq and Targeted NGS	3.10	7.81	7.81
CGPLBR68	TEC-Seq and Targeted NGS	3.40	10.41	10.41
CGPLBR41	TEC-Seq	4.50	11.56	11.56
CGPLOV20	TEC-Seq and Targeted NGS	4.20	5.67	5.67
CGPLLU145	TEC-Seq	3.00	10.30	10.30
CGPLLU178	TEC-Seq and Targeted NGS	3.00	10.78	10.78
CGPLH75	TEC-Seq	4.00	3.87	3.87
CGPLOV24	TEC-Seq	4.20	10.71	10.71
CGPLBR63	TEC-Seq and Targeted NGS	4.00	6.19	6.19
CGPLBR74	TEC-Seq and Targeted NGS	4.50	8.13	8.13
CGPLBR100	TEC-Seq and Targeted NGS	4.00	4.25	4.25
CGPLLU147	TEC-Seq and Targeted NGS	3.80	6.72	6.72
CGCRC311	TEC-Seq and Targeted NGS	8.50	3.91	3.91
CGPLBR73	TEC-Seq and Targeted NGS	3.30	14.69	14.69
CGPLOV25	TEC-Seq	4.80	6.78	6.78
CGPLBR40	TEC-Seq and Targeted NGS	4.60	15.69	15.69
CGCRC312	TEC-Seq and Targeted NGS	8.90	2.91	2.91
CGPLLU169	TEC-Seq and Targeted NGS	4.20	13.70	13.70
CGPLLU197	TEC-Seq and Targeted NGS	4.40	6.87	6.87
CGPLH81	TEC-Seq	4.00	5.16	5.16
CGPLBR99	TEC-Seq and Targeted NGS	4.50	6.78	6.78
CGPLBR44	TEC-Seq and Targeted NGS	3.70	12.81	12.81
CGPLBR59	TEC-Seq and Targeted NGS	4.10	8.24	8.24
CGPLOV10	TEC-Seq and Targeted NGS	3.40	7.09	7.09

CGCRC291	TEC-Seq and Targeted NGS	7.90	7.80	7.80
CGPLLU179	TEC-Seq and Targeted NGS	3.40	12.13	12.13
CGCRC321	TEC-Seq and Targeted NGS	9.30	4.25	4.25
CGCRC298	TEC-Seq and Targeted NGS	4.20	14.09	14.09
CGCRC292	TEC-Seq and Targeted NGS	7.90	6.73	6.73
CGPLLU204	TEC-Seq and Targeted NGS	4.10	10.64	10.64
CGPLLU170	TEC-Seq	4.50	12.46	12.46
CGPLBR61	TEC-Seq	4.10	13.25	13.25
CGPLBR102	TEC-Seq	3.60	13.67	13.67
CGPLOV12	TEC-Seq	3.20	12.44	12.44
CGPLBR43	TEC-Seq	4.20	13.35	13.35
CGPLLU14	TEC-Seq	2.00	2.55	2.55
CGCRC297	TEC-Seq and Targeted NGS	4.40	8.83	8.83
CGPLLU4	TEC-Seq	4.50	5.18	5.18
CGPLH83	TEC-Seq	4.00	5.04	5.04
CGPLLU146	TEC-Seq and Targeted NGS	3.60	13.72	13.72
CGPLOV2	TEC-Seq and Targeted NGS	4.09	9.66	9.66
CGPLBR91	TEC-Seq and Targeted NGS	3.20	22.41	22.41
CGCRC295	TEC-Seq and Targeted NGS	8.50	5.02	5.02
CGCRC314	TEC-Seq and Targeted NGS	8.50	5.77	5.77
CGPLBR67	TEC-Seq and Targeted NGS	3.70	15.59	15.59
CGPLBR80	TEC-Seq and Targeted NGS	4.30	9.70	9.70
CGPLH77	TEC-Seq	4.00	5.89	5.89
CGPLLU205	TEC-Seq and Targeted NGS	4.00	18.56	18.56
CGCRC300	TEC-Seq and Targeted NGS	4.30	10.48	10.48
CGPLOV44	TEC-Seq	4.50	8.79	8.79
CGPLH90	TEC-Seq	3.60	9.79	9.79
CGPLLU214	TEC-Seq	4.80	7.45	7.45

CGPLOV6	TEC-Seq and Targeted NGS	3.50	11.03	11.03
CGPLLU207	TEC-Seq and Targeted NGS	4.00	17.29	17.29
CGPLBR64	TEC-Seq and Targeted NGS	4.50	9.42	9.42
CGPLLU208	TEC-Seq and Targeted NGS	3.00	24.34	24.34
CGPLBR77	TEC-Seq and Targeted NGS	4.65	14.58	14.58
CGPLOV11	TEC-Seq and Targeted NGS	3.40	17.35	17.35
CGPLOV22	TEC-Seq and Targeted NGS	4.60	17.42	17.42
CGPLOV28	TEC-Seq	3.20	10.74	10.74
CGCRC303	TEC-Seq and Targeted NGS	9.20	7.65	7.65
CGPLH91	TEC-Seq	3.60	7.81	7.81
CGPLLU177	TEC-Seq and Targeted NGS	3.90	19.07	19.07
CGPLLU168	TEC-Seq and Targeted NGS	4.30	19.38	19.38
CGCRC299	TEC-Seq and Targeted NGS	8.80	10.18	10.18
CGPLLU198	TEC-Seq and Targeted NGS	4.20	14.09	14.09
CGPLLU5	TEC-Seq and Targeted NGS	4.50	7.91	7.91
CGPLLU174	TEC-Seq and Targeted NGS	3.00	18.16	18.16
CGCRC313	TEC-Seq and Targeted NGS	8.70	6.65	6.65
CGPLBR93	TEC-Seq	3.30	27.94	27.94
CGPLLU173	TEC-Seq	4.50	13.76	13.76
CGPLLU206	TEC-Seq and Targeted NGS	3.50	18.24	18.24
CGPLBR82	TEC-Seq and Targeted NGS	4.75	23.39	23.39
CGPLLU115	TEC-Seq and Targeted NGS	5.00	10.80	10.80
CGCRC305	TEC-Seq and Targeted NGS	8.60	9.10	9.10
CGPLOV43	TEC-Seq	4.40	9.09	9.09
CGPLH55	TEC-Seq	4.00	7.35	7.35
CGPLOV46	TEC-Seq	4.10	8.97	8.97
CGPLLU180	TEC-Seq and Targeted NGS	3.20	19.31	19.31
CGPLLU175	TEC-Seq and Targeted NGS	4.40	16.84	16.84

CGPLOV27	TEC-Seq	4.00	19.39	19.39
CGPLLU30	TEC-Seq and Targeted NGS	5.00	14.20	14.20
CGCRC315	TEC-Seq and Targeted NGS	8.60	9.67	9.67
CGPLLU6	TEC-Seq and Targeted NGS	4.39	11.57	11.57
CGPLOV1	TEC-Seq and Targeted NGS	4.70	9.98	9.98
CGPLOV41	TEC-Seq	4.40	10.03	10.03
CGPLBR104	TEC-Seq	4.70	19.89	19.89
CGPLLU165	TEC-Seq and Targeted NGS	4.50	20.13	20.13
CGPLLU144	TEC-Seq and Targeted NGS	3.50	31.51	31.51
CGPLOV16	TEC-Seq and Targeted NGS	4.50	27.28	27.28
CGPLOV50	TEC-Seq	4.50	8.89	8.89
CGPLBR86	TEC-Seq and Targeted NGS	3.70	19.89	19.89
CGPLLU9	TEC-Seq	6.00	12.48	12.48
CGPLOV19	TEC-Seq and Targeted NGS	5.00	23.46	23.46
CGPLOV31	TEC-Seq	4.00	14.45	14.45
CGPLLU66	TEC-Seq and Targeted NGS	5.00	3.40	3.40
CGPLH50	TEC-Seq	4.00	7.05	7.05
CGPLLU203	TEC-Seq and Targeted NGS	4.20	26.24	26.24
CGCRC340	TEC-Seq and Targeted NGS	3.50	9.57	9.57
CGPLLU29	TEC-Seq	5.00	8.00	8.00
CGPLH59	TEC-Seq	4.00	6.03	6.03
CGPLLU54	TEC-Seq and Targeted NGS	4.00	9.75	9.75
CGPLLU119	TEC-Seq and Targeted NGS	5.00	16.00	16.00
CGPLBR96	TEC-Seq and Targeted NGS	3.80	24.45	24.45
CGPLOV14	TEC-Seq and Targeted NGS	3.70	24.43	24.43
CGPLLU116	TEC-Seq and Targeted NGS	5.00	17.20	17.20
CGPLH60	TEC-Seq	4.00	1.48	1.48
CGCRC304	TEC-Seq and Targeted NGS	4.10	30.19	30.19

CGPLLU162	TEC-Seq and Targeted NGS	3.10	40.32	40.32
CGCRC307	TEC-Seq and Targeted NGS	8.50	14.26	14.26
CGPLLU65	TEC-Seq and Targeted NGS	5.00	12.20	12.20
CGPLOV47	TEC-Seq	4.50	19.35	19.35
CGPLLU19	TEC-Seq and Targeted NGS	3.50	12.46	12.46
CGCRC317	TEC-Seq and Targeted NGS	8.80	16.08	16.08
CGPLOV13	TEC-Seq and Targeted NGS	3.80	27.00	27.00
CGCRC294	TEC-Seq and Targeted NGS	8.40	18.87	18.87
CGCRC296	TEC-Seq and Targeted NGS	4.30	31.24	31.24
CGPLLU31	TEC-Seq and Targeted NGS	4.80	4.38	4.38
CGCRC320	TEC-Seq and Targeted NGS	4.50	30.37	30.37
CGPLOV38	TEC-Seq	2.40	34.29	34.29
CGPLOV49	TEC-Seq	4.20	16.48	16.48
CGPLLU202	TEC-Seq and Targeted NGS	4.40	24.72	24.72
CGPLLU138	TEC-Seq and Targeted NGS	5.00	4.60	4.60
CGCRC309	TEC-Seq and Targeted NGS	8.50	17.46	17.46
CGPLLU135	TEC-Seq and Targeted NGS	5.00	13.80	13.80
CGPLH45	TEC-Seq	4.00	10.85	10.85
CGPLLU117	TEC-Seq and Targeted NGS	5.00	7.00	7.00
CGPLOV26	TEC-Seq	4.50	27.90	27.90
CGPLOV23	TEC-Seq	5.00	26.73	26.73
CGPLOV32	TEC-Seq	3.20	27.36	27.36
CGPLLU64	TEC-Seq and Targeted NGS	5.00	15.60	15.60
CGCRC306	TEC-Seq and Targeted NGS	4.50	24.31	24.31
CGPLH48	TEC-Seq	4.00	6.38	6.38
CGCRC318	TEC-Seq and Targeted NGS	9.80	18.24	18.24
CGPLBR101	TEC-Seq	4.00	37.88	37.88
CGPLLU9	TEC-Seq	6.00	9.78	9.78

CGPLH52	TEC-Seq	4.00	9.90	9.90
CGPLH56	TEC-Seq	4.00	5.20	5.20
CGCRC302	TEC-Seq and Targeted NGS	4.30	52.13	52.13
CGPLLU20	TEC-Seq and Targeted NGS	4.39	15.49	15.49
CGPLLU59	TEC-Seq	3.00	12.00	12.00
CGPLH37	TEC-Seq	4.00	9.73	9.73
CGPLBR97	TEC-Seq and Targeted NGS	4.20	43.21	43.21
CGCRC308	TEC-Seq and Targeted NGS	4.30	46.87	46.87
CGPLH54	TEC-Seq	4.00	14.18	14.18
CGPLH53	TEC-Seq	4.00	4.78	4.78
CGPLH57	TEC-Seq	4.00	7.15	7.15
CGPLBR88	TEC-Seq and Targeted NGS	3.60	49.75	49.75
CGCRC316	TEC-Seq and Targeted NGS	4.90	52.16	52.16
CGPLBR66	TEC-Seq and Targeted NGS	4.80	24.11	24.11
CGPLLU137	TEC-Seq and Targeted NGS	5.00	19.80	19.80
CGPLH49	TEC-Seq	4.00	6.60	6.60
CGCRC339	TEC-Seq and Targeted NGS	4.50	15.56	15.56
CGPLBR92	TEC-Seq and Targeted NGS	3.10	81.00	81.00
CGPLLU118	TEC-Seq and Targeted NGS	4.00	13.50	13.50
CGPLH58	TEC-Seq	4.00	5.55	5.55
CGPLOV21	TEC-Seq and Targeted NGS	4.30	56.32	56.32
CGPLOV7	TEC-Seq and Targeted NGS	5.00	10.80	10.80
CGCRC334	TEC-Seq and Targeted NGS	4.50	21.36	21.36
CGPLOV48	TEC-Seq	3.50	22.8	22.80
CGPLH46	TEC-Seq	4.00	8.25	8.25
CGPLH62	TEC-Seq	4.00	2.28	2.28
CGPLLU28	TEC-Seq and Targeted NGS	5.00	30.20	30.20
CGPLLU161	TEC-Seq	4.00	83.04	62.50

CGPLH43	TEC-Seq	4.00	8.50	8.50
CGPLLU163	TEC-Seq and Targeted NGS	5.00	54.03	50.00
CGPLH47	TEC-Seq	4.00	7.43	7.43
CGPLOV8	TEC-Seq and Targeted NGS	5.00	14.80	14.80
CGPLLU166	TEC-Seq and Targeted NGS	5.00	46.14	46.14
CGPLLU209	TEC-Seq and Targeted NGS	5.50	53.95	45.45
CGCRC319	TEC-Seq and Targeted NGS	4.20	53.54	53.54
CGPLOV37	TEC-Seq	3.20	46.88	46.88
CGPLLU139	TEC-Seq and Targeted NGS	5.00	22.00	22.00
CGPLH63	TEC-Seq	4.00	10.10	10.10
CGPLH64	TEC-Seq	4.00	8.03	8.03
CGPLLU136	TEC-Seq and Targeted NGS	5.00	25.80	25.80
CGPLOV3	TEC-Seq and Targeted NGS	4.51	13.53	13.53
CGPLH42	TEC-Seq	4.00	14.30	14.30
CGPLLU47	TEC-Seq	7.00	22.29	22.29
CGCRC342	TEC-Seq	3.90	56.09	56.09
CGPLLU57	TEC-Seq	3.00	11.43	11.43
CGPLH51	TEC-Seq	4.00	7.85	7.85
CGPLLU21	TEC-Seq and Targeted NGS	2.00	44.00	44.00
CGPLLU44	TEC-Seq	5.00	35.08	35.08
CGPLH40	TEC-Seq	4.00	2.70	2.70
CGPLH35	TEC-Seq	4.00	13.15	13.15
CGPLLU26	TEC-Seq and Targeted NGS	5.00	4.60	4.60
CGPLOV42	TEC-Seq	4.20	49.51	49.51
CGPLH39	TEC-Seq	5.00	3.08	3.08
CGPLLU44	TEC-Seq	5.00	43.40	43.40
CGPLLU57	TEC-Seq	3.00	25.00	25.00
CGPLLU59	TEC-Seq	3.00	10.13	10.13

CGPLOV5	TEC-Seq	4.50	36.42	36.42
CGCRC310	TEC-Seq and Targeted NGS	4.60	72.12	72.12
CGPLLU47	TEC-Seq	8.00	24.00	24.00
CGPLOV40	TEC-Seq	1.60	193.6	156.25
CGPLH41	TEC-Seq	4.00	4.73	4.73
CGPLLU22	TEC-Seq and Targeted NGS	5.00	44.00	44.00
CGPLOV9	TEC-Seq and Targeted NGS	5.00	8.4	8.40
CGPLH36	TEC-Seq	4.00	13.00	13.00
CGPLH44	TEC-Seq	4.00	9.08	9.08
CGPLLU63	TEC-Seq and Targeted NGS	5.00	41.40	41.40
CGPLLU164	TEC-Seq and Targeted NGS	4.80	79.54	52.08
CGPLH61	TEC-Seq	4.00	7.15	7.15
CGPLLU23	TEC-Seq and Targeted NGS	5.00	29.00	29.00
CGPLLU2	TEC-Seq and Targeted NGS	4.50	44.22	44.22
CGPLLU67	TEC-Seq and Targeted NGS	5.00	80.00	80.00
CGPLH38	TEC-Seq	4.00	8.38	8.38
CGPLLU14	TEC-Seq	3.00	14.33	14.33
CGCRC338	TEC-Seq and Targeted NGS	2.30	109.76	109.76
CGCRC335	TEC-Seq and Targeted NGS	4.60	59.07	59.07
CGPLLU3	TEC-Seq and Targeted NGS	4.50	52.76	52.76
CGPLBR87	TEC-Seq and Targeted NGS	3.60	277.39	69.44
CGCRC333	TEC-Seq and Targeted NGS	4.00	113.88	113.88
CGCRC341	TEC-Seq	4.60	156.62	156.62
CGPLLU68	TEC-Seq	4.00	120.25	120.25
CGPLBR83	TEC-Seq and Targeted NGS	3.70	100.17	100.17
CGCRC337	TEC-Seq and Targeted NGS	3.40	208.66	208.66
CGCRC336	TEC-Seq and Targeted NGS	4.40	211.74	211.74
CGPLLU1	TEC-Seq and Targeted NGS	5.12	223.07	223.07

CGPLOV4	TEC-Seq	4.00	273.90	273.90

Table 3.3. Genes analyzed using TEC-Seq.

Gene	Region Analyzed	Gene Category*
ABL1	Specific Exons	Cancer Driver Gene
AKT1	Specific Exons	Cancer Driver Gene
ALK	Full Coding Region	Cancer Driver Gene
APC	Specific Exons	Cancer Driver Gene
AR	Full Coding Region	Cancer Driver Gene
ATM	Specific Exons	Cancer Driver and Clonal Hematopoiesis Gene
BRAF	Full Coding Region	Cancer Driver Gene
CDH1	Specific Exons	Cancer Driver Gene
CDK4	Full Coding Region	Cancer Driver Gene
CDK6	Full Coding Region	Cancer Driver Gene
CDKN2A	Specific Exons	Cancer Driver Gene
CSF1R	Specific Exons	Cancer Driver Gene
CTNNB1	Specific Exons	Cancer Driver Gene
DNMT3A	Specific Exons	Cancer Driver and Clonal Hematopoiesis Gene
EGFR	Full Coding Region	Cancer Driver Gene
ERBB2	Specific Exons	Cancer Driver Gene
ERBB4	Full Coding Region	Cancer Driver Gene
ESR1	Full Coding Region	Cancer Driver Gene
EZH2	Specific Exons	Cancer Driver Gene
FBXW7	Specific Exons	Cancer Driver Gene
FGFR1	Specific Exons	Cancer Driver Gene
FGFR2	Specific Exons	Cancer Driver Gene
FGFR3	Specific Exons	Cancer Driver Gene
FLT3	Specific Exons	Cancer Driver Gene

GNA11	Specific Exons	Cancer Driver Gene
GNAQ	Specific Exons	Cancer Driver Gene
GNAS	Specific Exons	Cancer Driver and Clonal Hematopoiesis Gene
HNF1A	Specific Exons	Cancer Driver Gene
HRAS	Full Coding Region	Cancer Driver Gene
IDH1	Specific Exons	Cancer Driver and Clonal Hematopoiesis Gene
IDH2	Specific Exons	Cancer Driver and Clonal Hematopoiesis Gene
JAK2	Full Coding Region	Cancer Driver and Clonal Hematopoiesis Gene
JAK3	Specific Exons	Cancer Driver Gene
KDR	Specific Exons	Cancer Driver Gene
KIT	Full Coding Region	Cancer Driver Gene
KRAS	Full Coding Region	Cancer Driver Gene
MAP2K1	Specific Exons	Cancer Driver Gene
MET	Specific Exons	Cancer Driver Gene
MLH1	Specific Exons	Cancer Driver Gene
MPL	Specific Exons	Cancer Driver Gene
MYC	Specific Exons	Cancer Driver Gene
NPM1	Specific Exons	Cancer Driver Gene
NRAS	Full Coding Region	Cancer Driver Gene
PDGFRA	Full Coding Region	Cancer Driver Gene
PIK3CA	Full Coding Region	Cancer Driver Gene
PIK3R1	Specific Exons	Cancer Driver Gene
PTEN	Full Coding Region	Cancer Driver Gene
PTPN11	Specific Exons	Cancer Driver Gene
RB1	Specific Exons	Cancer Driver Gene
RET	Specific Exons	Cancer Driver Gene
SMAD4	Specific Exons	Cancer Driver Gene
SMARCB1	Specific Exons	Cancer Driver Gene

SMO	Specific Exons	Cancer Driver Gene
SRC	Specific Exons	Cancer Driver Gene
STK11	Full Coding Region	Cancer Driver Gene
TERT	Specific Exons	Cancer Driver Gene
TP53	Full Coding Region	Cancer Driver Gene
VHL	Specific Exons	Cancer Driver Gene

* Analyzed genes inlcuded those that have been commonly implicated in cancer *(22)*. Specific genes (DNMT3A, IDH1, and IDH2) or certain alterations with genes (ATM amino acid residue 3008, GNAS amino acid residue 202 and JAK2 amino acid residue 617) were considered related to blood cell proliferation *(25-27)*.

Table 3.4. Cancer cases containing alterations in driver genes.

Tissue Type	Cases in COSMIC	Detectable Cases*	Detectable Fraction
Breast	1,002	719	72%
Colorectal	1,248	1,071	86%
Lung	1,198	932	78%
Ovarian	647	524	81%

*Detectable cases indicate those with at least one alteration from the cancer driver genes analyzed.

Table 3.5. Summary of TEC-Seq validation.

Sample Type*	Gene Symbol	Nucleotide	Amino Acid (protein)	Dilution	Expected Mutant Allele Fraction (%)	Observed Mutant Allele Fraction (%)	Mutation Detection	Interpretation	Total Distinct Coverage	Distinct Mutant Coverage	Hotspot Alteration
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	20.00%	15.32%	13.03%	Detected	True Positive	3322	433	Yes
CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	20.00%	19.94%	12.10%	Detected	True Positive	7977	965	Yes
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	20.00%	15.32%	12.34%	Detected	True Positive	1418	175	Yes
CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	20.00%	19.94%	13.50%	Detected	True Positive	1474	199	Yes
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	0.50%	0.77%	2.64%	Detected	True Positive	2651	70	Yes
CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	0.50%	1.00%	2.66%	Detected	True Positive	8482	226	Yes
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	2.00%	1.53%	1.41%	Detected	True Positive	4249	60	Yes

CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	2.00%	1.99%	1.16%	Detected	True Positive	10583	123	Yes
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	0.20%	0.31%	0.79%	Detected	True Positive	3305	26	Yes
CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	0.20%	0.40%	0.77%	Detected	True Positive	10351	80	Yes
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	1.00%	0.77%	0.71%	Detected	True Positive	4505	32	Yes
CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	1.00%	1.00%	0.50%	Detected	True Positive	10377	52	Yes
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	0.10%	0.15%	0.38%	Detected	True Positive	4222	16	Yes
CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	0.10%	0.20%	0.28%	Detected	True Positive	11181	31	Yes
Mutant Pool	NRAS	chr1_115256529-115256529_T_C	61Q>R	1.00%	1.15%	0.41%	Detected	True Positive	2706	11	Yes
Mutant Pool	NRAS	chr1_115258744-115258744_C_T	13G>D	1.00%	1.15%	0.48%	Detected	True Positive	5263	25	Yes

Mutant Pool	NRAS	chr1_115258747-115258747_C_A	12G>V	1.00%	0.53%	0.29%	Detected	True Positive	5210	15	Yes
Mutant Pool	KRAS	chr12_25378562-25378562_C_T	146A>T	1.00%	1.40%	0.89%	Detected	True Positive	2698	24	Yes
Mutant Pool	KRAS	chr12_25380276-25380276_T_A	61Q>L	1.00%	0.76%	0.31%	Detected	True Positive	3197	10	Yes
Mutant Pool	KRAS	chr12_25398284-25398284_C_T	12G>D	1.00%	0.85%	0.37%	Detected	True Positive	2669	10	Yes
Mutant Pool	ERBB2	chr17_37880998-37880998__TGT	776G>VC	1.00%	0.87%	0.54%	Detected	True Positive	8572	46	No
Mutant Pool	BRAF	chr7_140453136-140453136_A_T	600V>E	1.00%	1.55%	1.83%	Detected	True Positive	2840	52	Yes
Mutant Pool	EGFR	chr7_55241707-55241707_G_A	719G>S	1.00%	0.83%	0.73%	Detected	True Positive	3814	28	Yes
Mutant Pool	EGFR	chr7_55242466-55242474_GAATTAAGA_	746ELR>-	1.00%	1.25%	0.88%	Detected	True Positive	4547	40	No
Mutant Pool	EGFR	chr7_55249071-55249071_C_T	790T>M	1.00%	0.85%	1.03%	Detected	True Positive	9868	102	Yes

Mutant Pool	EGFR	chr7_55259515-55259515_T_G	858L>R	1.00%	0.79%	1.12%	Detected	True Positive	6081	68	Yes
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	5.00%	7.66%	18.11%	Detected	True Positive	2623	475	Yes
CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	5.00%	9.97%	18.57%	Detected	True Positive	3252	604	Yes
Mutant Pool	NRAS	chr1_115256529-115256529_T_C	61Q>R	0.50%	0.58%	0.41%	Detected	True Positive	1932	8	Yes
Mutant Pool	NRAS	chr1_115258744-115258744_C_T	13G>D	0.50%	0.57%	0.55%	Detected	True Positive	3988	22	Yes
Mutant Pool	NRAS	chr1_115258747-115258747_C_A	12G>V	0.50%	0.27%	0.25%	Detected	True Positive	3974	10	Yes
Mutant Pool	KRAS	chr12_25378562-25378562_C_T	146A>T	0.50%	0.70%	0.56%	Detected	True Positive	1773	10	Yes
Mutant Pool	KRAS	chr12_25380276-25380276_T_A	61Q>L	0.50%	0.38%	0.26%	Detected	True Positive	2274	6	Yes
Mutant Pool	KRAS	chr12_25398284-25398284_C_T	12G>D	0.50%	0.42%	0.36%	Detected	True Positive	1680	6	Yes

Mutant Pool	ERBB2	chr17_37880998-37880998__TGT	776G>VC	0.50%	0.44%	0.38%	Detected	True Positive	7964	30	No
Mutant Pool	BRAF	chr7_140453136-140453136_A_T	600V>E	0.50%	0.78%	0.83%	Detected	True Positive	1802	15	Yes
Mutant Pool	EGFR	chr7_55241707-55241707_G_A	719G>S	0.50%	0.41%	0.58%	Detected	True Positive	2746	16	Yes
Mutant Pool	EGFR	chr7_55242466-55242474_GAATTAAGA_	746ELR>-	0.50%	0.63%	1.21%	Detected	True Positive	3233	39	No
Mutant Pool	EGFR	chr7_55249071-55249071_C_T	790T>M	0.50%	0.43%	0.77%	Detected	True Positive	9640	74	Yes
Mutant Pool	EGFR	chr7_55259515-55259515_T_G	858L>R	0.50%	0.39%	0.55%	Detected	True Positive	5092	28	Yes
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	2.00%	3.06%	5.80%	Detected	True Positive	2466	143	Yes
CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	2.00%	3.99%	5.76%	Detected	True Positive	3400	196	Yes
Mutant Pool	NRAS	chr1_115256529-115256529_T_C	61Q>R	0.20%	0.23%	0.23%	Detected	True Positive	2651	6	Yes

Mutant Pool	NRAS	chr1_115258744-115258744_C_T	13G>D	0.20%	0.23%	0.07%	Detected	True Positive	5379	4	Yes
Mutant Pool	KRAS	chr12_25378562-25378562_C_T	146A>T	0.20%	0.28%	0.16%	Detected	True Positive	2510	4	Yes
Mutant Pool	KRAS	chr12_25398284-25398284_C_T	12G>D	0.20%	0.17%	0.17%	Detected	True Positive	2289	4	Yes
Mutant Pool	ERBB2	chr17_37880998-37880998__TGT	776G>VC	0.20%	0.17%	0.21%	Detected	True Positive	9489	20	No
Mutant Pool	BRAF	chr7_140453136-140453136_A_T	600V>E	0.20%	0.31%	0.41%	Detected	True Positive	2416	10	Yes
Mutant Pool	EGFR	chr7_55241707-55241707_G_A	719G>S	0.20%	0.17%	0.11%	Detected	True Positive	3493	4	Yes
Mutant Pool	EGFR	chr7_55242466-55242474_GAATTAAGA_	746ELR>-	0.20%	0.25%	0.42%	Detected	True Positive	4014	17	No
Mutant Pool	EGFR	chr7_55249071-55249071_C_T	790T>M	0.20%	0.17%	0.22%	Detected	True Positive	10070	22	Yes
Mutant Pool	EGFR	chr7_55259515-55259515_T_G	858L>R	0.20%	0.16%	0.38%	Detected	True Positive	6590	25	Yes

Mutant Pool	NRAS	chr1_115258747-115258747_C_A	12G>V	0.20%	0.11%	0.06%	Not Detected	False Negative	5380	3	Yes
Mutant Pool	KRAS	chr12_25380276-25380276_T_A	61Q>L	0.20%	0.15%	NA	Not Detected	False Negative	NA	NA	Yes
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	1.00%	1.53%	2.96%	Detected	True Positive	2665	79	Yes
CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	1.00%	1.99%	3.30%	Detected	True Positive	3149	104	Yes
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	2.00%	1.53%	1.39%	Detected	True Positive	4666	65	Yes
CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	2.00%	1.99%	1.31%	Detected	True Positive	9546	125	Yes
Mutant Pool	NRAS	chr1_115258744-115258744_C_T	13G>D	0.10%	0.11%	0.12%	Detected	True Positive	5104	6	Yes
Mutant Pool	KRAS	chr12_25378562-25378562_C_T	146A>T	0.10%	0.14%	0.21%	Detected	True Positive	2357	5	Yes
Mutant Pool	KRAS	chr12_25380276-25380276_T_A	61Q>L	0.10%	0.08%	0.15%	Detected	True Positive	2732	4	Yes

Mutant Pool	KRAS	chr12_25398284-25398284_C_T	12G>D	0.10%	0.08%	0.17%	Detected	True Positive	2334	4	Yes
Mutant Pool	BRAF	chr7_140453136-140453136_A_T	600V>E	0.10%	0.16%	0.13%	Detected	True Positive	2300	3	Yes
Mutant Pool	EGFR	chr7_55241707-55241707_G_A	719G>S	0.10%	0.08%	0.23%	Detected	True Positive	3472	8	Yes
Mutant Pool	EGFR	chr7_55242466-55242474_GAATTAAGA_	746ELR>-	0.10%	0.13%	0.29%	Detected	True Positive	3825	11	No
Mutant Pool	EGFR	chr7_55249071-55249071_C_T	790T>M	0.10%	0.09%	0.17%	Detected	True Positive	10159	17	Yes
Mutant Pool	EGFR	chr7_55259515-55259515_T_G	858L>R	0.10%	0.08%	0.13%	Detected	True Positive	6255	8	Yes
Mutant Pool	NRAS	chr1_115258747-115258747_C_A	12G>V	0.10%	0.05%	0.06%	Not Detected	False Negative	5111	3	Yes
Mutant Pool	NRAS	chr1_115256529-115256529_T_C	61Q>R	0.10%	0.12%	NA	Not Detected	False Negative	NA	NA	Yes
Mutant Pool	ERBB2	chr17_37880998-37880998__TGT	776G>VC	0.10%	0.09%	NA	Not Detected	False Negative	NA	NA	No

CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	0.50%	0.77%	1.34%	Detected	True Positive	2318	31	Yes
CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	0.50%	1.00%	1.16%	Detected	True Positive	2929	34	Yes
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	1.00%	0.77%	1.09%	Detected	True Positive	4844	53	Yes
CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	1.00%	1.00%	1.05%	Detected	True Positive	10306	108	Yes
Mutant Pool	NRAS	chr1_115256529-115256529_T_C	61Q>R	5.00%	5.77%	4.55%	Detected	True Positive	660	30	Yes
Mutant Pool	NRAS	chr1_115258744-115258744_C_T	13G>D	5.00%	5.75%	6.81%	Detected	True Positive	1219	83	Yes
Mutant Pool	NRAS	chr1_115258747-115258747_C_A	12G>V	5.00%	2.67%	2.70%	Detected	True Positive	1223	33	Yes
Mutant Pool	KRAS	chr12_25378562-25378562_C_T	146A>T	5.00%	7.02%	5.24%	Detected	True Positive	1011	53	Yes
Mutant Pool	KRAS	chr12_25380276-25380276_T_A	61Q>L	5.00%	3.80%	4.12%	Detected	True Positive	1214	50	Yes

Mutant Pool	KRAS	chr12_25398284-25398284_C_T	12G>D	5.00%	4.25%	5.96%	Detected	True Positive	1174	70	Yes
Mutant Pool	ERBB2	chr17_37880998-37880998__TGT	776G>VC	5.00%	4.37%	5.14%	Detected	True Positive	1479	76	No
Mutant Pool	BRAF	chr7_140453136-140453136_A_T	600V>E	5.00%	7.76%	8.35%	Detected	True Positive	1761	147	Yes
Mutant Pool	EGFR	chr7_55241707-55241707_G_A	719G>S	5.00%	4.14%	3.42%	Detected	True Positive	2020	69	Yes
Mutant Pool	EGFR	chr7_55242466-55242474_GAATTAAGA_	746ELR>-	5.00%	6.26%	6.51%	Detected	True Positive	2459	160	No
Mutant Pool	EGFR	chr7_55249071-55249071_C_T	790T>M	5.00%	4.26%	4.24%	Detected	True Positive	2617	111	Yes
Mutant Pool	EGFR	chr7_55259515-55259515_T_G	858L>R	5.00%	3.95%	5.13%	Detected	True Positive	2455	126	Yes
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	5.00%	7.66%	20.16%	Detected	True Positive	2465	497	Yes
CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	5.00%	9.97%	18.71%	Detected	True Positive	3378	632	Yes

Mutant Pool	NRAS	chr1_115256529-115256529_T_C	61Q>R	2.00%	2.31%	1.58%	Detected	True Positive	886	14	Yes
Mutant Pool	NRAS	chr1_115258744-115258744_C_T	13G>D	2.00%	2.30%	1.93%	Detected	True Positive	1398	27	Yes
Mutant Pool	NRAS	chr1_115258747-115258747_C_A	12G>V	2.00%	1.07%	2.13%	Detected	True Positive	1408	30	Yes
Mutant Pool	KRAS	chr12_25378562-25378562_C_T	146A>T	2.00%	2.81%	2.20%	Detected	True Positive	1225	27	Yes
Mutant Pool	KRAS	chr12_25380276-25380276_T_A	61Q>L	2.00%	1.52%	2.06%	Detected	True Positive	1411	29	Yes
Mutant Pool	KRAS	chr12_25398284-25398284_C_T	12G>D	2.00%	1.70%	2.77%	Detected	True Positive	1407	39	Yes
Mutant Pool	ERBB2	chr17_37880998-37880998__TGT	776G>VC	2.00%	1.75%	1.19%	Detected	True Positive	1677	20	No
Mutant Pool	BRAF	chr7_140453136-140453136_A_T	600V>E	2.00%	3.10%	4.95%	Detected	True Positive	1575	78	Yes
Mutant Pool	EGFR	chr7_55241707-55241707_G_A	719G>S	2.00%	1.66%	1.86%	Detected	True Positive	1829	34	Yes

Mutant Pool	EGFR	chr7_55242466-55242474_GAATTAAGA_	746ELR>-	2.00%	2.50%	4.00%	Detected	True Positive	1977	79	No
Mutant Pool	EGFR	chr7_55249071-55249071_C_T	790T>M	2.00%	1.70%	2.35%	Detected	True Positive	2257	53	Yes
Mutant Pool	EGFR	chr7_55259515-55259515_T_G	858L>R	2.00%	1.58%	3.26%	Detected	True Positive	2238	73	Yes
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	2.00%	3.06%	5.50%	Detected	True Positive	2889	159	Yes
CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	2.00%	3.99%	4.89%	Detected	True Positive	3295	161	Yes
Mutant Pool	NRAS	chr1_115256529-115256529_T_C	61Q>R	1.00%	1.15%	0.36%	Detected	True Positive	845	3	Yes
Mutant Pool	NRAS	chr1_115258744-115258744_C_T	13G>D	1.00%	1.15%	1.13%	Detected	True Positive	1410	16	Yes
Mutant Pool	NRAS	chr1_115258747-115258747_C_A	12G>V	1.00%	0.53%	0.56%	Detected	True Positive	1425	8	Yes
Mutant Pool	KRAS	chr12_25378562-25378562_C_T	146A>T	1.00%	1.40%	1.11%	Detected	True Positive	1261	14	Yes

Mutant Pool	KRAS	chr12_25380276-25380276_T_A	61Q>L	1.00%	0.76%	1.14%	Detected	True Positive	1400	16	Yes
Mutant Pool	KRAS	chr12_25398284-25398284_C_T	12G>D	1.00%	0.85%	1.32%	Detected	True Positive	1360	18	Yes
Mutant Pool	ERBB2	chr17_37880998-37880998__TGT	776G>VC	1.00%	0.87%	0.82%	Detected	True Positive	1706	14	No
Mutant Pool	BRAF	chr7_140453136-140453136_A_T	600V>E	1.00%	1.55%	2.84%	Detected	True Positive	1479	42	Yes
Mutant Pool	EGFR	chr7_55241707-55241707_G_A	719G>S	1.00%	0.83%	1.15%	Detected	True Positive	1478	17	Yes
Mutant Pool	EGFR	chr7_55242466-55242474_GAATTAAGA_	746ELR>-	1.00%	1.25%	3.10%	Detected	True Positive	1711	53	No
Mutant Pool	EGFR	chr7_55249071-55249071_C_T	790T>M	1.00%	0.85%	1.75%	Detected	True Positive	1768	31	Yes
Mutant Pool	EGFR	chr7_55259515-55259515_T_G	858L>R	1.00%	0.79%	1.50%	Detected	True Positive	1665	25	Yes
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	1.00%	1.53%	2.86%	Detected	True Positive	2693	77	Yes

CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	1.00%	1.99%	3.03%	Detected	True Positive	3363	102	Yes
Mutant Pool	NRAS	chr1_115256529-115256529_T_C	61Q>R	1.00%	1.15%	1.22%	Detected	True Positive	2301	28	Yes
Mutant Pool	NRAS	chr1_115258744-115258744_C_T	13G>D	1.00%	1.15%	0.91%	Detected	True Positive	4528	41	Yes
Mutant Pool	NRAS	chr1_115258747-115258747_C_A	12G>V	1.00%	0.53%	0.53%	Detected	True Positive	4525	24	Yes
Mutant Pool	KRAS	chr12_25378562-25378562_C_T	146A>T	1.00%	1.40%	1.16%	Detected	True Positive	2074	24	Yes
Mutant Pool	KRAS	chr12_25380276-25380276_T_A	61Q>L	1.00%	0.76%	0.60%	Detected	True Positive	2656	16	Yes
Mutant Pool	KRAS	chr12_25398284-25398284_C_T	12G>D	1.00%	0.85%	0.86%	Detected	True Positive	2095	18	Yes
Mutant Pool	ERBB2	chr17_37880998-37880998__TGT	776G>VC	1.00%	0.87%	0.83%	Detected	True Positive	8463	70	No
Mutant Pool	BRAF	chr7_140453136-140453136_A_T	600V>E	1.00%	1.55%	2.93%	Detected	True Positive	2250	66	Yes

Mutant Pool	EGFR	chr7_55241707-55241707_G_A	719G>S	1.00%	0.83%	1.47%	Detected	True Positive	3617	53	Yes
Mutant Pool	EGFR	chr7_55242466-55242474_GAATTAAGA_	746ELR>-	1.00%	1.25%	2.26%	Detected	True Positive	4644	105	No
Mutant Pool	EGFR	chr7_55249071-55249071_C_T	790T>M	1.00%	0.85%	1.65%	Detected	True Positive	10973	181	Yes
Mutant Pool	EGFR	chr7_55259515-55259515_T_G	858L>R	1.00%	0.79%	1.51%	Detected	True Positive	6603	100	Yes
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	100.00%	-	76.58%	Detected	True Positive	538	412	Yes
CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	100.00%	-	99.70%	Detected	True Positive	2019	2013	Yes
Mutant Pool	NRAS	chr1_115256529-115256529_T_C	61Q>R	0.50%	0.58%	0.52%	Detected	True Positive	965	5	Yes
Mutant Pool	NRAS	chr1_115258744-115258744_C_T	13G>D	0.50%	0.57%	0.57%	Detected	True Positive	1752	10	Yes
Mutant Pool	NRAS	chr1_115258747-115258747_C_A	12G>V	0.50%	0.27%	0.17%	Detected	True Positive	1736	3	Yes

Mutant Pool	KRAS	chr12_25378562-25378562_C_T	146A>T	0.50%	0.70%	0.39%	Detected	True Positive	1294	5	Yes
Mutant Pool	KRAS	chr12_25380276-25380276_T_A	61Q>L	0.50%	0.38%	0.87%	Detected	True Positive	1502	13	Yes
Mutant Pool	KRAS	chr12_25398284-25398284_C_T	12G>D	0.50%	0.42%	0.34%	Detected	True Positive	1490	5	Yes
Mutant Pool	ERBB2	chr17_37880998-37880998__TGT	776G>VC	0.50%	0.44%	0.63%	Detected	True Positive	1747	11	No
Mutant Pool	BRAF	chr7_140453136-140453136_A_T	600V>E	0.50%	0.78%	1.45%	Detected	True Positive	1381	20	Yes
Mutant Pool	EGFR	chr7_55241707-55241707_G_A	719G>S	0.50%	0.41%	0.83%	Detected	True Positive	1447	12	Yes
Mutant Pool	EGFR	chr7_55242466-55242474_GAATTAAGA_	746ELR>-	0.50%	0.63%	1.16%	Detected	True Positive	1726	20	No
Mutant Pool	EGFR	chr7_55249071-55249071_C_T	790T>M	0.50%	0.43%	0.83%	Detected	True Positive	2038	17	Yes
Mutant Pool	EGFR	chr7_55259515-55259515_T_G	858L>R	0.50%	0.39%	1.01%	Detected	True Positive	1885	19	Yes

CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	0.50%	0.77%	1.31%	Detected	True Positive	2210	29	Yes
CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	0.50%	1.00%	1.34%	Detected	True Positive	2995	40	Yes
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	10.00%	7.66%	8.31%	Detected	True Positive	1228	102	Yes
CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	10.00%	9.97%	7.22%	Detected	True Positive	1329	96	Yes
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	10.00%	7.66%	10.69%	Detected	True Positive	1076	115	Yes
CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	10.00%	9.97%	8.30%	Detected	True Positive	1338	111	Yes
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	60.00%	45.95%	44.31%	Detected	True Positive	1607	712	Yes
CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	60.00%	59.82%	47.05%	Detected	True Positive	4884	2298	Yes
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	25.00%	38.29%	55.61%	Detected	True Positive	624	347	Yes

CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	25.00%	49.85%	62.87%	Detected	True Positive	2769	1741	Yes
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	40.00%	30.63%	38.11%	Detected	True Positive	1257	479	Yes
CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	40.00%	39.88%	40.23%	Detected	True Positive	4514	1816	Yes
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	20.00%	30.63%	48.98%	Detected	True Positive	831	407	Yes
CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	20.00%	39.88%	53.94%	Detected	True Positive	3298	1779	Yes
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	60.00%	45.95%	29.40%	Detected	True Positive	2000	588	Yes
CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	60.00%	59.82%	32.58%	Detected	True Positive	5547	1807	Yes
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	5.00%	7.66%	17.69%	Detected	True Positive	2481	439	Yes
CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	5.00%	9.97%	17.97%	Detected	True Positive	7366	1324	Yes

CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	60.00%	45.95%	42.29%	Detected	True Positive	1336	565	Yes
CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	60.00%	59.82%	42.94%	Detected	True Positive	1423	611	Yes
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	40.00%	30.63%	23.89%	Detected	True Positive	2704	646	Yes
CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	40.00%	39.88%	20.96%	Detected	True Positive	5910	1239	Yes
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	2.00%	3.06%	8.58%	Detected	True Positive	2447	210	Yes
CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	2.00%	3.99%	8.71%	Detected	True Positive	7809	680	Yes
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	40.00%	30.63%	30.50%	Detected	True Positive	1459	445	Yes
CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	40.00%	39.88%	26.52%	Detected	True Positive	1463	388	Yes
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	20.00%	15.32%	10.11%	Detected	True Positive	3473	351	Yes

CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	20.00%	19.94%	9.68%	Detected	True Positive	8319	805	Yes
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	1.00%	1.53%	4.27%	Detected	True Positive	3749	160	Yes
CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	1.00%	1.99%	4.67%	Detected	True Positive	9312	435	Yes
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	20.00%	15.32%	15.12%	Detected	True Positive	1435	217	Yes
CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	20.00%	19.94%	12.21%	Detected	True Positive	1687	206	Yes
Mutant Pool	NRAS	chr1_115256529-115256529_T_C	61Q>R	100.00%	NA	5.41%	Detected	True Positive	1424	77	Yes
Mutant Pool	NRAS	chr1_115258744-115258744_C_T	13G>D	100.00%	NA	5.39%	Detected	True Positive	2711	146	Yes
Mutant Pool	NRAS	chr1_115258747-115258747_C_A	12G>V	100.00%	NA	2.51%	Detected	True Positive	2714	68	Yes
Mutant Pool	KRAS	chr12_25378562-25378562_C_T	146A>T	100.00%	NA	6.57%	Detected	True Positive	1293	85	Yes

Mutant Pool	KRAS	chr12_25380276-25380276_T_A	61Q>L	100.00%	NA	3.56%	Detected	True Positive	1685	60	Yes
Mutant Pool	KRAS	chr12_25398284-25398284_C_T	12G>D	100.00%	NA	3.98%	Detected	True Positive	1206	48	Yes
Mutant Pool	ERBB2	chr17_37880998-37880998__TGT	776G>VC	100.00%	NA	4.09%	Detected	True Positive	6618	271	No
Mutant Pool	BRAF	chr7_140453136-140453136_A_T	600V>E	100.00%	NA	7.26%	Detected	True Positive	1817	132	Yes
Mutant Pool	EGFR	chr7_55241707-55241707_G_A	719G>S	100.00%	NA	3.88%	Detected	True Positive	3944	153	Yes
Mutant Pool	EGFR	chr7_55242466-55242474_GAATTAAGA_	746ELR>-	100.00%	NA	5.86%	Detected	True Positive	4623	271	No
Mutant Pool	EGFR	chr7_55249071-55249071_C_T	790T>M	100.00%	NA	3.99%	Detected	True Positive	11759	469	Yes
Mutant Pool	EGFR	chr7_55259515-55259515_T_G	858L>R	100.00%	NA	3.70%	Detected	True Positive	7032	260	Yes
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	0.50%	0.77%	2.61%	Detected	True Positive	3486	91	Yes

CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	0.50%	1.00%	2.45%	Detected	True Positive	9375	230	Yes
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	60.00%	45.95%	44.99%	Detected	True Positive	1336	601	Yes
CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	60.00%	59.82%	46.00%	Detected	True Positive	1413	650	Yes
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	0.20%	0.31%	0.57%	Detected	True Positive	3306	19	Yes
CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	0.20%	0.40%	0.51%	Detected	True Positive	9081	46	Yes
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	40.00%	30.63%	29.44%	Detected	True Positive	1505	443	Yes
CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	40.00%	39.88%	27.94%	Detected	True Positive	1571	439	Yes
CGBR4C	PIK3CA	chr3_178952085-178952085_A_G	1047H>R	0.10%	0.15%	0.29%	Detected	True Positive	5179	15	Yes
CGBR4C	TP53	chr17_7578442-7578442_T_C	163Y>C	0.10%	0.20%	0.39%	Detected	True Positive	12397	48	Yes

*Mutant pools represent a mixture of cell lines obtained from ATCC and combined in ratios to reflect the mutant allele frequency. The cell lines include CCL-237, CRL-2158, CRL-2547, CRL-7585, CRL-9068, CRL-2177, CCL-231, CRL-2871, CRL-5908, CRL-5908, CCL-224, and CRL-5894. Dilution replicates can be identified by distinct sample type, dilution, DNA yield, and observed mutant allele fraction.

Table 3.6. Summary of TEC-Seq validation sequencing statistics.

Sample Type*	Analysis Type	Genome Build	DNA Yield (ng)	Read Length	Bases Sequenced	Bases Mapped to Genome	Percent Mapped to Genome	Bases Mapped to Target Regions	Percent Mapped to Target Regions	Total Coverage	Distinct Coverage
CGBR4C	TEC-Seq	HG19	250	100	6856574800	6812015300	99%	3214675331	47%	31980	12660
CGBR4C	TEC-Seq	HG19	25	100	6735169400	6651278500	99%	2612823217	39%	25984	2156
CGBR4C	TEC-Seq	HG19	250	100	6710441800	6611461900	99%	2594449575	39%	25764	10461
CGBR4C	TEC-Seq	HG19	250	100	7114561400	7041572000	99%	3198864566	45%	31659	10081
CGBR4C	TEC-Seq	HG19	250	100	6227890400	6169346900	99%	2823618330	46%	28033	10958
CGBR4C	TEC-Seq	HG19	250	100	7190500800	7112278700	99%	3193164477	45%	31592	9350
CGBR4C	TEC-Seq	HG19	250	100	7044900400	6982583400	99%	3229966262	46%	32052	10665
Mutant Pool	TEC-Seq	HG19	250	100	7360101200	7291843600	99%	3569490069	49%	35090	10074
CGBR4C	TEC-Seq	HG19	25	100	6763442800	6730757000	100%	3211592781	48%	31851	5149
Mutant Pool	TEC-Seq	HG19	250	100	7138154800	7038364300	99%	2966510847	42%	29544	11353
CGBR4C	TEC-Seq	HG19	25	100	7222610600	7176256200	99%	3400025435	47%	33721	3772
Mutant Pool	TEC-Seq	HG19	250	100	7089526000	7022804100	99%	3307747509	47%	32957	10527
Mutant Pool	TEC-Seq	HG19	250	100	7089526000	7022804100	99%	3307747509	47%	32957	10527
CGBR4C	TEC-Seq	HG19	25	100	6922188600	6872546100	99%	3158459299	46%	31326	3523
CGBR4C	TEC-Seq	HG19	250	100	6989895800	6934399400	99%	3347919878	48%	33138	9126
Mutant Pool	TEC-Seq	HG19	250	100	7515707000	7445146400	99%	3467813924	47%	34525	11325
CGBR4C	TEC-Seq	HG19	25	100	6901678400	6846752700	99%	3093886686	45%	30679	2945
CGBR4C	TEC-Seq	HG19	250	100	7649029000	7568166000	99%	3348483905	44%	33106	10230

Mutant Pool	TEC-Seq	HG19	25	100	7028013400	6990286200	99%	3657071276	52%	36433	2260
CGBR4C	TEC-Seq	HG19	25	100	6361754800	6329308300	99%	2979822154	47%	29641	5256
Mutant Pool	TEC-Seq	HG19	25	100	7000288400	6947587900	99%	3389157517	49%	33746	2215
CGBR4C	TEC-Seq	HG19	25	100	6530419600	6486680200	99%	3021521069	47%	30041	3962
Mutant Pool	TEC-Seq	HG19	25	100	6574107400	6523160400	99%	3123803265	48%	31109	1963
CGBR4C	TEC-Seq	HG19	25	100	6751453800	6680328100	99%	2838275125	42%	28125	3534
Mutant Pool	TEC-Seq	HG19	250	100	6888519600	6839602800	99%	3399663697	50%	33754	10098
CGBR4C	TEC-Seq	HG19	250	100	9328679400	9301652800	100%	4425280583	48%	43863	17086
Mutant Pool	TEC-Seq	HG19	25	100	6043536000	6003423400	99%	2899146002	48%	28872	1993
CGBR4C	TEC-Seq	HG19	25	100	5951876000	5886480200	99%	2490875896	42%	24765	3181
CGBR4C	TEC-Seq	HG19	25	100	7032340600	6986282200	99%	3133755150	45%	31111	1819
CGBR4C	TEC-Seq	HG19	25	100	6718561600	6677846600	99%	3035627349	45%	30107	1627
CGBR4C	TEC-Seq	HG19	250	100	8402040800	8362876000	100%	3890439877	47%	38555	13466
CGBR4C	TEC-Seq	HG19	250	100	8312266800	8273298600	100%	3850085281	47%	38164	16139
CGBR4C	TEC-Seq	HG19	250	100	7623931600	7578468100	99%	3435971145	45%	34092	14205
CGBR4C	TEC-Seq	HG19	250	100	7914309600	7875670900	100%	3653166119	46%	36204	15328
CGBR4C	TEC-Seq	HG19	250	100	7498391600	7435573600	99%	3582296548	48%	35502	11256
CGBR4C	TEC-Seq	HG19	250	100	8089340600	8036693300	99%	3787340645	47%	37548	14257
CGBR4C	TEC-Seq	HG19	25	100	7129435800	7093160200	99%	3231584828	46%	32109	3268
CGBR4C	TEC-Seq	HG19	250	100	7556623000	7479077700	99%	3431817875	46%	33986	10652

CGBR4C	TEC-Seq	HG19	250	100	6409081000	6366777900	99%	3022620692	47%	29975	11921
CGBR4C	TEC-Seq	HG19	25	100	6717712400	6677656200	99%	3030471882	45%	30118	2678
CGBR4C	TEC-Seq	HG19	250	100	7395864800	7306951100	99%	3308676352	45%	32774	10615
CGBR4C	TEC-Seq	HG19	250	100	7175702200	7126131000	99%	3358722525	47%	33287	11117
CGBR4C	TEC-Seq	HG19	25	100	7038038000	6989836500	99%	3230463272	46%	32110	2318
Mutant Pool	TEC-Seq	HG19	250	100	6334363200	6295098000	99%	3362894228	53%	33478	9227
CGBR4C	TEC-Seq	HG19	250	100	6964052000	6884217700	99%	2922966719	42%	28980	9915
CGBR4C	TEC-Seq	HG19	25	100	6391984800	6363907800	100%	2949774853	46%	29325	3179
CGBR4C	TEC-Seq	HG19	250	100	6476025200	6400463500	99%	2706820015	42%	26826	9558
CGBR4C	TEC-Seq	HG19	25	100	6864653400	6824745400	99%	3115598042	46%	30962	2950
CGBR4C	TEC-Seq	HG19	250	100	8167591600	8103370000	99%	3853829182	48%	38208	11502

*Mutant pools represent a mixture of cell lines obtained from ATCC and combined in ratios to reflect the mutant allele frequency. The cell lines include CCL-237, CRL-2158, CRL-2547, CRL-7585, CRL-9068, CRL-2177, CCL-231, CRL-2871, CRL-5908, CRL-5908, CCL-224, and CRL-5894. Dilution replicates can be identified by distinct sample type, dilution, DNA yield, and observed mutant allele fraction.

Table 3.7. Summary of genomic analyses.

Patient	Patient Type	Sample Type	Analysis Type	Read Length	Bases in Target Region	Bases Mapped to Genome	Bases Mapped to Target Regions	Percent Mapped to Target Regions	Total Coverage	Distinct Coverage	Tumor Normal Match (%)
CGPLBR100	Breast Cancer	cfDNA	TEC-Seq	100	80930	7299964400	3750278051	51%	44794	3249	NA
CGPLBR100	Breast Cancer	Germline DNA	Targeted NGS	150	639291	587752350	303296797	52%	466	316	100%
CGPLBR100	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	989328900	456255327	46%	702	259	100%
CGPLBR101	Breast Cancer	cfDNA	TEC-Seq	100	80930	7420822800	3810365416	51%	45565	9784	NA
CGPLBR102	Breast Cancer	cfDNA	TEC-Seq	100	80930	6679304900	3269688319	49%	38679	7613	NA
CGPLBR103	Breast Cancer	cfDNA	TEC-Seq	100	80930	7040304400	3495542468	50%	41786	6748	NA
CGPLBR104	Breast Cancer	cfDNA	TEC-Seq	100	80930	7188389200	3716096781	52%	44316	9448	NA
CGPLBR38	Breast Cancer	cfDNA	TEC-Seq	100	80930	7810293900	4057576306	52%	48098	9868	NA
CGPLBR38	Breast Cancer	Germline DNA	Targeted NGS	150	639291	607162800	295989325	49%	453	312	100%
CGPLBR38	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	1300314150	653992005	50%	998	529	100%
CGPLBR39	Breast Cancer	cfDNA	TEC-Seq	100	80930	7745701500	3805623239	49%	45084	11065	NA
CGPLBR39	Breast Cancer	Germline DNA	Targeted NGS	150	639291	536928000	258238473	48%	395	284	100%
CGPLBR39	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	1462893000	727470248	50%	1104	560	100%

CGPLBR40	Breast Cancer	cfDNA	TEC-Seq	100	80930	7558990500	3652442341	48%	43333	12948	NA
CGPLBR40	Breast Cancer	Germline DNA	Targeted NGS	150	639291	504208500	270658416	54%	410	298	100%
CGPLBR40	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	1265950500	761635668	60%	1154	457	100%
CGPLBR41	Breast Cancer	cfDNA	TEC-Seq	100	80930	7900994600	3836600101	49%	45535	10847	NA
CGPLBR42	Breast Cancer	cfDNA	TEC-Seq	100	80930	7756986400	3456255655	45%	40980	7211	NA
CGPLBR43	Breast Cancer	cfDNA	TEC-Seq	100	80930	7561881700	3880346064	51%	46098	8588	NA
CGPLBR44	Breast Cancer	cfDNA	TEC-Seq	100	80930	7017744200	3269110569	47%	38672	8344	NA
CGPLBR44	Breast Cancer	Germline DNA	Targeted NGS	150	639291	574791000	316417055	55%	484	331	100%
CGPLBR44	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	759286350	357135705	47%	542	112	100%
CGPLBR48	Breast Cancer	cfDNA	TEC-Seq	100	80930	5629044200	2611554623	46%	30860	8652	NA
CGPLBR48	Breast Cancer	Germline DNA	Targeted NGS	150	639291	635913150	357101607	56%	547	387	100%
CGPLBR48	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	1096215750	558883146	51%	857	289	100%
CGPLBR49	Breast Cancer	cfDNA	TEC-Seq	100	80930	5784711600	2673457893	46%	31274	10429	NA
CGPLBR49	Breast Cancer	Germline DNA	Targeted NGS	150	639291	594631950	337522575	57%	512	347	100%
CGPLBR49	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	1273922850	773715963	61%	1174	396	100%

CGPLBR53	Breast Cancer	cfDNA	TEC-Seq	100	80930	7223682000	3635203812	50%	43212	4722	NA
CGPLBR53	Breast Cancer	Germline DNA	Targeted NGS	150	639291	588261000	341304903	58%	518	348	100%
CGPLBR53	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	1543663050	928939469	60%	1408	651	100%
CGPLBR55	Breast Cancer	cfDNA	TEC-Seq	100	80930	8309154900	4306956261	52%	51143	8328	NA
CGPLBR55	Breast Cancer	Germline DNA	Targeted NGS	150	639291	428455950	119796448	28%	180	117	100%
CGPLBR55	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	1281568800	786549957	61%	1179	544	100%
CGPLBR57	Breast Cancer	cfDNA	TEC-Seq	100	80930	8636181000	4391502618	51%	52108	5857	NA
CGPLBR57	Breast Cancer	Germline DNA	Targeted NGS	150	639291	549441900	314057412	57%	481	330	100%
CGPLBR57	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	1014956550	514492105	51%	787	218	100%
CGPLBR59	Breast Cancer	cfDNA	TEC-Seq	100	80930	8799457700	4152328555	47%	49281	5855	NA
CGPLBR59	Breast Cancer	Germline DNA	Targeted NGS	150	639291	677024100	354381703	52%	537	346	100%
CGPLBR59	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	1502988750	722936771	48%	1075	206	100%
CGPLBR61	Breast Cancer	cfDNA	TEC-Seq	100	80930	8163706700	3952010628	48%	46755	8522	NA
CGPLBR63	Breast Cancer	cfDNA	TEC-Seq	100	80930	7020533100	3542447304	50%	41956	4773	NA
CGPLBR63	Breast Cancer	Germline DNA	Targeted NGS	150	639291	504208500	270658416	54%	410	298	100%

CGPLBR63	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	1265950500	761635668	60%	1154	457	100%
CGPLBR64	Breast Cancer	cfDNA	TEC-Seq	100	80930	7300630900	3292529227	45%	38909	5202	NA
CGPLBR64	Breast Cancer	Germline DNA	Targeted NGS	150	639291	413363850	193561159	47%	291	186	100%
CGPLBR64	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	1511564550	760271556	50%	1137	119	100%
CGPLBR66	Breast Cancer	cfDNA	TEC-Seq	100	80930	6963339500	3554735108	51%	42011	6399	NA
CGPLBR66	Breast Cancer	Germline DNA	Targeted NGS	150	639291	603047250	339194896	56%	513	366	100%
CGPLBR66	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	1263813900	736742733	58%	1119	626	100%
CGPLBR67	Breast Cancer	cfDNA	TEC-Seq	100	80930	8264353900	3686093696	45%	43516	7752	NA
CGPLBR67	Breast Cancer	Germline DNA	Targeted NGS	150	639291	699562950	409741164	59%	623	410	100%
CGPLBR67	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	1218949500	732812428	60%	1112	326	100%
CGPLBR68	Breast Cancer	cfDNA	TEC-Seq	100	80930	7629312300	4078969547	53%	48389	7402	NA
CGPLBR68	Breast Cancer	Germline DNA	Targeted NGS	150	639291	672,427,650	353,985,085	53%	539	332	100%
CGPLBR68	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	1,320,628,050	797,283,712	60%	1215	649	100%
CGPLBR69	Breast Cancer	cfDNA	TEC-Seq	100	80930	7571501500	3857354512	51%	45322	7047	NA
CGPLBR69	Breast Cancer	Germline DNA	Targeted NGS	150	639291	591797550	327570487	55%	500	349	100%

CGPLBR69	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	1254192750	670305026	53%	1016	331	100%
CGPLBR70	Breast Cancer	cfDNA	TEC-Seq	100	80930	7251760700	3641333708	50%	43203	8884	NA
CGPLBR70	Breast Cancer	Germline DNA	Targeted NGS	150	639291	552791700	315920897	57%	483	335	100%
CGPLBR70	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	985165050	518833636	53%	789	284	100%
CGPLBR71	Breast Cancer	cfDNA	TEC-Seq	100	80930	8515402600	4496696391	53%	53340	6805	NA
CGPLBR71	Breast Cancer	Germline DNA	Targeted NGS	150	639291	675318450	386958218	57%	591	389	100%
CGPLBR71	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	1408808850	841106831	60%	1273	525	100%
CGPLBR72	Breast Cancer	cfDNA	TEC-Seq	100	80930	8556946900	4389761697	51%	52081	5632	NA
CGPLBR72	Breast Cancer	Germline DNA	Targeted NGS	150	639291	381478350	195981864	51%	295	181	100%
CGPLBR72	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	1980049200	1206509350	61%	1829	494	100%
CGPLBR73	Breast Cancer	cfDNA	TEC-Seq	100	80930	7959392300	4006933338	50%	47555	8791	NA
CGPLBR73	Breast Cancer	Germline DNA	Targeted NGS	150	639291	1061212350	537918502	51%	824	229	100%
CGPLBR73	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	511075500	289986972	57%	446	316	100%
CGPLBR74	Breast Cancer	cfDNA	TEC-Seq	100	80930	8524536400	4063900599	48%	48252	7013	NA
CGPLBR74	Breast Cancer	Germline DNA	Targeted NGS	150	639291	696478500	204182619	29%	311	218	100%

CGPLBR74	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	1444542150	831504089	58%	1266	520	100%
CGPLBR75	Breast Cancer	cfDNA	TEC-Seq	100	80930	8260379100	3960599885	48%	46955	6319	NA
CGPLBR75	Breast Cancer	Germline DNA	Targeted NGS	150	639291	658358550	242257252	37%	370	244	100%
CGPLBR75	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	1209742200	615931952	51%	926	418	100%
CGPLBR76	Breast Cancer	cfDNA	TEC-Seq	100	80930	7774235200	3893622420	50%	46192	9628	NA
CGPLBR76	Breast Cancer	Germline DNA	Targeted NGS	150	639291	409580550	161155508	39%	236	148	100%
CGPLBR76	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	1404856200	808020500	58%	1222	300	100%
CGPLBR77	Breast Cancer	cfDNA	TEC-Seq	100	80930	7572797600	3255963429	43%	38568	8263	NA
CGPLBR77	Breast Cancer	Germline DNA	Targeted NGS	150	639291	537431700	305330922	57%	470	332	100%
CGPLBR77	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	1297056000	507301668	39%	770	356	100%
CGPLBR80	Breast Cancer	cfDNA	TEC-Seq	100	80930	6845325800	3147476693	46%	37201	5595	NA
CGPLBR80	Breast Cancer	Germline DNA	Targeted NGS	150	639291	610652400	273064709	45%	413	269	100%
CGPLBR80	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	1744826850	861765758	49%	1264	384	100%
CGPLBR82	Breast Cancer	cfDNA	TEC-Seq	100	80930	8236705200	4170465005	51%	49361	12319	NA
CGPLBR82	Breast Cancer	Germline DNA	Targeted NGS	150	639291	721861350	404540286	56%	616	393	100%

CGPLBR82	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	1162764150	528197785	45%	795	347	100%
CGPLBR83	Breast Cancer	cfDNA	TEC-Seq	100	80930	7434568100	3676855019	49%	43628	5458	NA
CGPLBR83	Breast Cancer	Germline DNA	Targeted NGS	150	639291	NA	NA	NA	NA	NA	NA
CGPLBR83	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	NA	NA	NA	NA	NA	NA
CGPLBR86	Breast Cancer	cfDNA	TEC-Seq	100	80930	7616282500	3644791327	48%	43490	7048	NA
CGPLBR86	Breast Cancer	Germline DNA	Targeted NGS	150	639291	365127450	175449534	48%	256	165	100%
CGPLBR86	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	1374528450	752315631	55%	1136	226	100%
CGPLBR87	Breast Cancer	cfDNA	TEC-Seq	100	80930	6194021300	3004882010	49%	35765	5306	NA
CGPLBR87	Breast Cancer	Germline DNA	Targeted NGS	150	639291	625919850	324734673	52%	493	305	100%
CGPLBR87	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	918553500	175004335	19%	265	157	100%
CGPLBR88	Breast Cancer	cfDNA	TEC-Seq	100	80930	6071567200	2847926237	47%	33945	10319	NA
CGPLBR88	Breast Cancer	Germline DNA	Targeted NGS	150	639291	474856950	101788416	21%	156	108	100%
CGPLBR88	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	1068209550	551606147	52%	846	394	100%
CGPLBR91	Breast Cancer	cfDNA	TEC-Seq	100	80930	7192457700	3480203404	48%	41570	9912	NA
CGPLBR91	Breast Cancer	Germline DNA	Targeted NGS	150	639291	649309800	260265354	40%	397	292	100%

CGPLBR91	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	1115092650	563337213	51%	865	391	100%
CGPLBR92	Breast Cancer	cfDNA	TEC-Seq	100	80930	7678981800	3600279233	47%	42975	13580	NA
CGPLBR92	Breast Cancer	Germline DNA	Targeted NGS	150	639291	614417400	332144854	54%	505	301	100%
CGPLBR92	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	1231916700	741434698	60%	1136	478	100%
CGPLBR93	Breast Cancer	cfDNA	TEC-Seq	100	80930	7605717800	3998713397	53%	47866	10329	NA
CGPLBR96	Breast Cancer	cfDNA	TEC-Seq	100	80930	6297446700	2463064737	39%	29341	7937	NA
CGPLBR96	Breast Cancer	Germline DNA	Targeted NGS	150	639291	601662300	226863962	38%	347	245	100%
CGPLBR96	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	1018240950	545472202	54%	834	227	100%
CGPLBR97	Breast Cancer	cfDNA	TEC-Seq	100	80930	7114921600	3557069027	50%	42488	10712	NA
CGPLBR97	Breast Cancer	Germline DNA	Targeted NGS	150	639291	635832150	330334979	52%	505	339	100%
CGPLBR97	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	1081210050	602942749	56%	916	373	100%
CGPLBR99	Breast Cancer	cfDNA	TEC-Seq	100	80930	6946513800	3223603304	46%	38391	5412	NA
CGPLBR99	Breast Cancer	Germline DNA	Targeted NGS	150	639291	1114707300	583697712	52%	892	438	100%
CGPLBR99	Breast Cancer	Tumor DNA	Targeted NGS	150	639291	601828650	322598549	54%	496	312	100%
CGCRC291	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	7501485600	3771359756	50%	44345	10359	NA

CGCRC291	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	1074846000	479885292	45%	733	485	100%
CGCRC291	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	1012582500	454866080	45%	696	448	100%
CGCRC292	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	6736035200	3098886973	46%	36448	8603	NA
CGCRC292	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	997109550	448775788	45%	685	452	100%
CGCRC292	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	1058966700	467350094	44%	711	456	100%
CGCRC293	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	6300244000	2818734206	45%	33117	5953	NA
CGCRC293	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	956307150	428412364	45%	653	427	100%
CGCRC293	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	962764950	424030808	44%	647	422	100%
CGCRC294	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	7786872600	3911796709	50%	46016	12071	NA
CGCRC294	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	941706900	399120498	42%	609	410	100%
CGCRC294	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	1028456550	439156889	43%	669	450	100%
CGCRC295	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	8240660200	3478059753	42%	40787	5826	NA
CGCRC295	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	1045862700	456802680	44%	697	469	100%
CGCRC295	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	928288500	421984627	45%	644	423	100%
CGCRC296	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	5718556500	2898549356	51%	33912	10180	NA

CGCRC296	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	726412050	329483654	45%	503	338	100%
CGCRC296	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	796948200	343513710	43%	524	344	100%
CGCRC297	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	7550826100	3717222432	49%	43545	5870	NA
CGCRC297	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	686847900	305825281	45%	467	313	100%
CGCRC297	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	816835050	294168635	36%	448	299	100%
CGCRC298	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	12501036400	6096393764	49%	71196	9617	NA
CGCRC298	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	915267600	391756000	43%	601	401	100%
CGCRC298	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	864069900	404835020	47%	621	396	100%
CGCRC299	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	7812602900	4121569690	53%	48098	10338	NA
CGCRC299	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	962294850	410383252	43%	622	414	100%
CGCRC299	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	1020461700	424930722	42%	643	439	100%
CGCRC300	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	8648090300	3962285136	46%	46364	5756	NA
CGCRC300	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	1088902200	464279410	43%	711	464	100%
CGCRC300	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	831117750	368422619	44%	564	376	100%
CGCRC301	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	7538758100	3695480348	49%	43024	6618	NA

CGCRC301	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	915540150	400988996	44%	615	414	100%
CGCRC301	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	973842300	423804347	44%	650	404	100%
CGCRC302	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	8573658300	4349420574	51%	51006	13799	NA
CGCRC302	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	811854000	351266173	43%	538	359	100%
CGCRC302	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	1115477250	483033596	43%	739	466	100%
CGCRC303	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	5224046400	2505714343	48%	29365	8372	NA
CGCRC303	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	1060418400	442109304	42%	676	446	100%
CGCRC303	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	1176646650	493560885	42%	756	490	100%
CGCRC304	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	5762112600	2942170530	51%	34462	10208	NA
CGCRC304	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	1007139450	431389782	43%	660	433	100%
CGCRC304	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	1057360350	445316426	42%	681	441	100%
CGCRC305	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	7213384100	3726953480	52%	43516	8589	NA
CGCRC305	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	1246122600	632083848	51%	957	623	100%
CGCRC305	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	986335800	418119169	42%	634	406	100%
CGCRC306	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	7075579700	3552441899	50%	41507	7372	NA

CGCRC306	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	1018205250	425416271	42%	651	415	100%
CGCRC306	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	895480950	384530177	43%	589	373	100%
CGCRC307	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	7572687100	3492191519	46%	40793	9680	NA
CGCRC307	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	913508250	387133404	42%	591	396	100%
CGCRC307	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	939545250	417034584	44%	637	419	100%
CGCRC308	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	7945738000	3895908986	49%	45224	11809	NA
CGCRC308	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	938120700	425166128	45%	651	419	100%
CGCRC308	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	1074720000	457076684	43%	699	481	100%
CGCRC309	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	8487455800	3921079811	46%	45736	10739	NA
CGCRC309	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	1025337600	447153051	44%	679	443	100%
CGCRC309	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	1077028800	468056542	43%	712	456	100%
CGCRC310	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	9003580500	4678812441	52%	54713	11139	NA
CGCRC310	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	980782650	427168989	44%	648	419	100%
CGCRC310	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	1456285350	624050285	43%	953	602	100%
CGCRC311	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	6528162700	3276653864	50%	38324	6044	NA

CGCRC311	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	846414300	367848055	43%	563	373	100%
CGCRC311	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	1046356050	469927791	45%	719	461	100%
CGCRC312	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	7683294300	3316719187	43%	38652	4622	NA
CGCRC312	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	1045262100	465476158	45%	712	454	100%
CGCRC312	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	1045012950	452684790	43%	693	448	100%
CGCRC313	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	5874099200	2896148722	49%	33821	6506	NA
CGCRC313	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	1411402200	622602217	44%	934	597	100%
CGCRC313	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	1357397850	594057235	44%	894	571	100%
CGCRC314	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	6883148500	3382767492	49%	39414	6664	NA
CGCRC314	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	948293550	408431471	43%	625	409	100%
CGCRC314	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	1059318450	470533351	44%	720	451	100%
CGCRC315	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	7497252500	3775556051	50%	44034	8666	NA
CGCRC315	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	944269800	470119319	50%	714	469	100%
CGCRC315	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	1329922500	668919352	50%	1022	633	100%
CGCRC316	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	10684720400	5533857153	52%	64693	14289	NA

CGCRC316	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	1102555350	549138041	50%	837	551	100%
CGCRC316	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	1008207750	469494561	47%	715	473	100%
CGCRC317	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	7086877600	3669434216	52%	43538	10944	NA
CGCRC317	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	1099990350	547860243	50%	835	542	100%
CGCRC317	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	1107316350	560348663	51%	855	552	100%
CGCRC318	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	6880041100	3326357413	48%	39077	11571	NA
CGCRC318	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	990188550	489300022	49%	746	483	100%
CGCRC318	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	1434731100	691861596	48%	1050	669	100%
CGCRC319	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	7485342900	3982677483	53%	47327	10502	NA
CGCRC319	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	1098523800	536937209	49%	816	533	100%
CGCRC319	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	1258206600	610731063	49%	930	624	100%
CGCRC320	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	7058703200	3450648135	49%	40888	10198	NA
CGCRC320	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	881784300	449537819	51%	680	431	100%
CGCRC320	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	1067872350	538856991	50%	815	514	100%
CGCRC321	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	7203625900	3633396892	50%	43065	6499	NA

CGCRC321	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	1068635550	502245626	47%	768	486	100%
CGCRC321	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	1114618650	514924981	46%	785	499	100%
CGCRC332	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	7202969100	3758323705	52%	44580	3243	NA
CGCRC332	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	758356050	406546836	54%	621	354	100%
CGCRC332	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	1274461350	744251805	58%	1135	508	100%
CGCRC333	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	8767144700	4199126827	48%	49781	8336	NA
CGCRC333	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	560436600	241285888	43%	368	225	100%
CGCRC333	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	1407707550	796386336	57%	1212	501	100%
CGCRC334	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	7771869100	3944578280	51%	46518	5014	NA
CGCRC334	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	562592100	287288732	51%	439	304	100%
CGCRC334	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	1236847050	701921622	57%	1070	507	100%
CGCRC335	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	7972524600	4064901201	51%	48308	6151	NA
CGCRC335	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	733030800	391132272	53%	596	355	100%
CGCRC335	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	1123386600	441750380	39%	675	340	100%
CGCRC336	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	8597346400	4333410573	50%	51390	7551	NA

CGCRC336	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	450245550	228507680	51%	351	246	100%
CGCRC336	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	1112596650	647572679	58%	995	495	100%
CGCRC337	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	7399611700	3800666199	51%	45083	8092	NA
CGCRC337	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	457483050	216543896	47%	330	228	100%
CGCRC337	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	1206110850	665588725	55%	997	574	100%
CGCRC338	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	8029493700	4179383804	52%	49380	5831	NA
CGCRC338	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	654071250	285478494	44%	434	257	100%
CGCRC338	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	1156258950	519228652	45%	780	361	100%
CGCRC339	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	7938963600	4095555110	52%	48397	3808	NA
CGCRC339	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	NA	NA	NA	NA	NA	NA
CGCRC339	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	NA	NA	NA	NA	NA	NA
CGCRC340	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	7214889500	3706643098	51%	43805	3014	NA
CGCRC340	Colorectal Cancer	Germline DNA	Targeted NGS	150	639291	579149550	234017537	40%	356	234	100%
CGCRC340	Colorectal Cancer	Tumor DNA	Targeted NGS	150	639291	1056358800	500654866	47%	761	363	100%
CGCRC341	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	8803159200	3668208527	42%	43106	11957	NA

CGCRC342	Colorectal Cancer	cfDNA	TEC-Seq	100	80930	8478811500	3425540889	40%	40328	9592	NA
CGPLH35	Healthy	cfDNA	TEC-Seq	100	80930	6919126300	2312758764	33%	25570	1989	NA
CGPLH36	Healthy	cfDNA	TEC-Seq	100	80930	6089923400	2038548115	33%	22719	1478	NA
CGPLH37	Healthy	cfDNA	TEC-Seq	100	80930	5557270200	1935301929	35%	21673	2312	NA
CGPLH38	Healthy	cfDNA	TEC-Seq	100	80930	5771193800	1963294894	34%	21816	784	NA
CGPLH39	Healthy	cfDNA	TEC-Seq	100	80930	6002281900	2209984880	37%	24784	563	NA
CGPLH40	Healthy	cfDNA	TEC-Seq	100	80930	6773660700	2713539772	40%	30611	409	NA
CGPLH41	Healthy	cfDNA	TEC-Seq	100	80930	5660677000	1997748737	35%	23006	583	NA
CGPLH42	Healthy	cfDNA	TEC-Seq	100	80930	5792045400	2388036949	41%	27197	2523	NA
CGPLH43	Healthy	cfDNA	TEC-Seq	100	80930	5568321700	2017813329	36%	23228	1650	NA
CGPLH44	Healthy	cfDNA	TEC-Seq	100	80930	6636969300	2424276812	37%	27040	1023	NA
CGPLH45	Healthy	cfDNA	TEC-Seq	100	80930	8485593200	2770176078	33%	32829	3114	NA
CGPLH46	Healthy	cfDNA	TEC-Seq	100	80930	5083171100	1899395790	37%	21821	1678	NA
CGPLH47	Healthy	cfDNA	TEC-Seq	100	80930	6016388500	2062392156	34%	23459	1431	NA
CGPLH48	Healthy	cfDNA	TEC-Seq	100	80930	4958945900	1809825992	36%	20702	1698	NA

CGPLH49	Healthy	cfDNA	TEC-Seq	100	80930	7953812200	2511365904	32%	27006	1440	NA
CGPLH50	Healthy	cfDNA	TEC-Seq	100	80930	6989407600	2561288100	37%	29177	2591	NA
CGPLH51	Healthy	cfDNA	TEC-Seq	100	80930	7862073200	2525091396	32%	29999	1293	NA
CGPLH52	Healthy	cfDNA	TEC-Seq	100	80930	6939636800	2397922699	35%	27029	2501	NA
CGPLH53	Healthy	cfDNA	TEC-Seq	100	80930	7563547300	2316943911	31%	24210	1109	NA
CGPLH54	Healthy	cfDNA	TEC-Seq	100	80930	10611934700	2290823134	22%	27175	3306	NA
CGPLH55	Healthy	cfDNA	TEC-Seq	100	80930	9912569200	2521962244	25%	27082	3161	NA
CGPLH56	Healthy	cfDNA	TEC-Seq	100	80930	5777591900	2023874863	35%	22916	1301	NA
CGPLH57	Healthy	cfDNA	TEC-Seq	100	80930	9234904800	1493926244	16%	15843	1655	NA
CGPLH58	Healthy	cfDNA	TEC-Seq	100	80930	7571923100	2169535037	29%	22576	1174	NA
CGPLH59	Healthy	cfDNA	TEC-Seq	100	80930	9726052100	2987875484	31%	35427	2143	NA
CGPLH60	Healthy	cfDNA	TEC-Seq	100	80930	6812180400	2141533749	31%	23217	493	NA
CGPLH61	Healthy	cfDNA	TEC-Seq	100	80930	7701716900	2225623104	29%	23858	724	NA
CGPLH62	Healthy	cfDNA	TEC-Seq	100	80930	7565636200	2042450491	27%	22021	453	NA
CGPLH63	Healthy	cfDNA	TEC-Seq	100	80930	8696405000	2521574759	29%	26689	1851	NA

CGPLH64	Healthy	cfDNA	TEC-Seq	100	80930	5438852600	996198502	18%	11477	1443	NA
CGPLH75	Healthy	cfDNA	TEC-Seq	100	80930	3446444000	1505718480	44%	17805	3016	NA
CGPLH76	Healthy	cfDNA	TEC-Seq	100	80930	7499116400	3685762725	49%	43682	4643	NA
CGPLH77	Healthy	cfDNA	TEC-Seq	100	80930	6512408400	2537359345	39%	30280	3131	NA
CGPLH78	Healthy	cfDNA	TEC-Seq	100	80930	7642949300	3946069680	52%	46316	5358	NA
CGPLH79	Healthy	cfDNA	TEC-Seq	100	80930	7785475700	3910639227	50%	45280	6714	NA
CGPLH80	Healthy	cfDNA	TEC-Seq	100	80930	7918361500	3558236955	45%	42171	5062	NA
CGPLH81	Healthy	cfDNA	TEC-Seq	100	80930	6646268900	3112369850	47%	37119	3678	NA
CGPLH82	Healthy	cfDNA	TEC-Seq	100	80930	7744065000	3941700596	51%	46820	5723	NA
CGPLH83	Healthy	cfDNA	TEC-Seq	100	80930	6957686000	1447603106	21%	17280	2875	NA
CGPLH84	Healthy	cfDNA	TEC-Seq	100	80930	8326493200	3969908122	48%	47464	3647	NA
CGPLH85	Healthy	cfDNA	TEC-Seq	100	80930	8713042600	4461252536	51%	53246	3721	NA
CGPLH86	Healthy	cfDNA	TEC-Seq	100	80930	8664194700	4470145091	52%	53398	5094	NA
CGPLH90	Healthy	cfDNA	TEC-Seq	100	80930	7516078800	3841504088	51%	45907	4414	NA
CGPLH91	Healthy	cfDNA	TEC-Seq	100	80930	7356314100	3796192344	52%	45369	3333	NA

CGPLLU1	Lung Cancer	cfDNA	TEC-Seq	100	80930	6239458800	2329606202	37%	26443	5581	NA
CGPLLU1	Lung Cancer	Germline DNA	Targeted NGS	150	639291	677237100	268756748	40%	408	289	100%
CGPLLU1	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1689013800	769286196	46%	1152	721	100%
CGPLLU115	Lung Cancer	cfDNA	TEC-Seq	100	80930	10979564000	3536107395	32%	38888	5934	NA
CGPLLU115	Lung Cancer	Germline DNA	Targeted NGS	150	639291	673939500	254019687	38%	386	263	100%
CGPLLU115	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1249447950	555225070	44%	842	534	100%
CGPLLU116	Lung Cancer	cfDNA	TEC-Seq	100	80930	8427732900	2666386481	32%	31382	7168	NA
CGPLLU116	Lung Cancer	Germline DNA	Targeted NGS	150	639291	536485350	194819603	36%	296	210	100%
CGPLLU116	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1226021550	543715026	44%	826	520	100%
CGPLLU117	Lung Cancer	cfDNA	TEC-Seq	100	80930	7722272300	2377605802	31%	27869	2500	NA
CGPLLU117	Lung Cancer	Germline DNA	Targeted NGS	150	639291	568698450	214221003	38%	327	227	100%
CGPLLU117	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1346899650	607087256	45%	924	448	100%
CGPLLU118	Lung Cancer	cfDNA	TEC-Seq	100	80930	8821329900	3017869157	34%	35234	2876	NA
CGPLLU118	Lung Cancer	Germline DNA	Targeted NGS	150	639291	824984250	340283945	41%	515	353	100%
CGPLLU118	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1541356200	667852108	43%	1007	667	100%

CGPLLU119	Lung Cancer	cfDNA	TEC-Seq	100	80930	8224786400	2826168731	34%	32615	6973	NA
CGPLLU119	Lung Cancer	Germline DNA	Targeted NGS	150	639291	622004250	238879678	38%	362	254	100%
CGPLLU119	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1248973650	509082004	41%	770	513	100%
CGPLLU135	Lung Cancer	cfDNA	TEC-Seq	100	80930	7479506200	2465400664	33%	29106	4986	NA
CGPLLU135	Lung Cancer	Germline DNA	Targeted NGS	150	639291	689515950	271757497	39%	411	288	100%
CGPLLU135	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1340064900	618218568	46%	939	554	100%
CGPLLU136	Lung Cancer	cfDNA	TEC-Seq	100	80930	9255976600	3044006428	33%	35637	5742	NA
CGPLLU136	Lung Cancer	Germline DNA	Targeted NGS	150	639291	570675300	226580792	40%	344	239	100%
CGPLLU136	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	802359600	428240472	53%	650	165	100%
CGPLLU137	Lung Cancer	cfDNA	TEC-Seq	100	80930	9182200700	2707533740	29%	32066	5436	NA
CGPLLU137	Lung Cancer	Germline DNA	Targeted NGS	150	639291	677915850	256959903	38%	390	275	100%
CGPLLU137	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1363517850	612337794	45%	933	594	100%
CGPLLU138	Lung Cancer	cfDNA	TEC-Seq	100	80930	8658694500	2845274314	33%	33349	1669	NA
CGPLLU138	Lung Cancer	Germline DNA	Targeted NGS	150	639291	764916750	375266555	49%	571	390	100%
CGPLLU138	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1504663500	842387976	56%	1277	720	100%

CGPLLU139	Lung Cancer	cfDNA	TEC-Seq	100	80930	8269549200	2779580391	34%	32503	5050	NA
CGPLLU139	Lung Cancer	Germline DNA	Targeted NGS	150	639291	680111250	353773681	52%	532	197	100%
CGPLLU139	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1390145700	801181359	58%	1207	650	100%
CGPLLU14	Lung Cancer	cfDNA, serial blood draw 1	TEC-Seq	100	80930	7398028800	1699011387	23%	20075	778	NA
CGPLLU14	Lung Cancer	cfDNA, serial blood draw 2	TEC-Seq	100	80930	5656145300	2098827088	37%	23699	995	NA
CGPLLU144	Lung Cancer	cfDNA	TEC-Seq	100	80930	8716827400	4216576624	48%	49370	10771	NA
CGPLLU144	Lung Cancer	Germline DNA	Targeted NGS	150	639291	695037000	361563821	52%	550	366	100%
CGPLLU144	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1200620250	549449256	46%	830	401	100%
CGPLLU145	Lung Cancer	cfDNA	TEC-Seq	100	80930	8925580700	4416873069	49%	51680	6262	NA
CGPLLU146	Lung Cancer	cfDNA	TEC-Seq	100	80930	8506844200	4195033049	49%	49084	6968	NA
CGPLLU146	Lung Cancer	Germline DNA	Targeted NGS	150	639291	608225100	316448889	52%	483	274	100%
CGPLLU146	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1116711600	390267100	35%	592	383	100%
CGPLLU147	Lung Cancer	cfDNA	TEC-Seq	100	80930	7416300600	3530746046	48%	41302	4691	NA
CGPLLU147	Lung Cancer	Germline DNA	Targeted NGS	150	639291	1174428600	536650801	46%	808	517	100%

CGPLLU147	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	808104900	399673367	49%	608	388	100%
CGPLLU161	Lung Cancer	cfDNA	TEC-Seq	100	80930	7789148700	3280139772	42%	38568	12229	NA
CGPLLU162	Lung Cancer	cfDNA	TEC-Seq	100	80930	7625462000	3470147667	46%	40918	10099	NA
CGPLLU162	Lung Cancer	Germline DNA	Targeted NGS	150	639291	429861300	151297004	35%	226	188	100%
CGPLLU162	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1334133150	732531538	55%	1111	619	100%
CGPLLU163	Lung Cancer	cfDNA	TEC-Seq	100	80930	8019293200	3946533983	49%	46471	12108	NA
CGPLLU163	Lung Cancer	Germline DNA	Targeted NGS	150	639291	524,208,300	200,482,808	38%	301	236	100%
CGPLLU163	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1,352,545,050	696,512,666	51%	1020	583	100%
CGPLLU164	Lung Cancer	cfDNA	TEC-Seq	100	80930	8110030900	3592748235	44%	42161	6947	NA
CGPLLU164	Lung Cancer	Germline DNA	Targeted NGS	150	639291	509906100	220862753	43%	322	241	100%
CGPLLU164	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1305960150	678924309	52%	1003	598	100%
CGPLLU165	Lung Cancer	cfDNA	TEC-Seq	100	80930	8389514600	4147501817	49%	48770	8996	NA
CGPLLU165	Lung Cancer	Germline DNA	Targeted NGS	150	639291	535155150	224433873	42%	328	150	100%
CGPLLU165	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1063652400	607101865	57%	912	211	100%
CGPLLU166	Lung Cancer	cfDNA	TEC-Seq	100	80930	8040278000	3908624400	49%	45999	11030	NA

CGPLLU166	Lung Cancer	Germline DNA	Targeted NGS	150	639291	NA	NA	NA	NA	NA	NA
CGPLLU166	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	NA	NA	NA	NA	NA	NA
CGPLLU168	Lung Cancer	cfDNA	TEC-Seq	100	80930	7690630000	3868237773	50%	45625	9711	NA
CGPLLU168	Lung Cancer	Germline DNA	Targeted NGS	150	639291	510783000	285892642	56%	438	164	100%
CGPLLU168	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1285271400	685373694	53%	1037	661	100%
CGPLLU169	Lung Cancer	cfDNA	TEC-Seq	100	80930	9378353000	4800407624	51%	56547	10261	NA
CGPLLU169	Lung Cancer	Germline DNA	Targeted NGS	150	639291	591105000	300994824	51%	457	326	100%
CGPLLU169	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1285960650	685383054	53%	1041	666	100%
CGPLLU170	Lung Cancer	cfDNA	TEC-Seq	100	80930	7276494400	3543816325	49%	41280	8803	NA
CGPLLU173	Lung Cancer	cfDNA	TEC-Seq	100	80930	7592835800	2879843635	38%	34033	6924	NA
CGPLLU174	Lung Cancer	cfDNA	TEC-Seq	100	80930	7481844600	3067532518	41%	36321	6137	NA
CGPLLU174	Lung Cancer	Germline DNA	Targeted NGS	150	639291	2072668200	1190204964	57%	1786	303	100%
CGPLLU174	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	645803700	355337091	55%	535	350	100%
CGPLLU175	Lung Cancer	cfDNA	TEC-Seq	100	80930	8532324200	4002541569	47%	47084	7862	NA
CGPLLU175	Lung Cancer	Germline DNA	Targeted NGS	150	639291	618550950	339691335	55%	517	358	100%

CGPLLU175	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1449611550	855214102	59%	1308	391	100%
CGPLLU176	Lung Cancer	cfDNA	TEC-Seq	100	80930	8143905000	4054098929	50%	47708	5588	NA
CGPLLU176	Lung Cancer	Germline DNA	Targeted NGS	150	639291	610189200	337041659	55%	516	332	100%
CGPLLU176	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1095470850	567775337	52%	871	434	100%
CGPLLU177	Lung Cancer	cfDNA	TEC-Seq	100	80930	8421611300	4197108809	50%	49476	8780	NA
CGPLLU177	Lung Cancer	Germline DNA	Targeted NGS	150	639291	659891250	372266268	56%	566	373	100%
CGPLLU177	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1472590050	834893525	57%	1267	715	100%
CGPLLU178	Lung Cancer	cfDNA	TEC-Seq	100	80930	8483124700	4169577489	49%	48580	6445	NA
CGPLLU178	Lung Cancer	Germline DNA	Targeted NGS	150	639291	694574700	380911029	55%	577	380	100%
CGPLLU178	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1148297400	667857382	58%	1030	547	100%
CGPLLU179	Lung Cancer	cfDNA	TEC-Seq	100	80930	7774358700	3304915738	43%	38768	6862	NA
CGPLLU179	Lung Cancer	Germline DNA	Targeted NGS	150	639291	1535136750	925596393	60%	1405	341	100%
CGPLLU179	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	664354500	339072044	51%	515	330	100%
CGPLLU180	Lung Cancer	cfDNA	TEC-Seq	100	80930	8192813800	3937552475	48%	46498	6568	NA
CGPLLU180	Lung Cancer	Germline DNA	Targeted NGS	150	639291	1498872600	781889116	52%	1190	639	100%

CGPLLU180	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1086715200	600798880	55%	916	193	100%
CGPLLU19	Lung Cancer	cfDNA	TEC-Seq	100	80930	7263539200	2547333392	35%	28493	3441	NA
CGPLLU19	Lung Cancer	Germline DNA	Targeted NGS	150	639291	591053700	223344073	38%	341	172	100%
CGPLLU19	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1214483550	519092020	43%	787	315	100%
CGPLLU197	Lung Cancer	cfDNA	TEC-Seq	100	80930	7996779200	3082397881	39%	36381	5388	NA
CGPLLU197	Lung Cancer	Germline DNA	Targeted NGS	150	639291	491469750	173278101	35%	266	132	100%
CGPLLU197	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	768799800	406238720	53%	619	111	100%
CGPLLU198	Lung Cancer	cfDNA	TEC-Seq	100	80930	7175247200	3545719100	49%	42008	6817	NA
CGPLLU198	Lung Cancer	Germline DNA	Targeted NGS	150	639291	571152750	243139384	43%	369	259	100%
CGPLLU198	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	NA	NA	NA	NA	NA	NA
CGPLLU2	Lung Cancer	cfDNA	TEC-Seq	100	80930	9495304600	3389293546	36%	40802	4853	NA
CGPLLU2	Lung Cancer	Germline DNA	Targeted NGS	150	639291	499588050	197204704	39%	300	221	100%
CGPLLU2	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1632543150	749366432	46%	1128	609	100%
CGPLLU20	Lung Cancer	cfDNA	TEC-Seq	100	80930	12481073200	994477545	8%	10329	4129	NA
CGPLLU20	Lung Cancer	Germline DNA	Targeted NGS	150	639291	574141350	228443607	40%	347	248	100%

CGPLLU20	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1150028400	506414454	44%	758	475	100%
CGPLLU202	Lung Cancer	cfDNA	TEC-Seq	100	80930	6840112800	3427820669	50%	40670	7951	NA
CGPLLU202	Lung Cancer	Germline DNA	Targeted NGS	150	639291	645258450	207925335	32%	316	209	100%
CGPLLU202	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1293518100	670182891	52%	1023	504	100%
CGPLLU203	Lung Cancer	cfDNA	TEC-Seq	100	80930	7468749900	3762726574	50%	44500	9917	NA
CGPLLU203	Lung Cancer	Germline DNA	Targeted NGS	150	639291	557531850	297213725	53%	453	303	100%
CGPLLU203	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1198142100	596099136	50%	910	563	100%
CGPLLU204	Lung Cancer	cfDNA	TEC-Seq	100	80930	7445026400	3703545153	50%	44317	6856	NA
CGPLLU204	Lung Cancer	Germline DNA	Targeted NGS	150	639291	1178585700	582456480	49%	883	341	100%
CGPLLU204	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	661557450	348927002	53%	525	351	100%
CGPLLU205	Lung Cancer	cfDNA	TEC-Seq	100	80930	9205429100	4350573991	47%	51627	9810	NA
CGPLLU205	Lung Cancer	Germline DNA	Targeted NGS	150	639291	704826750	297031777	42%	453	294	100%
CGPLLU205	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1259501400	676224830	54%	1028	545	100%
CGPLLU206	Lung Cancer	cfDNA	TEC-Seq	100	80930	7397914600	3635210205	49%	43016	7124	NA
CGPLLU206	Lung Cancer	Germline DNA	Targeted NGS	150	639291	587897550	260243234	44%	396	265	100%

CGPLLU206	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	936519600	84546885	9%	129	78	100%
CGPLLU207	Lung Cancer	cfDNA	TEC-Seq	100	80930	7133043900	3736258011	52%	44291	8499	NA
CGPLLU207	Lung Cancer	Germline DNA	Targeted NGS	150	639291	635760000	252119850	40%	384	263	100%
CGPLLU207	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1122284550	498538490	44%	759	438	100%
CGPLLU208	Lung Cancer	cfDNA	TEC-Seq	100	80930	7346976400	3855814032	52%	45782	8940	NA
CGPLLU208	Lung Cancer	Germline DNA	Targeted NGS	150	639291	549011700	306386280	56%	472	327	100%
CGPLLU208	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1061510250	574889056	54%	882	370	100%
CGPLLU209	Lung Cancer	cfDNA	TEC-Seq	100	80930	6723337800	3362944595	50%	39531	11946	NA
CGPLLU209	Lung Cancer	Germline DNA	Targeted NGS	150	639291	578241150	293725997	51%	450	302	100%
CGPLLU209	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1041774600	505999221	49%	770	406	100%
CGPLLU21	Lung Cancer	cfDNA	TEC-Seq	100	80930	7869841100	1317965426	17%	14215	3501	NA
CGPLLU21	Lung Cancer	Germline DNA	Targeted NGS	150	639291	589913250	196975420	33%	301	223	100%
CGPLLU21	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1301333700	584929403	45%	894	523	100%
CGPLLU214	Lung Cancer	cfDNA	TEC-Seq	100	80930	6691661600	3406240415	51%	40249	4462	NA
CGPLLU22	Lung Cancer	cfDNA	TEC-Seq	100	80930	7574547100	2108841152	28%	21572	6544	NA

CGPLLU22	Lung Cancer	Germline DNA	Targeted NGS	150	639291	745022400	191686179	26%	291	211	100%
CGPLLU22	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1223740200	573490645	47%	870	427	100%
CGPLLU23	Lung Cancer	cfDNA	TEC-Seq	100	80930	7019961300	2276529333	32%	24728	3670	NA
CGPLLU23	Lung Cancer	Germline DNA	Targeted NGS	150	639291	621765750	243401140	39%	373	224	100%
CGPLLU23	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1154575200	558861771	48%	855	333	100%
CGPLLU26	Lung Cancer	cfDNA	TEC-Seq	100	80930	8423085100	2399668113	28%	25687	861	NA
CGPLLU26	Lung Cancer	Germline DNA	Targeted NGS	150	639291	826125900	249126153	30%	361	268	100%
CGPLLU26	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	NA	NA	NA	NA	NA	NA
CGPLLU28	Lung Cancer	cfDNA	TEC-Seq	100	80930	8381515900	2342788295	28%	24533	7424	NA
CGPLLU28	Lung Cancer	Germline DNA	Targeted NGS	150	639291	642068100	269574172	42%	407	321	100%
CGPLLU28	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1253304300	536280022	43%	809	474	100%
CGPLLU29	Lung Cancer	cfDNA	TEC-Seq	100	80930	7475284900	2387903088	32%	25788	3573	NA
CGPLLU3	Lung Cancer	cfDNA	TEC-Seq	100	80930	5628696000	2034607883	36%	22950	5122	NA
CGPLLU3	Lung Cancer	Germline DNA	Targeted NGS	150	639291	600536700	243416472	41%	371	195	100%
CGPLLU3	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1283482650	587505537	46%	884	376	100%

CGPLLU30	Lung Cancer	cfDNA	TEC-Seq	100	80930	8699392200	2448312466	28%	26625	7523	NA
CGPLLU30	Lung Cancer	Germline DNA	Targeted NGS	150	639291	592419600	254080805	43%	384	289	100%
CGPLLU30	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	534800550	247881687	46%	376	105	100%
CGPLLU31	Lung Cancer	cfDNA	TEC-Seq	100	80930	4407055300	1196657814	27%	13701	1573	NA
CGPLLU31	Lung Cancer	Germline DNA	Targeted NGS	150	639291	658089150	222043322	34%	334	231	100%
CGPLLU31	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1250134500	557178929	45%	841	426	100%
CGPLLU4	Lung Cancer	cfDNA	TEC-Seq	100	80930	8456184600	3386153208	40%	39896	3346	NA
CGPLLU44	Lung Cancer	cfDNA, serial blood draw 1	TEC-Seq	100	80930	9744751800	2002428243	21%	21635	6924	NA
CGPLLU44	Lung Cancer	cfDNA, serial blood draw 2	TEC-Seq	100	80930	9622990500	3284300067	34%	39152	7788	NA
CGPLLU47	Lung Cancer	cfDNA, serial blood draw 1	TEC-Seq	100	80930	8463284700	2682429672	32%	28887	6048	NA
CGPLLU47	Lung Cancer	cfDNA, serial blood draw 2	TEC-Seq	100	80930	7710341500	3072111376	40%	36516	6875	NA
CGPLLU5	Lung Cancer	cfDNA	TEC-Seq	100	80930	6598676600	2581497896	39%	29200	4018	NA
CGPLLU5	Lung Cancer	Germline DNA	Targeted NGS	150	639291	566575050	218575022	39%	332	241	100%

CGPLLU5	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1128992550	543920310	48%	823	266	100%
CGPLLU54	Lung Cancer	cfDNA	TEC-Seq	100	80930	8486375600	2682295256	32%	31317	3453	NA
CGPLLU54	Lung Cancer	Germline DNA	Targeted NGS	150	639291	324943500	108468275	33%	167	122	100%
CGPLLU54	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	2039507400	391222046	45%	593	123	100%
CGPLLU57	Lung Cancer	cfDNA, serial blood draw 1	TEC-Seq	100	80930	9291392600	3154714157	34%	37406	1502	NA
CGPLLU57	Lung Cancer	cfDNA, serial blood draw 2	TEC-Seq	100	80930	8244793600	2507050777	30%	29132	2655	NA
CGPLLU59	Lung Cancer	cfDNA, serial blood draw 1	TEC-Seq	100	80930	10938574300	692400779	6%	8033	1026	NA
CGPLLU59	Lung Cancer	cfDNA, serial blood draw 2	TEC-Seq	100	80930	5079072900	2009883319	40%	23141	2173	NA
CGPLLU6	Lung Cancer	cfDNA	TEC-Seq	100	80930	7056975000	2654750988	38%	30203	5250	NA
CGPLLU6	Lung Cancer	Germline DNA	Targeted NGS	150	639291	498756750	194335181	39%	295	224	100%
CGPLLU6	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1438016400	668585167	46%	1013	523	100%
CGPLLU63	Lung Cancer	cfDNA	TEC-Seq	100	80930	7668552500	1044408270	14%	12325	5802	NA
CGPLLU63	Lung Cancer	Germline DNA	Targeted NGS	150	639291	667,878,450	309,382,277	46%	470	280	100%

CGPLLU63	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1,284,084,450	536,973,644	42%	816	334	100%
CGPLLU64	Lung Cancer	cfDNA	TEC-Seq	100	80930	8088897900	3083234373	38%	36108	5320	NA
CGPLLU64	Lung Cancer	Germline DNA	Targeted NGS	150	639291	652960200	296973185	45%	450	309	100%
CGPLLU64	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1415779950	574175656	41%	874	558	100%
CGPLLU65	Lung Cancer	cfDNA	TEC-Seq	100	80930	8699641600	2679059287	31%	30935	4831	NA
CGPLLU65	Lung Cancer	Germline DNA	Targeted NGS	150	639291	656203200	256082240	39%	387	271	100%
CGPLLU65	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1226734500	555791782	45%	841	299	100%
CGPLLU66	Lung Cancer	cfDNA	TEC-Seq	100	80930	10396598700	3774873437	36%	43806	1603	NA
CGPLLU66	Lung Cancer	Germline DNA	Targeted NGS	150	639291	600045150	239464814	40%	363	250	100%
CGPLLU66	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1498678200	638138245	43%	968	547	100%
CGPLLU67	Lung Cancer	cfDNA	TEC-Seq	100	80930	7967355300	2652881488	33%	30996	9590	NA
CGPLLU67	Lung Cancer	Germline DNA	Targeted NGS	150	639291	596848350	230451277	39%	349	242	100%
CGPLLU67	Lung Cancer	Tumor DNA	Targeted NGS	150	639291	1148746200	492136159	43%	747	388	100%
CGPLLU68	Lung Cancer	cfDNA	TEC-Seq	100	80930	7414515500	2339316921	32%	27348	7876	NA
CGPLLU9	Lung Cancer	cfDNA, serial blood draw 1	TEC-Seq	100	80930	7865706800	2818163586	36%	32789	7118	NA

CGPLLU9	Lung Cancer	cfDNA, serial blood draw 2	TEC-Seq	100	80930	7632488500	2833849772	37%	33369	3716	NA
CGPLOV1	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	6674383100	2571045006	39%	30944	4752	NA
CGPLOV1	Ovarian Cancer	Germline DNA	Targeted NGS	150	639291	739803600	260650215	35%	394	254	100%
CGPLOV1	Ovarian Cancer	Tumor DNA	Targeted NGS	150	639291	1335971850	581484581	44%	880	416	100%
CGPLOV10	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	7073534200	3402308123	48%	39820	4059	NA
CGPLOV10	Ovarian Cancer	Germline DNA	Targeted NGS	150	639291	639810000	321433448	50%	487	334	100%
CGPLOV10	Ovarian Cancer	Tumor DNA	Targeted NGS	150	639291	1252083750	539790788	43%	821	350	100%
CGPLOV11	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	6924062200	3324593050	48%	38796	7185	NA
CGPLOV11	Ovarian Cancer	Germline DNA	Targeted NGS	150	639291	703802550	353129590	50%	538	359	100%
CGPLOV11	Ovarian Cancer	Tumor DNA	Targeted NGS	150	639291	1225966350	534080567	44%	813	238	100%
CGPLOV12	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	6552080100	3181854993	49%	37340	6114	NA
CGPLOV13	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	6796755500	3264897084	48%	38340	7931	NA
CGPLOV13	Ovarian Cancer	Germline DNA	Targeted NGS	150	639291	818085300	401088839	49%	607	389	100%
CGPLOV13	Ovarian Cancer	Tumor DNA	Targeted NGS	150	639291	1330495800	655509142	49%	993	414	100%
CGPLOV14	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	7856573900	3408425065	43%	39997	7712	NA

CGPLOV14	Ovarian Cancer	Germline DNA	Targeted NGS	150	639291	791921100	385336441	49%	583	401	100%
CGPLOV14	Ovarian Cancer	Tumor DNA	Targeted NGS	150	639291	1297177950	591287827	46%	894	541	100%
CGPLOV15	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	7239201500	3322285607	46%	38953	6644	NA
CGPLOV15	Ovarian Cancer	Germline DNA	Targeted NGS	150	639291	701500350	360045925	51%	551	360	100%
CGPLOV15	Ovarian Cancer	Tumor DNA	Targeted NGS	150	639291	1150070100	452417090	39%	692	378	100%
CGPLOV16	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	8570755900	4344288233	51%	51009	11947	NA
CGPLOV16	Ovarian Cancer	Germline DNA	Targeted NGS	150	639291	734391000	314221694	43%	480	237	100%
CGPLOV16	Ovarian Cancer	Tumor DNA	Targeted NGS	150	639291	968859000	495142955	51%	754	202	100%
CGPLOV17	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	6910310400	2805243492	41%	32828	4307	NA
CGPLOV17	Ovarian Cancer	Germline DNA	Targeted NGS	150	639291	734076450	345696639	47%	529	357	100%
CGPLOV17	Ovarian Cancer	Tumor DNA	Targeted NGS	150	639291	1315736700	713860251	54%	1089	500	100%
CGPLOV18	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	8173037600	4064432407	50%	47714	5182	NA
CGPLOV18	Ovarian Cancer	Germline DNA	Targeted NGS	150	639291	583473150	302593210	52%	463	317	100%
CGPLOV18	Ovarian Cancer	Tumor DNA	Targeted NGS	150	639291	891864900	557764532	63%	853	197	100%
CGPLOV19	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	7732198900	3672564399	47%	43020	11127	NA

CGPLOV19	Ovarian Cancer	Germline DNA	Targeted NGS	150	639291	653914950	257169658	39%	393	254	100%
CGPLOV19	Ovarian Cancer	Tumor DNA	Targeted NGS	150	639291	1238679450	672750557	54%	1023	327	100%
CGPLOV2	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	9365390900	3705407220	40%	43375	5495	NA
CGPLOV2	Ovarian Cancer	Germline DNA	Targeted NGS	150	639291	750964800	323428781	43%	489	309	100%
CGPLOV2	Ovarian Cancer	Tumor DNA	Targeted NGS	150	639291	1286955600	606105596	47%	916	297	100%
CGPLOV20	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	7559602000	3678700179	49%	43230	4872	NA
CGPLOV20	Ovarian Cancer	Germline DNA	Targeted NGS	150	639291	589255200	291459229	49%	443	316	100%
CGPLOV20	Ovarian Cancer	Tumor DNA	Targeted NGS	150	639291	1163379600	632169517	54%	964	430	100%
CGPLOV21	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	8949032900	4616255499	52%	54012	12777	NA
CGPLOV21	Ovarian Cancer	Germline DNA	Targeted NGS	150	639291	540460650	262969134	49%	401	274	100%
CGPLOV21	Ovarian Cancer	Tumor DNA	Targeted NGS	150	639291	1373819100	743538836	54%	1136	341	100%
CGPLOV22	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	8680136500	4049934586	47%	46912	9715	NA
CGPLOV22	Ovarian Cancer	Germline DNA	Targeted NGS	150	639291	588115950	289776467	49%	443	297	100%
CGPLOV22	Ovarian Cancer	Tumor DNA	Targeted NGS	150	639291	1316281800	696435612	53%	1061	408	100%
CGPLOV23	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	6660696600	3422631774	51%	40810	9460	NA

CGPLOV24	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	8634287200	4272258165	49%	50736	8689	NA
CGPLOV25	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	6978295000	3390206388	49%	40188	5856	NA
CGPLOV26	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	7041038300	3728879661	53%	44341	8950	NA
CGPLOV27	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	7036348000	3126621660	44%	36944	8226	NA
CGPLOV3	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	5666658900	1644257965	29%	18549	2703	NA
CGPLOV3	Ovarian Cancer	Germline DNA	Targeted NGS	150	639291	565973400	199491765	35%	296	198	100%
CGPLOV3	Ovarian Cancer	Tumor DNA	Targeted NGS	150	639291	1661075700	780726926	47%	1173	581	100%
CGPLOV4	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	7652138800	2958089057	39%	34791	3453	NA
CGPLOV5	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	6846268800	2569276432	38%	30795	5514	NA
CGPLOV6	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	5802806700	2285641930	39%	25954	4766	NA
CGPLOV6	Ovarian Cancer	Germline DNA	Targeted NGS	150	639291	778602000	297048515	38%	452	296	100%
CGPLOV6	Ovarian Cancer	Tumor DNA	Targeted NGS	150	639291	1453338900	778245944	54%	1187	307	100%
CGPLOV7	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	5104003900	1911759262	37%	22053	2832	NA
CGPLOV7	Ovarian Cancer	Germline DNA	Targeted NGS	150	639291	690537300	211116154	31%	316	208	100%
CGPLOV7	Ovarian Cancer	Tumor DNA	Targeted NGS	150	639291	1415413950	681644124	48%	1017	429	100%

CGPLOV8	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	6247060500	2119191732	34%	23377	3541	NA
CGPLOV8	Ovarian Cancer	Germline DNA	Targeted NGS	150	639291	555609900	288758931	52%	443	232	100%
CGPLOV8	Ovarian Cancer	Tumor DNA	Targeted NGS	150	639291	1546324800	782429622	51%	1169	545	100%
CGPLOV9	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	4440232900	1135063183	26%	12542	1199	NA
CGPLOV9	Ovarian Cancer	Germline DNA	Targeted NGS	150	639291	558193200	276211578	49%	413	235	100%
CGPLOV9	Ovarian Cancer	Tumor DNA	Targeted NGS	150	639291	1346789400	603639598	45%	919	596	100%
CGPLOV28	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	7429236900	3753051715	51%	45430	4155	NA
CGPLOV31	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	8981384000	4621838729	51%	55429	5458	NA
CGPLOV32	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	9344536800	4737698323	51%	57234	6165	NA
CGPLOV37	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	8158083200	4184432898	51%	50648	6934	NA
CGPLOV38	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	8654435400	4492987085	52%	53789	6124	NA
CGPLOV40	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	9868640700	4934400809	50%	59049	7721	NA
CGPLOV41	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	7689013600	3861448829	50%	46292	4469	NA
CGPLOV42	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	9836516300	4864154366	49%	58302	7632	NA
CGPLOV43	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	8756507100	4515479918	52%	54661	4310	NA

CGPLOV44	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	7576310800	4120933922	54%	49903	4969	NA
CGPLOV46	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	9346036300	5037820346	54%	61204	3927	NA
CGPLOV47	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	10880620200	5491357828	50%	66363	6895	NA
CGPLOV48	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	7658787800	3335991337	44%	40332	4066	NA
CGPLOV49	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	10076208000	5519656698	55%	67117	5097	NA
CGPLOV50	Ovarian Cancer	cfDNA	TEC-Seq	100	80930	8239290400	4472380276	54%	54150	3836	NA

*NA denotes data not available.

Table 3.8. Alterations in blood cell proliferation genes in healthy individuals and cancer patients.

Patient	Gene Symbol	Nucleotide	Genome Build	Amino Acid (Protein)	Mutant Allele Fraction	Total Distinct Coverage	Distinct Mutant Coverage	Alteration Detected in Matched Blood Cells	Hotspot Alteration
CGCRC304	ATM	chr11_108236086-108236086_C_T	HG19	3008R>C	0.43%	6303	27	Yes	No
CGCRC316	ATM	chr11_108236087-108236087_G_C	HG19	3008R>P	0.13%	9855	13	Yes	No
CGPLLU31	DNMT3A	chr2_25321027-25321027_T_A	HG18	1555-2A>T	0.58%	2580	15	Yes	No
CGPLLU22	DNMT3A	chr2_25317105-25317105_T_C	HG18	2083-2A>G	3.59%	3675	132	Yes	No
CGCRC303	DNMT3A	chr2_25463508-25463508_C_T	HG19	2173+1G>A	0.17%	4172	7	No	No
CGPLLU177	DNMT3A	chr2_25463508-25463508_C_T	HG19	2173+1G>A	0.13%	5299	7	No	No
CGPLBR42	DNMT3A	chr2_25463320-25463320_C_	HG19	2174-1delG	0.99%	3145	31	Not Assessed	No
CGPLLU175	DNMT3A	chr2_25457290-25457290_C_T	HG19	2598-1G>A	0.21%	5281	11	No	No
CGPLLU163	DNMT3A	chr2_25467494-25467494_A_C	HG19	528Y>D	0.15%	7953	12	Yes	No
CGPLBR96	DNMT3A	chr2_25467484-25467484_T_C	HG19	531D>G	5.81%	3685	214	Yes	No
CGPLBR104	DNMT3A	chr2_25467437-25467437_G_A	HG19	547L>F	0.35%	5141	18	Not Assessed	No
CGPLLU214	DNMT3A	chr2_25467428-25467428_C_T	HG19	550G>R	0.37%	2429	9	Not Assessed	No
CGPLLU205	DNMT3A	chr2_25463596-25463596_G_A	HG19	696Q>X	3.47%	7468	259	Yes	No
CGCRC318	DNMT3A	chr2_25463589-25463589_C_T	HG19	698W>X	0.25%	6888	17	Yes	No
CGPLBR44	DNMT3A	chr2_25463568-25463568_A_G	HG19	705I>T	0.41%	5409	22	Yes	No
CGPLLU68	DNMT3A	chr2_25317070-25317070_C_T	HG18	706G>R	0.21%	5365	11	Not Assessed	No
CGPLLU173	DNMT3A	chr2_25463563-25463563_C_T	HG19	707G>S	0.25%	4814	12	Not Assessed	No
CGCRC299	DNMT3A	chr2_25463553-25463553_C_G	HG19	710C>S	0.12%	8240	10	Yes	No
CGCRC298	DNMT3A	chr2_25463541-25463541_G_C	HG19	714S>C	0.11%	7980	9	No	No
CGPLBR104	DNMT3A	chr2_25463541-25463541_G_C	HG19	714S>C	0.14%	6497	9	Not Assessed	No
CGPLLU174	DNMT3A	chr2_25463537-25463537_G_C	HG19	715I>M	0.26%	4567	12	Yes	No
CGCRC300	DNMT3A	chr2_25463524-25463524_G_C	HG19	720R>G	0.15%	4663	7	No	No
CGCRC319	DNMT3A	chr2_25463316-25463316_C_A	HG19	726G>V	0.62%	4519	28	Yes	No
CGPLLU67	DNMT3A	chr2_25316811-25316811_C_T	HG18	729R>Q	0.24%	4202	10	No	No
CGPLBR42	DNMT3A	chr2_25463298-25463300_AAG_	HG19	731FF>F	0.31%	3584	11	Not Assessed	No
CGPLH51	DNMT3A	chr2_25316802-25316804_AAG_	HG18	731FF>F	1.39%	1227	17	NA	No
CGPLH84	DNMT3A	chr2_25463298-25463300_AAG_	HG19	731FF>F	0.42%	2151	9	NA	No
CGCRC299	DNMT3A	chr2_25463289-25463289_T_C	HG19	735Y>C	0.30%	5307	16	Yes	No
CGPLLU176	DNMT3A	chr2_25463289-25463289_T_C	HG19	735Y>C	0.21%	3292	7	Yes	No

CGPLLU65	DNMT3A	chr2_25316793-25316793_T_C	HG18	735Y>C	3.24%	3668	119	Yes	No
CGPLOV8	DNMT3A	chr2_25316793-25316793_T_G	HG18	735Y>S	0.24%	3725	9	No	No
CGPLBR61	DNMT3A	chr2_25463288-25463288_G_T	HG19	735Y>X	0.29%	4162	12	Not Assessed	No
CGPLLU205	DNMT3A	chr2_25463287-25463287_G_A	HG19	736R>C	0.70%	5295	37	Yes	No
CGPLLU57	DNMT3A	chr2_25316790-25316790_C_T	HG18	736R>H	0.48%	1678	8	Not Assessed	No
CGPLLU57	DNMT3A	chr2_25316790-25316790_C_T	HG18	736R>H	0.51%	2921	15	Not Assessed	No
CGPLBR59	DNMT3A	chr2_25463287-25463287_G_T	HG19	736R>S	0.27%	4069	11	No	No
CGPLLU168	DNMT3A	chr2_25463287-25463287_G_T	HG19	736R>S	0.39%	5088	20	No	No
CGPLLU146	DNMT3A	chr2_25463283-25463283_A_T	HG19	737L>H	0.84%	4052	34	Yes	No
CGCRC314	DNMT3A	chr2_25463280-25463280_A_T	HG19	738L>Q	2.50%	4008	100	Yes	No
CGPLBR55	DNMT3A	chr2_25463266-25463266_G_A	HG19	743P>S	0.18%	5118	9	No	No
CGPLLU176	DNMT3A	chr2_25463245-25463245_G_A	HG19	750P>S	0.92%	3700	34	Yes	No
CGPLLU175	DNMT3A	chr2_25463230-25463230_A_G	HG19	755F>L	0.15%	5365	8	Yes	No
CGCRC303	DNMT3A	chr2_25463229-25463229_A_G	HG19	755F>S	0.21%	3838	8	No	No
CGPLH38	DNMT3A	chr2_25316688-25316688_G_A	HG18	770S>L	3.09%	970	30	NA	No
CGPLBR57	DNMT3A	chr2_25463182-25463182_G_A	HG19	771R>X	0.37%	4321	16	Yes	No
CGPLH63	DNMT3A	chr2_25316686-25316686_G_A	HG18	771R>X	1.26%	2061	26	NA	No
CGPLLU173	DNMT3A	chr2_25463182-25463182_G_A	HG19	771R>X	4.18%	3854	161	Not Assessed	No
CGPLBR69	DNMT3A	chr2_25463172-25463172_T_A	HG19	774E>V	0.29%	4168	12	No	No
CGPLLU179	DNMT3A	chr2_25457252-25457252_T_C	HG19	879N>D	0.38%	4213	16	Yes	No
CGPLLU197	DNMT3A	chr2_25457252-25457252_T_C	HG19	879N>D	0.38%	3721	14	No	No
CGPLBR57	DNMT3A	chr2_25457243-25457243_G_A	HG19	882R>C	1.17%	5025	59	Yes	Yes
CGPLH42	DNMT3A	chr2_25310747-25310747_G_A	HG18	882R>C	0.78%	2561	20	NA	Yes
CGPLH76	DNMT3A	chr2_25457243-25457243_G_A	HG19	882R>C	5.28%	3446	182	NA	Yes
CGPLH84	DNMT3A	chr2_25457243-25457243_G_A	HG19	882R>C	0.16%	2534	4	NA	Yes
CGPLLU118	DNMT3A	chr2_25310747-25310747_G_A	HG18	882R>C	7.60%	3317	252	Yes	Yes
CGPLLU177	DNMT3A	chr2_25457243-25457243_G_A	HG19	882R>C	0.29%	5872	17	No	Yes
CGPLLU197	DNMT3A	chr2_25457243-25457243_G_A	HG19	882R>C	0.16%	3843	6	No	Yes
CGCRC298	DNMT3A	chr2_25457242-25457242_C_T	HG19	882R>H	0.08%	7666	6	Yes	Yes
CGCRC308	DNMT3A	chr2_25457242-25457242_C_T	HG19	882R>H	0.06%	7259	4	No	Yes
CGCRC311	DNMT3A	chr2_25457242-25457242_C_T	HG19	882R>H	0.86%	4992	43	No	Yes
CGCRC321	DNMT3A	chr2_25457242-25457242_C_T	HG19	882R>H	0.08%	5297	4	No	Yes
CGPLBR101	DNMT3A	chr2.fa:25457242-25457242_C_T	HG19	882R>H	0.08%	13567	5	Not Assessed	Yes
CGPLBR44	DNMT3A	chr2_25457242-25457242_C_T	HG19	882R>H	1.82%	4777	87	Yes	Yes
CGPLBR59	DNMT3A	chr2.fa:25457242-25457242_C_T	HG19	882R>H	0.08%	7113	4	No	Yes

CGPLBR67	DNMT3A	chr2_25457242-25457242_C_T	HG19	882R>H	0.11%	5579	6	Yes	Yes
CGPLBR87	DNMT3A	chr2_25457242-25457242_C_T	HG19	882R>H	0.31%	3228	10	No	Yes
CGPLBR97	DNMT3A	chr2_25457242-25457242_C_T	HG19	882R>H	0.11%	6299	7	Yes	Yes
CGPLOV11	DNMT3A	chr2_25457216-25457221_GGCCCA_	HG19	889LGR>R	2.59%	5019	130	No	No
CGPLLU174	DNMT3A	chr2_25457216-25457216_G_A	HG19	891R>W	0.29%	4133	12	Yes	No
CGPLLU177	DNMT3A	chr2_25457197-25457197_A_C	HG19	897V>G	1.53%	5541	85	Yes	No
CGPLLU161	DNMT3A	chr2_25457191-25457191_C_T	HG19	899R>H	0.13%	5502	7	Not Assessed	No
CGPLLU139	DNMT3A	chr2_25320920-25320920_A_	HG18	C554Afs*97	0.24%	3821	9	No	No
CGCRC338	DNMT3A	chr2_25467443-25467444_CA_	HG19	E545Gfs*32	0.15%	5322	8	No	No
CGCRC296	DNMT3A	chr2_25463301-25463301_A_	HG19	F731Sfs*48	0.27%	3774	10	No	No
CGPLBR72	DNMT3A	chr2_25463301-25463301_A_	HG19	F731Sfs*48	0.74%	3761	28	No	No
CGPLLU22	DNMT3A	chr2_25316794-25316795_AG_	HG18	F734Lfs*6	2.75%	2876	79	Yes	No
CGCRC314	DNMT3A	chr2_25467513-25467513_A_	HG19	L522Wfs*129	0.15%	5440	8	No	No
CGPLLU57	DNMT3A	chr2_25316676-25316679_TCGA_	HG18	L773Rfs*5	0.50%	2380	12	Not Assessed	No
CGPLBR63	DNMT3A	chr2_25463596-25463596__G	HG19	Q696Pfs*17	0.28%	3248	9	No	No
CGPLLU6	DNMT3A	chr2_25310695-25310695_C_	HG18	R899Pfs*7	0.18%	4517	8	No	No
CGPLBR92	DNMT3A	chr2_25463572-25463572_C_	HG19	V704*	0.16%	8195	13	Yes	No
CGPLLU174	DNMT3A	chr2_25463532-25463536_TTGAC_	HG19	V716Pfs*16	0.27%	4474	12	Yes	No
CGPLLU68	DNMT3A	chr2_25310792-25310792_C_	HG18	V867Yfs*14	0.33%	4600	15	Not Assessed	No
CGPLH55	DNMT3A	chr2_25320975-25320975_G_	HG18	Y536Tfs*115	0.24%	2858	7	NA	No
CGCRC295	IDH1	chr2_209113196-209113196_C_A	HG19	104G>V	0.34%	5362	18	Yes	No
CGPLBR59	IDH2	chr15_90631934-90631934_C_T	HG19	140R>Q	0.20%	3420	7	No	Yes
CGPLOV9	IDH2	chr15_88432939-88432939_G_A	HG18	140R>W	0.47%	1279	6	Yes	No
CGPLLU136	JAK2	chr9_5063770-5063770_G_T	HG18	617V>F	0.32%	2799	9	No	Yes
CGPLLU146	JAK2	chr9_5073770-5073770_G_T	HG19	617V>F	0.25%	4051	10	No	Yes
CGPLOV32	DNMT3A	chr2_25463507-25463507_A_G	HG19	2173+2T>C	0.15%	5213	8	Not Assessed	No
CGPLOV38	DNMT3A	chr2_25463289-25463289_T_G	HG19	735Y>S	0.33%	4852	16	Not Assessed	No
CGPLOV42	DNMT3A	chr2_25463286-25463286_C_T	HG19	736R>H	0.16%	6361	10	Not Assessed	No
CGPLOV43	DNMT3A	chr2_25463248-25463248_G_A	HG19	749R>C	7.08%	3797	269	Not Assessed	No
CGPLOV43	DNMT3A	chr2_25463524-25463524_G_A	HG19	720R>C	0.30%	3956	12	Not Assessed	No
CGPLOV47	DNMT3A	chr2_25457243-25457243_G_A	HG19	882R>C	1.77%	6156	109	Not Assessed	Yes
CGPLOV40	DNMT3A	chr2.fa:25457243-25457243_G_A	HG19	882R>C	0.06%	8509	5	Not Assessed	Yes

Table 3.9. Cancer patients detected using TEC-Seq.

Cancer Type	Patients (n)	Patients with ctDNA Alterations (n)	Fraction of patients with ctDNA Alterations (%)
Colorectal			
I	8	4	50%
II	9	8	89%
III	10	9	90%
IV	15	14	93%
I-IV	42	35	83%
Lung			
I	29	13	45%
II	32	23	72%
III	4	3	75%
IV	6	5	83%
I-IV	71	44	62%
Ovarian			
I	24	16	67%
II	4	3	75%
III	8	6	75%
IV	6	5	83%
I-IV	42	30	71%
Breast			
I	3	2	67%
II	29	17	59%
III	13	6	46%
IV	0	NA	NA
I-IV	45	25	56%
All			
I, II	138	86	62%
III, IV	62	48	77%
I-IV	200	134	67%

Table 3.10. Germline alterations identified in cfDNA.

Patient	Gene Symbol	Nucleotide	Genome Build	Amino Acid (Protein)	Mutant Allele Fraction*	Total Distinct Coverage	Distinct Mutant Coverage	Alteration Detected in Matched White Blood Cells
CGCRC292	EGFR	chr7_55248982-55248982_C_G	HG19	2284-4C>G	31.99%	8385	2682	Yes
CGCRC296	EGFR	chr7_55266472-55266472_G_A	HG19	922E>K	30.48%	9919	3023	Yes
CGCRC297	KIT	chr4_55524233-55524233_C_T	HG19	18L>F	41.39%	4921	2037	Yes
CGCRC306	ERBB4	chr2_212251596-212251596_G_A	HG19	1155R>X	38.70%	4530	1753	Yes
CGCRC306	PDGFRA	chr4_55130065-55130065_C_G	HG19	200T>S	34.78%	6000	2087	Yes
CGCRC308	EGFR	chr7_55259485-55259485_C_T	HG19	848P>L	27.69%	9481	2625	Yes
CGCRC313	FBXW7	chr4_153332911-153332911__AGG	HG19	15T>TL	38.42%	5331	2048	Yes
CGCRC320	KIT	chr4_55524233-55524233_C_T	HG19	18L>F	34.76%	6997	2432	Yes
CGCRC321	EGFR	chr7_55229225-55229225_C_A	HG19	511S>Y	41.86%	6457	2703	Yes
CGCRC334	EGFR	chr7_55238900-55238900_C_T	HG19	638T>M	35.28%	2832	999	Yes
CGCRC337	APC	chr5_112162851-112162851_G_A	HG19	485M>I	46.26%	4825	2232	Yes
CGCRC342	ALK	chr2_29606646-29606646_G_A	HG19	412R>C	48.98%	5878	2879	Not Assessed
CGCRC342	APC	chr5_112175211-112175211_T_A	HG19	1307I>K	45.67%	4705	2149	Not Assessed
CGCRC342	TP53	chr17_7579316-7579316__C	HG19	C124Wfs*25	63.52%	1576	1001	Not Assessed
CGPLBR40	AR	chrX_66766163-66766163_C_G	HG19	392P>R	28.99%	7141	2070	Yes
CGPLBR41	MET	chr7_116423378-116423378_T_G	HG19	1236V>G	36.19%	3987	1443	Not Assessed
CGPLBR42	KIT	chr4_55594248-55594248_A_T	HG19	651M>L	39.02%	4600	1795	Not Assessed
CGPLBR42	STK11	chr19_1223125-1223125_C_G	HG19	354F>L	42.32%	3840	1625	Not Assessed
CGPLBR43	ERBB4	chr2_212566755-212566755_T_C	HG19	476T>A	39.79%	5411	2153	Not Assessed
CGPLBR48	ALK	chr2_29436901-29436901_C_T	HG19	1231R>Q	34.61%	4938	1709	Yes
CGPLBR59	STK11	chr19_1223125-1223125_C_G	HG19	354F>L	44.53%	4393	1956	Yes
CGPLBR61	STK11	chr19_1223125-1223125_C_G	HG19	354F>L	40.14%	5598	2247	Not Assessed
CGPLBR63	FGFR3	chr4_1806188-1806188_A_G	HG19	403K>E	34.82%	2967	1033	Yes
CGPLBR67	ESR1	chr6_152129399-152129399_T_C	HG19	118S>P	33.30%	4643	1546	Not Assessed
CGPLBR69	CTNNB1	chr3_41266092-41266092_A_C	HG19	30Y>S	41.74%	5287	2207	Yes
CGPLBR69	IDH1	chr2_209108158-209108158_A_T	HG19	231Y>N	41.66%	3317	1382	Yes

CGPLBR70	APC	chr5_112176022-112176022_A_C	HG19	1577E>D	40.28%	4057	1634	Yes
CGPLBR72	APC	chr5_112175886-112175886_A_G	HG19	1532D>G	44.03%	3607	1588	Yes
CGPLBR73	ERBB4	chr2_212652833-212652833_G_T	HG19	158A>E	35.58%	4461	1587	Yes
CGPLBR74	AR	chrX_66788865-66788865_G_T	HG19	20+1G>T	36.23%	4770	1728	Yes
CGPLBR76	KDR	chr4_55946310-55946310_C_T	HG19	1290S>N	36.57%	4607	1685	Yes
CGPLBR79	STK11	chr19_1223125-1223125_C_G	HG19	354F>L	45.17%	2267	1024	Not Assessed
CGPLBR83	AR	chrX_66937328-66937328_A_G	HG19	728N>D	42.66%	4646	1982	Yes
CGPLBR83	ERBB4	chr2_212543783-212543783_T_G	HG19	539Y>S	44.91%	2035	914	Yes
CGPLBR86	SMARCB1	chr22_24159126-24159126_A_G	HG19	795+3A>G	43.38%	3926	1703	Yes
CGPLBR86	STK11	chr19_1223125-1223125_C_G	HG19	354F>L	42.32%	4421	1871	Yes
CGPLBR87	AR	chrX_66931310-66931310_G_A	HG19	651S>N	42.94%	2762	1186	Yes
CGPLBR88	APC	chr5_112174665-112174665_T_C	HG19	1125V>A	31.19%	5775	1801	Yes
CGPLBR93	ALK	chr2_29451911-29451911_T_C	HG19	885D>G	34.03%	5078	1728	Not Assessed
CGPLBR93	JAK2	chr9_5090536-5090536_T_C	HG19	951I>T	41.23%	3810	1571	Not Assessed
CGPLBR97	PDGFRA	chr4_55136880-55136880_C_A	HG19	401A>D	34.12%	6134	2093	Yes
CGPLH36	STK11	chr19_1174125-1174125_C_G	HG18	354F>L	47.41%	1447	686	NA
CGPLH38	ATM	chr11_107623008-107623008_C_T	HG18	337R>C	42.42%	396	168	NA
CGPLH41	ALK	chr2_29310038-29310038_A_G	HG18	795I>T	41.58%	606	252	NA
CGPLH42	ALK	chr2_29269863-29269863_T_C	HG18	1532N>D	45.86%	2608	1196	NA
CGPLH42	FLT3	chr13_27506341-27506341_T_C	HG18	572Y>C	47.36%	1588	752	NA
CGPLH43	STK11	chr19_1174125-1174125_C_G	HG18	354F>L	45.16%	1384	625	NA
CGPLH46	ALK	chr2_29996673-29996673_C_A	HG18	119E>D	46.86%	1242	582	NA
CGPLH47	ALK	chr2_29269863-29269863_T_C	HG18	1532N>D	46.78%	1678	785	NA
CGPLH48	ALK	chr2_29327569-29327569_C_T	HG18	704A>T	45.58%	1505	686	NA
CGPLH48	APC	chr5_112203557-112203557_A_C	HG18	1456K>T	44.92%	1672	751	NA
CGPLH50	APC	chr5_112202635-112202635_G_A	HG18	1149E>K	47.29%	2252	1065	NA
CGPLH51	DNMT3A	chr4_55292273-55292273_A_G	HG18	722M>V	46.62%	1109	517	NA
CGPLH55	STK11	chr19_1177588-1177588_G_A	HG18	415R>H	46.11%	1503	693	NA
CGPLH58	STK11	chr19_1174125-1174125_C_G	HG18	354F>L	47.55%	1060	504	NA
CGPLH59	ATM	chr11_107680672-107680672_G_A	HG18	1853D>N	43.55%	1621	706	NA
CGPLH61	STK11	chr19_1174125-1174125_C_G	HG18	354F>L	42.48%	565	240	NA

CGPLH64	ATM	chr11_107627849-107627849_A_T	HG18	561Q>H	47.13%	942	444	NA
CGPLH76	APC	chr5_112179623-112179623_G_T	HG19	2778A>S	47.22%	3530	1667	NA
CGPLH76	STK11	chr19_1223125-1223125_C_G	HG19	354F>L	43.93%	2859	1256	NA
CGPLH77	PDGFRA	chr4_55138642-55138642_C_T	HG19	440T>M	43.63%	3516	1534	NA
CGPLH91	TP53	chr17_7577612-7577612_G_A	HG19	673-4C>T	45.32%	1390	630	NA
CGPLLU115	ERBB4	chr2_212003965-212003965_C_A	HG18	865A>S	40.24%	5030	2024	Yes
CGPLLU116	APC	chr5_112203604-112203604_G_A	HG18	1472V>I	39.15%	6117	2395	Yes
CGPLLU118	ALK	chr2_29996561-29996561_G_A	HG18	157P>S	45.16%	2655	1199	Yes
CGPLLU118	APC	chr5_112203139-112203139_G_C	HG18	1317E>Q	47.73%	2793	1333	Yes
CGPLLU119	ATM	chr11_107680672-107680672_G_A	HG18	1853D>N	34.56%	2951	1020	Yes
CGPLLU136	ALK	chr2_29269863-29269863_T_C	HG18	1532N>D	40.61%	5555	2256	Yes
CGPLLU136	PDGFRA	chr4_54833314-54833314_G_A	HG18	1238-4G>A	39.68%	5993	2378	Yes
CGPLLU147	PDGFRA	chr4_55130065-55130065_C_G	HG19	200T>S	43.47%	3391	1474	Yes
CGPLLU164	STK11	chr19_1223125-1223125_C_G	HG19	354F>L	42.52%	4567	1942	Yes
CGPLLU165	STK11	chr19_1223125-1223125_C_G	HG19	354F>L	36.62%	7248	2654	Yes
CGPLLU166	CDKN2A	chr9_21974529-21974529_T_C	HG19	100R>G	35.62%	6125	2182	Yes
CGPLLU175	ATM	chr11_108117798-108117798_C_T	HG19	337R>C	43.84%	3741	1640	Yes
CGPLLU179	APC	chr5_112179123-112179123_C_T	HG19	2611T>I	39.91%	3112	1242	Yes
CGPLLU207	ALK	chr2_29606625-29606625_A_G	HG19	419F>L	34.58%	6469	2237	Yes
CGPLLU208	EGFR	chr7_55220281-55220281_G_A	HG19	224R>H	39.34%	6075	2390	Yes
CGPLLU209	EGFR	chr7_55231493-55231493_A_G	HG19	567M>V	30.41%	8339	2536	Yes
CGPLLU209	STK11	chr19_1223125-1223125_C_G	HG19	354F>L	26.84%	7365	1977	Yes
CGPLLU214	STK11	chr19_1223125-1223125_C_G	HG19	354F>L	44.26%	2822	1249	Not Assessed
CGPLLU26	ALK	chr2_29771364-29771364_A_G	HG18	270F>L	46.69%	906	423	Yes
CGPLLU26	APC	chr5_112203139-112203139_G_C	HG18	1317E>Q	49.94%	811	405	Yes
CGPLLU28	ATM	chr11_107680672-107680672_G_A	HG18	1853D>N	29.16%	2507	731	Yes
CGPLLU30	ALK	chr2_29996561-29996561_G_A	HG18	157P>S	35.57%	3399	1209	Not Assessed
CGPLLU30	ATM	chr11_107680672-107680672_G_A	HG18	1853D>N	30.78%	2752	847	Not Assessed
CGPLLU30	ERBB4	chr2_212278315-212278315_C_T	HG18	391V>I	31.90%	2301	734	Not Assessed
CGPLLU3	ATM	chr11_107680672-107680672_G_A	HG18	1853D>N	6.62%	1950	129	Yes
CGPLLU65	APC	chr5_112207522-112207522_G_T	HG18	2778A>S	44.64%	4715	2105	Yes

CGPLLU66	ERBB2	chr17_35121793-35121793_C_T	HG18	330R>W	42.17%	1513	638	Yes
CGPLLU68	RET	chr10_42930095-42930095_C_G	HG18	681Q>E	36.15%	5948	2150	Not Assessed
CGPLLU6	JAK2	chr9_5012112-5012112_A_G	HG18	42Y>C	42.68%	3997	1706	Yes
CGPLOV11	TP53	chr17_7579499-7579499_G_A	HG19	63A>V	37.77%	4226	1596	Yes
CGPLOV13	PDGFRA	chr4_55136880-55136880_C_A	HG19	401A>D	37.98%	5721	2173	Yes
CGPLOV17	PDGFRA	chr4_55161380-55161380_G_A	HG19	1071D>N	44.10%	4370	1927	Yes
CGPLOV18	APC	chr5_112174665-112174665_T_C	HG19	1125V>A	40.81%	4700	1918	Yes
CGPLOV19	AR	chrX_66765516-66765516_C_A	HG19	176S>R	65.29%	10349	6757	Yes
CGPLOV19	FGFR3	chr4_1806188-1806188_A_G	HG19	403K>E	23.80%	7734	1841	Yes
CGPLOV1	APC	chr5_112205737-112205737_T_A	HG18	2183S>T	40.48%	2473	1001	Yes
CGPLOV20	EGFR	chr7_55221714-55221714_A_G	HG19	253K>R	44.05%	5092	2243	Yes
CGPLOV21	STK11	chr19_1223125-1223125_C_G	HG19	354F>L	7.68%	11974	919	Yes
CGPLOV25	APC	chr5_112177982-112177982_A_T	HG19	2231I>F	43.86%	3785	1660	Not Assessed
CGPLOV2	STK11	chr19_1174125-1174125_C_G	HG18	354F>L	44.29%	4213	1866	Yes
CGPLLU44	APC	chr5_112201775-112201775_A_G	HG18	862N>S	41.71%	6004	2504	Not Assessed
CGPLLU44	APC	chr5_112201775-112201775_A_G	HG18	862N>S	41.00%	3984	1637	Not Assessed
CGPLLU47	KIT	chr4_55288221-55288221_A_C	HG18	541M>L	45.20%	3898	1762	Not Assessed
CGPLOV32	EGFR	chr7_55221714-55221714_A_G	HG19	253K>R	40.94%	7697	3151	Not Assessed
CGPLOV49	ALK	chr2_29450454-29450454_G_T	HG19	967T>N	37.87%	4814	1823	Not Assessed
CGPLOV49	CDK4	chr12_58145379-58145379_T_C	HG19	41N>S	45.58%	5448	2483	Not Assessed

*ATM D1853N and STK11 F354L alterations were considered germline as they were recurrent and the majority of alterations had MAF >25%

Table 3.11. Somatic alterations detected in cfDNA of cancer patients.

Patient	Gene Symbol	Nucleotide	Genome Build	Amino Acid (Protein)	Mutant Allele Fraction	Total Distinct Coverage	Distinct Mutant Coverage	Alteration Detected in Tumor[#]	Hotspot Alteration*
CGCRC309	AKT1	chr14_105246551-105246551_C_T	HG19	17E>K	2.70%	6154	166	Yes	Yes
CGPLLU147	ALK	chr2_29416343-29416343_C_T	HG19	1537G>E	0.94%	4034	38	Yes	No
CGPLBR97	ALK	chr2_30143420-30143420__GGA	HG19	36->S	0.14%	5060	7	Not Assessed	No
CGPLLU203	ALK	chr2_30143420-30143420__GGA	HG19	36->S	0.11%	6627	7	Not Assessed	No
CGPLOV14	ALK	chr2_30143420-30143420__GGA	HG19	36->S	0.12%	5784	7	Not Assessed	No
CGPLOV13	ALK	chr2_29551298-29551298_C_A	HG19	444W>C	0.12%	6814	8	Yes	No
CGPLBR73	ALK	chr2_29474053-29474053_A_G	HG19	708S>P	0.27%	6205	17	No	No
CGPLLU67	ALK	chr2_29308683-29308683_C_T	HG18	875G>R	13.19%	6597	870	No	No
CGCRC291	APC	chr5_112175069-112175069_C_T	HG19	1260Q>X	11.23%	4193	471	Yes	No

CGCRC336	APC	chr5_112175147-112175147_G_T	HG19	1286E>X	81.61%	2827	2307	Yes	No
CGCRC340	APC	chr5_112175207-112175207_G_T	HG19	1306E>X	22.57%	1750	395	Yes	Yes
CGCRC294	APC	chr5_112175390-112175390_C_T	HG19	1367Q>X	0.13%	5421	7	Yes	Yes
CGPLOV19	APC	chr5_112175423-112175423_C_T	HG19	1378Q>X	46.35%	5640	2614	Yes	Yes
CGCRC314	APC	chr5_112175426-112175426_G_T	HG19	1379E>X	0.38%	4523	17	Yes	Yes
CGCRC291	APC	chr5_112175639-112175639_C_T	HG19	1450R>X	11.05%	7518	831	Yes	Yes
CGCRC308	APC	chr5_112175729-112175729_C_T	HG19	1480Q>X	0.11%	6664	7	Yes	No
CGCRC310	APC	chr5_112175828-112175828_G_T	HG19	1513E>X	0.11%	7287	8	Yes	No
CGCRC310	APC	chr5_112175852-112175852_G_T	HG19	1521E>X	0.15%	6204	9	Yes	No
CGCRC294	APC	chr5_112116592-112116592_C_T	HG19	213R>X	0.14%	2952	4	Yes	Yes
CGCRC317	APC	chr5_112128143-112128143_C_T	HG19	216R>X	0.29%	1381	4	No	Yes

CGCRC342	APC	chr5_112151204-112151204_C_T	HG19	283R>X	21.93%	4286	940	Not Assessed	Yes
CGCRC313	APC	chr5_112173917-112173917_C_T	HG19	876R>X	0.07%	5390	4	Yes	Yes
CGCRC339	APC	chr5_112173917-112173917_C_T	HG19	876R>X	2.35%	2888	68	Yes	Yes
CGCRC312	APC	chr5_112174170-112174170_C_G	HG19	960S>X	0.59%	3580	21	Yes	No
CGCRC337	APC	chr5_112128170-112128170_G_	HG19	E225Kfs*68	39.28%	1423	559	Yes	No
CGCRC342	APC	chr5_112175208-112175208__A	HG19	I1307Nfs*8	30.72%	4756	1461	Not Assessed	No
CGCRC317	APC	chr5_112175438-112175438__A	HG19	M1383Nfs*3	0.14%	5705	8	Yes	No
CGCRC334	APC	chr5_112175294-112175294__A	HG19	S1335Kfs*7	16.24%	3208	521	Yes	No
CGCRC338	APC	chr5_112175536-112175536_T_	HG19	S1415Rfs*4	36.00%	5497	1979	Yes	No
CGPLBR96	AR	chrX_66765026-66765026_G_A	HG19	13R>Q	0.60%	3147	19	No	No
CGCRC317	ATM	chr11_108142132-108142132_T_C	HG19	1026W>R	0.23%	5713	13	Yes	No

CGCRC301	ATM	chr11_108199847-108199847_C_T	HG19	2397Q>X	0.21%	3865	8	No	No
CGPLBR70	ATM	chr11_108216546-108216546_G_A	HG19	2832R>H	0.36%	3622	13	No	No
CGPLLU117	ATM	chr11_107721788-107721788_G_T	HG18	2843A>S	0.47%	1899	9	No	No
CGPLLU144	ATM	chr11_108115727-108115727_C_T	HG19	292P>L	0.22%	2743	6	No	No
CGCRC304	ATM	chr11_108142134-108142134_G_A	HG19	3077+1G>A	0.27%	4827	13	No	No
CGPLBR42	ATM	chr11_108142134-108142134_G_A	HG19	3077+1G>A	0.37%	4299	16	Not Assessed	No
CGPLBR83	ATM	chr11_108117753-108117753_G_A	HG19	322E>K	0.28%	2130	6	No	No
CGPLBR70	ATM	chr11_108117756-108117756_A_G	HG19	323I>V	0.36%	2490	9	Not Assessed	No
CGPLLU146	ATM	chr11_108122699-108122699_A_T	HG19	581L>F	0.20%	4534	9	No	No
CGPLBR70	ATM	chr11_108115513-108115513_A_G	HG19	663-2A>G	0.26%	3448	9	Not Assessed	No
CGCRC342	BRAF	chr7_140624473-140624473__CAGGCT	HG19	11->SL	0.36%	3056	11	Not Assessed	No

CGCRC342	BRAF	chr7_140624413-140624413_C_G	HG19	31A>P	0.21%	3370	7	Not Assessed	No
CGCRC291	BRAF	chr7_140624404-140624409_CGGCGC_	HG19	32GAA>A	0.24%	2472	6	Not Assessed	No
CGPLLU162	BRAF	chr7_140494187-140494187_C_T	HG19	354R>Q	0.14%	5176	7	No	No
CGCRC302	BRAF	chr7_140453136-140453136_A_T	HG19	600V>E	0.12%	5002	6	Yes	Yes
CGCRC307	BRAF	chr7_140453136-140453136_A_T	HG19	600V>E	0.38%	4488	17	Yes	Yes
CGCRC309	BRAF	chr7_140453136-140453136_A_T	HG19	600V>E	3.00%	5192	156	Yes	Yes
CGCRC333	BRAF	chr7_140453136-140453136_A_T	HG19	600V>E	22.26%	7026	1564	Yes	Yes
CGCRC335	BRAF	chr7_140453136-140453136_A_T	HG19	600V>E	0.32%	3754	12	Yes	Yes
CGPLBR102	BRAF	chr7_140449173-140449173_G_A	HG19	636Q>X	0.21%	3302	7	Not Assessed	No
CGPLLU178	CDH1	chr16_68844164-68844164_C_T	HG19	251T>M	0.29%	4196	12	No	No
CGPLLU161	CDK6	chr7_92462557-92462557__GAACGGAGG	HG19	27V>VLRS	0.16%	5040	8	Not Assessed	No

CGPLLU162	CDK6	chr7_92462557-92462557__GAACGGAGG	HG19	27V>VLRS	0.14%	5095	7	No	No
CGPLBR43	CDK6	chr7_92462495-92462495_T_C	HG19	48Q>R	0.27%	3011	8	Not Assessed	No
CGPLBR88	CDK6	chr7_92462487-92462487_C_T	HG19	51E>K	0.13%	6043	8	No	No
CGPLBR88	CDK6	chr7_92462424-92462424_C_T	HG19	72E>K	0.38%	1570	6	Not Assessed	No
CGCRC306	CDKN2A	chr9_21971039-21971039_G_A	HG19	107R>C	8.02%	5925	475	Yes	No
CGCRC321	CDKN2A	chr9_21974792-21974792_G_A	HG19	12S>L	0.20%	3027	6	No	No
CGPLBR80	CDKN2A	chr9_21974792-21974792_G_A	HG19	12S>L	0.54%	1673	9	No	No
CGPLBR87	CDKN2A	chr9_21974792-21974792_G_A	HG19	12S>L	0.45%	1330	6	Not Assessed	No
CGPLLU161	CDKN2A	chr9_21974792-21974792_G_A	HG19	12S>L	0.20%	3007	6	Not Assessed	No
CGPLLU162	CDKN2A	chr9_21974792-21974792_G_A	HG19	12S>L	0.22%	3171	7	No	No
CGPLLU163	CDKN2A	chr9_21974792-21974792_G_A	HG19	12S>L	0.21%	3298	7	No	No

CGCRC316	CDKN2A	chr9_21974825-21974825_A_C	HG19	1M>R	5.74%	2577	148	Yes	No
CGPLLU47	CDKN2A	chr9_21964759-21964759_C_A	HG18	23G>V	10.30%	4385	450	Not Assessed	No
CGPLLU47	CDKN2A	chr9_21964759-21964759_C_A	HG18	23G>V	8.70%	2208	192	Not Assessed	No
CGPLLU9	CDKN2A	chr9_21964679-21964679_G_A	HG18	50Q>X	2.30%	2921	66	Not Assessed	No
CGPLLU9	CDKN2A	chr9_21964679-21964679_G_A	HG18	50Q>X	1.42%	4779	68	Not Assessed	No
CGPLOV8	CDKN2A	chr9_21961116-21961116_G_T	HG18	81P>H	0.22%	4020	9	Yes	No
CGPLLU209	CDKN2A	chr9_21971096-21971096_C_A	HG19	88E>X	9.13%	7127	651	Yes	Yes
CGPLLU206	CDKN2A	chr9_21968233-21968233_C_	HG19	D156Ifs	14.59%	2379	347	Yes	No
CGPLLU175	CDKN2A	chr9_21974768-21974774_GCGGCCG_	HG19	T18Rfs*6	7.14%	4807	343	Yes	No
CGPLLU67	CTNNB1	chr3_41241105-41241105_C_G	HG18	33S>C	0.11%	5442	6	Yes	Yes
CGCRC316	CTNNB1	chr3_41266113-41266113_C_G	HG19	37S>C	5.47%	8673	474	Yes	Yes

CGCRC292	CTNNB1	chr3_41266124-41266124_A_G	HG19	41T>A	0.13%	5936	8	Yes	Yes
CGPLOV22	CTNNB1	chr3_41266124-41266124_A_G	HG19	41T>A	0.34%	6983	24	Yes	Yes
CGCRC304	EGFR	chr7_55273068-55273068_A_T	HG19	1131T>S	0.22%	6977	15	No	No
CGPLLU9	EGFR	chr7_55196682-55196682_G_A	HG18	1499-4G>A	0.14%	6370	9	Not Assessed	No
CGCRC316	EGFR	chr7_55266407-55266407_C_T	HG19	2702-3C>T	0.11%	8499	9	No	No
CGPLLU144	EGFR	chr7_55224336-55224336_C_T	HG19	373P>S	0.16%	5066	8	Yes	No
CGPLOV15	EGFR	chr7_55225445-55225445_C_G	HG19	433H>D	0.19%	4815	9	No	No
CGCRC306	EGFR	chr7_55233103-55233103_A_G	HG19	618H>R	6.32%	4935	312	Yes	No
CGPLBR48	EGFR	chr7_55240762-55240762_G_A	HG19	669R>Q	0.18%	5013	9	No	No
CGPLBR87	EGFR	chr7_55240762-55240762_G_A	HG19	669R>Q	0.36%	3339	12	Not Assessed	No
CGPLLU14	EGFR	chr7_55209201-55209201_G_A	HG18	719G>S	0.90%	743	7	Not Assessed	Yes

CGPLLU166	EGFR	chr7_55242462-55242462_C_G	HG19	744I>M	0.12%	5608	7	Yes	No
CGPLLU65	EGFR	chr7_55209960-55209974_GAATTAAGAGAAGCA_	HG18	745KELREA>K	0.33%	3910	13	Yes	Yes
CGPLLU214	EGFR	chr7_55242466-55242480_GAATTAAGAGAAGCA_	HG19	745KELREA>K	0.68%	2950	20	Not Assessed	Yes
CGPLLU135	EGFR	chr7_55209959-55209973_GGAATTAAGAGAAGC_	HG18	745KELREA>T	0.19%	3699	7	Yes	Yes
CGPLLU136	EGFR	chr7_55209959-55209973_GGAATTAAGAGAAGC_	HG18	745KELREA>T	0.21%	4343	9	Yes	Yes
CGPLLU19	EGFR	chr7_55209959-55209973_GGAATTAAGAGAAGC_	HG18	745KELREA>T	1.76%	2890	51	Yes	Yes
CGPLLU207	EGFR	chr7_55242465-55242479_GGAATTAAGAGAAGC_	HG19	745KELREA>T	0.28%	4703	13	Yes	Yes
CGPLLU20	EGFR	chr7_55209959-55209973_GGAATTAAGAGAAGC_	HG18	745KELREA>T	0.29%	1712	5	Yes	Yes
CGPLLU202	EGFR	chr7.fa:55249071-55249071_C_T	HG19	790T>M	0.05%	13342	4	Yes	Yes
CGPLLU207	EGFR	chr7.fa:55249071-55249071_C_T	HG19	790T>M	0.09%	12421	6	No	Yes
CGPLLU14	EGFR	chr7_55216565-55216565_C_T	HG18	790T>M	0.40%	718	3	Not Assessed	Yes

CGPLLU14	EGFR	chr7_55216565-55216565_C_T	HG18	790T>M	0.33%	1208	4	Not Assessed	Yes
CGPLLU162	EGFR	chr7_55259515-55259515_T_G	HG19	858L>R	0.22%	6346	14	Yes	Yes
CGPLLU166	EGFR	chr7_55259515-55259515_T_G	HG19	858L>R	0.44%	7451	33	Yes	Yes
CGPLLU168	EGFR	chr7.fa:55259515-55259515_T_G	HG19	858L>R	0.07%	11196	4	Yes	Yes
CGPLLU198	EGFR	chr7_55259515-55259515_T_G	HG19	858L>R	0.52%	5176	27	Yes	Yes
CGPLLU208	EGFR	chr7_55259515-55259515_T_G	HG19	858L>R	0.86%	5686	49	Yes	Yes
CGPLLU14	EGFR	chr7_55227018-55227018_T_A	HG18	861L>Q	0.40%	737	3	Not Assessed	Yes
CGPLLU202	EGFR	chr7_55259544-55259544_G_T	HG19	868E>X	0.13%	5463	7	No	No
CGPLLU118	EGFR	chr7_55235571-55235571_C_T	HG18	973R>X	2.68%	3243	87	Yes	No
CGPLLU1	ERBB2	chr17_35121734-35121734_C_T	HG18	310S>F	0.30%	4342	13	Yes	No
CGPLLU146	ERBB2	chr17_37880982-37880982__GCATACGTGATG	HG19	771->AYVM	1.18%	6200	73	Yes	No

CGPLBR67	ERBB4	chr2_212285302-212285302_T_G	HG19	1000D>A	0.28%	3864	11	No	No
CGPLBR42	ERBB4	chr2_212248464-212248464_T_C	HG19	1268Y>C	0.21%	3817	8	Not Assessed	No
CGPLLU164	ERBB4	chr2_212248371-212248371_G_A	HG19	1299P>L	0.96%	3008	29	Yes	No
CGCRC307	ERBB4	chr2_213403205-213403205_G_A	HG19	17A>V	0.15%	5893	9	No	No
CGPLLU164	ERBB4	chr2_212587243-212587243_T_C	HG19	253N>S	0.22%	3234	7	No	No
CGPLLU170	ERBB4	chr2_212576859-212576859_G_A	HG19	347T>I	0.27%	5234	14	Not Assessed	No
CGPLLU144	ERBB4	chr2_212568841-212568841_C_T	HG19	426R>K	0.18%	4498	8	No	No
CGPLOV21	ERBB4	chr2_212530114-212530114_C_G	HG19	602S>T	14.36%	9659	1387	No	No
CGCRC333	ERBB4	chr2_212495194-212495194_T_G	HG19	691E>A	1.00%	3613	36	No	No
CGCRC320	ERBB4	chr2_212989479-212989479_G_A	HG19	78R>W	0.12%	5654	7	No	No
CGPLLU175	ERBB4	chr2_212288925-212288925_G_A	HG19	941Q>X	3.64%	4502	164	Yes	No

CGCRC332	ESR1	chr6_152129111-152129111_G_T	HG19	22E>X	0.38%	1847	7	Not Assessed	No
CGPLLU145	ESR1	chr6_152332868-152332868_G_A	HG19	392V>I	0.37%	5112	19	Not Assessed	No
CGPLBR102	ESR1	chr6_152415658-152415658_G_A	HG19	503R>Q	0.17%	6547	11	Not Assessed	No
CGCRC307	FBXW7	chr4_153249385-153249385_G_A	HG19	465R>C	0.31%	6399	20	Yes	Yes
CGCRC315	FBXW7	chr4_153247289-153247289_G_A	HG19	505R>C	0.25%	5691	14	Yes	Yes
CGCRC295	FBXW7	chr4_153259012-153259013_AT_	HG19	M268Dfs*18	0.50%	3377	17	No	No
CGCRC295	FBXW7	chr4_153251985-153251989_CTTTT_	HG19	R339Sfs*22	2.09%	4249	89	Yes	No
CGPLLU68	FGFR2	chr10_123269525-123269525_A_T	HG18	299S>R	0.12%	5905	7	Not Assessed	No
CGCRC305	GNA11	chr19_3118954-3118954_G_A	HG19	213R>Q	0.11%	7025	8	Yes	No
CGPLLU164	GNA11	chr19_3118919-3118919_C_T	HG19	606-3C>T	0.20%	4070	8	No	No
CGCRC307	GNAS	chr20_57484420-57484420_C_T	HG19	201R>C	0.24%	3271	8	Yes[#]	Yes

CGPLBR104	GNAS	chr20_57484420-57484420_C_T	HG19	201R>C	0.13%	3200	4	Not Assessed	Yes
CGPLBR55	GNAS	chr20_57484421-57484421_G_A	HG19	201R>H	0.68%	3247	22	Yes	Yes
CGPLBR97	GNAS	chr20_57484421-57484421_G_A	HG19	201R>H	0.13%	2995	4	Yes	Yes
CGPLLU165	GNAS	chr20_57484421-57484421_G_A	HG19	201R>H	0.16%	3676	6	Yes	Yes
CGPLLU169	HNF1A	chr12_121426820-121426820_C_T	HG19	171R>X	0.13%	7587	10	Yes	No
CGPLOV14	HNF1A	chr12_121431484-121431484_G_A	HG19	230E>K	0.14%	6643	9	No	No
CGPLLU174	JAK2	chr9_5050695-5050695_G_T	HG19	160D>Y	0.40%	2271	9	Yes	No
CGPLBR87	JAK2	chr9_5054591-5054591_C_T	HG19	215R>X	0.35%	2270	8	No	No
CGCRC307	JAK2	chr9_5080662-5080662_C_G	HG19	805L>V	0.56%	3419	19	No	No
CGPLOV6	JAK3	chr19_17808961-17808961_T_G	HG18	588H>P	2.55%	510	13	Not Assessed	No
CGPLOV13	KIT	chr4_55564516-55564516_G_A	HG19	135R>H	0.35%	4886	17	Yes	No

CGPLOV27	KIT	chr4_55594262-55594262_T_A	HG19	655N>K	0.18%	4360	8	Not Assessed	No
CGPLOV4	KIT	chr4_55292273-55292273_A_G	HG18	722M>V	1.39%	2945	41	Not Assessed	No
CGPLLU47	KIT	chr4_55292809-55292809_G_C	HG18	750R>T	0.70%	6376	42	Not Assessed	No
CGPLLU47	KIT	chr4_55292809-55292809_G_C	HG18	750R>T	0.56%	5187	29	Not Assessed	No
CGPLOV27	KIT	chr4_55599332-55599332_G_T	HG19	820D>Y	5.58%	4499	251	Not Assessed	No
CGPLLU204	KIT	chr4_55604659-55604659_G_A	HG19	956R>Q	0.26%	4533	12	No	No
CGCRC291	KRAS	chr12_25398284-25398284_C_G	HG19	12G>A	14.65%	4800	703	Yes	Yes
CGPLLU144	KRAS	chr12_25398285-25398285_C_A	HG19	12G>C	5.10%	4587	234	Yes	Yes
CGPLLU174	KRAS	chr12_25398285-25398285_C_A	HG19	12G>C	0.16%	3065	5	Yes	Yes
CGPLLU26	KRAS	chr12_25289552-25289552_C_A	HG18	12G>C	2.67%	675	18	Yes	Yes
CGCRC314	KRAS	chr12_25398284-25398284_C_T	HG19	12G>D	0.30%	2960	9	Yes	Yes

CGCRC319	KRAS	chr12_25398284-25398284_C_T	HG19	12G>D	0.11%	3731	4	Yes	Yes
CGCRC338	KRAS	chr12_25398284-25398284_C_T	HG19	12G>D	27.03%	2379	643	Yes	Yes
CGPLLU4	KRAS	chr12_25289551-25289551_C_T	HG18	12G>D	0.22%	2267	5	Not Assessed	Yes
CGCRC313	KRAS	chr12_25398285-25398285_C_T	HG19	12G>S	0.17%	2950	5	Yes	Yes
CGCRC310	KRAS	chr12_25398284-25398284_C_A	HG19	12G>V	0.13%	4448	6	Yes	Yes
CGCRC336	KRAS	chr12_25398284-25398284_C_A	HG19	12G>V	42.87%	2442	1047	Yes	Yes
CGPLLU177	KRAS	chr12_25398284-25398284_C_A	HG19	12G>V	2.49%	3941	98	Yes	Yes
CGPLOV2	KRAS	chr12_25289551-25289551_C_A	HG18	12G>V	0.34%	2940	10	Yes	Yes
CGPLLU44	KRAS	chr12_25289551-25289551_C_A	HG18	12G>V	9.09%	3532	321	Not Assessed	Yes
CGPLLU44	KRAS	chr12_25289551-25289551_C_A	HG18	12G>V	15.00%	2027	300	Not Assessed	Yes
CGCRC339	KRAS	chr12_25398281-25398281_C_T	HG19	13G>D	1.94%	1908	37	Yes	Yes

CGPLLU57	KRAS	chr12_25289548-25289548_C_T	HG18	13G>D	7.58%	1254	95	Not Assessed	Yes
CGPLLU57	KRAS	chr12_25289548-25289548_C_T	HG18	13G>D	8.67%	1903	165	Not Assessed	Yes
CGCRC292	KRAS	chr12_25378561-25378561_G_A	HG19	146A>V	1.41%	2757	39	No	Yes
CGCRC306	KRAS	chr12_25380277-25380277_G_T	HG19	61Q>K	7.30%	4232	309	Yes	Yes
CGCRC295	MAP2K1	chr15_66727455-66727455_G_T	HG19	57K>N	2.26%	5529	125	Not Assessed	No
CGCRC309	MLH1	chr3_37089132-37089132__GGC	HG19	618K>KA	0.89%	6423	57	No	No
CGCRC320	MLH1	chr3_37089132-37089132__GGC	HG19	618K>KA	0.64%	5640	36	No	No
CGPLBR82	MYC	chr8_128750755-128750755_C_T	HG19	98R>W	0.12%	7262	9	Yes	No
CGPLLU208	MYC	chr8_128750755-128750755_C_T	HG19	98R>W	0.17%	5293	9	No	No
CGCRC315	NRAS	chr1_115258747-115258747_C_T	HG19	12G>D	0.27%	5246	14	Yes	Yes
CGCRC312	NRAS	chr1_115256530-115256530_G_T	HG19	61Q>K	0.47%	2354	11	Yes	Yes

CGCRC295	PDGFRA	chr4_55124988-55124988_C_T	HG19	49+4C>T	0.45%	3805	17	No	No
CGPLLU170	PDGFRA	chr4_55124988-55124988_C_T	HG19	49+4C>T	0.31%	4769	15	Not Assessed	No
CGPLLU44	PDGFRA	chr4_54835769-54835769_C_T	HG18	553P>L	1.28%	4600	59	Not Assessed	No
CGPLLU44	PDGFRA	chr4_54835769-54835769_C_T	HG18	553P>L	1.00%	2934	31	Not Assessed	No
CGCRC342	PDGFRA	chr4_55144601-55144601_G_T	HG19	692S>I	0.25%	5704	14	Not Assessed	No
CGPLBR44	PDGFRA	chr4_55153609-55153609_G_A	HG19	859V>M	0.13%	6745	9	Yes	No
CGPLLU209	PDGFRA	chr4_55155052-55155052_G_A	HG19	921A>T	9.82%	5806	570	Yes	No
CGCRC339	PIK3CA	chr3_178952085-178952085_A_T	HG19	1047H>L	1.71%	2926	50	Yes	Yes
CGPLBR102	PIK3CA	chr3_178952085-178952085_A_G	HG19	1047H>R	0.25%	5274	13	Not Assessed	Yes
CGPLBR75	PIK3CA	chr3_178952085-178952085_A_G	HG19	1047H>R	0.14%	4869	7	Yes	Yes
CGPLBR76	PIK3CA	chr3_178952085-178952085_A_G	HG19	1047H>R	0.12%	7334	9	Yes	Yes

CGCRC334	PIK3CA	chr3_178916924-178916924_C_G	HG19	104P>R	3.85%	2934	113	No	No
CGPLBR55	PIK3CA	chr3_178921553-178921553_T_A	HG19	345N>K	0.42%	2157	9	Yes	Yes
CGPLLU44	PIK3CA	chr3_180399419-180399419_C_T	HG18	38R>C	0.18%	5470	10	Not Assessed	No
CGPLLU44	PIK3CA	chr3_180399419-180399419_C_T	HG18	38R>C	0.26%	3412	9	Not Assessed	No
CGCRC339	PIK3CA	chr3_178927457-178927457_G_T	HG19	407C>F	3.14%	1813	57	Yes	No
CGCRC298	PIK3CA	chr3_178927478-178927478_G_T	HG19	414G>V	0.55%	3657	20	No	No
CGPLLU9	PIK3CA	chr3_180399318-180399318_G_T	HG18	4R>L	1.70%	2500	43	Not Assessed	No
CGPLLU9	PIK3CA	chr3_180399318-180399318_G_T	HG18	4R>L	0.48%	2904	14	Not Assessed	No
CGCRC291	PIK3CA	chr3_178936082-178936082_G_A	HG19	542E>K	18.11%	3938	713	Yes	Yes
CGPLBR41	PIK3CA	chr3_178936082-178936082_G_A	HG19	542E>K	0.32%	3143	10	Not Assessed	Yes
CGCRC306	PIK3CA	chr3_178936092-178936092_A_C	HG19	545E>A	0.96%	3122	30	No	Yes

CGPLBR67	PIK3CA	chr3_178936091-178936091_G_A	HG19	545E>K	0.68%	3076	21	Yes	Yes
CGPLLU144	PIK3CA	chr3_178936091-178936091_G_A	HG19	545E>K	2.94%	3940	116	Yes	Yes
CGPLLU178	PIK3CA	chr3_178947145-178947145_C_T	HG19	861Q>X	0.17%	4012	7	No	No
CGCRC334	PIK3R1	chr5_67589576-67589593_AAAAAATTACATGAATAT_	HG19	446GKKLHEY>G	0.56%	1432	8	Yes	No
CGPLBR77	PTEN	chr10_89711891-89711891_G_T	HG19	170S>I	2.29%	3319	76	Yes	No
CGPLBR99	PTEN	chr10_89653785-89653785__TT	HG19	Y29Ffs*26	0.57%	2291	13	Not Assessed	No
CGPLLU47	RB1	chr13_47853511-47853511_G_C	HG18	542L>F	2.50%	2752	68	Not Assessed	No
CGPLLU47	RB1	chr13_47853511-47853511_G_C	HG18	542L>F	2.61%	2377	62	Not Assessed	No
CGPLLU180	RB1	chr13_48955578-48955578_C_G	HG19	565S>X	1.01%	3163	32	Yes	No
CGPLLU146	RB1	chr13_48937095-48937095_T_C	HG19	861+2T>C	0.87%	2868	25	Yes	No
CGPLOV3	SMAD4	chr18_46857030-46857030_C_T	HG18	445R>X	9.88%	1528	151	Yes	No

CGPLBR87	SMAD4	chr18_48604664-48604664_C_T	HG19	496R>C	0.40%	4551	18	No	No
CGCRC291	SMAD4	chr18_48591806-48591807_GT_	HG19	C324Ffs*6	22.69%	7307	1658	Yes	No
CGCRC307	SMARCB1	chr22_24145480-24145480_A_G	HG19	501-2A>G	0.34%	4728	16	Yes	No
CGPLLU180	SMARCB1	chr22_24159061-24159062_AT_	HG19	I245Rfs*35	2.39%	5697	136	Yes	No
CGPLLU164	STK11	chr19_1220629-1220629_C_A	HG19	216S>Y	1.23%	3416	42	Yes	No
CGPLLU180	STK11	chr19_1220691-1220691_G_T	HG19	237D>Y	2.43%	3377	82	Yes	No
CGPLLU26	STK11	chr19_1172998-1172998_C_T	HG18	305Q>X	1.05%	569	6	Yes	No
CGCRC291	STK11	chr19_1207027-1207027_C_T	HG19	39R>C	0.14%	5165	7	No	No
CGPLLU174	STK11	chr19_1220505-1220505_G_T	HG19	597+1G>T	0.33%	3050	10	Yes	No
CGCRC337	STK11	chr19_1220718-1220718_T_A	HG19	734+2T>A	0.12%	8482	10	No	No
CGPLLU177	STK11	chr19_1221266-1221266_G_	HG19	L263Ffs*24	3.22%	4414	142	Yes	No

CGPLLU209	TP53	chr17_7579389-7579389_G_A	HG19	100Q>X	9.97%	5258	524	Yes	No
CGCRC332	TP53	chr17_7579313-7579313_G_C	HG19	125T>R	19.98%	936	187	Yes	No
CGPLLU206	TP53	chr17_7578538-7578538_T_C	HG19	131N>S	0.21%	6283	13	No	No
CGPLBR99	TP53	chr17_7578535-7578537_TTG_	HG19	131NK>K	0.90%	4242	38	Yes	No
CGPLLU168	TP53	chr17.fa:7578524-7578524_G_A	HG19	136Q>X	0.06%	15310	5	Yes	Yes
CGCRC302	TP53	chr17_7578508-7578508_C_T	HG19	141C>Y	0.05%	11449	6	Yes	Yes
CGPLLU44	TP53	chr17.fa:7519233-7519233_C_T	HG18	141C>Y	0.09%	4511	4	Not Assessed	Yes
CGPLLU19	TP53	chr17_7519228-7519228_C_T	HG18	143V>M	0.12%	3288	4	No	Yes
CGPLLU14	TP53	chr17_7519186-7519186_C_A	HG18	157V>F	1.50%	750	11	Not Assessed	Yes
CGPLLU14	TP53	chr17_7519186-7519186_C_A	HG18	157V>F	0.51%	1366	7	Not Assessed	Yes
CGPLLU57	TP53	chr17_7519186-7519186_C_A	HG18	157V>F	6.51%	1459	95	Not Assessed	Yes

CGPLLU57	TP53	chr17_7519186-7519186_C_A	HG18	157V>F	6.32%	2628	166	Not Assessed	Yes
CGPLLU9	TP53	chr17_7519186-7519186_C_A	HG18	157V>F	1.70%	3932	68	Not Assessed	Yes
CGPLLU9	TP53	chr17_7519186-7519186_C_A	HG18	157V>F	0.77%	6528	50	Not Assessed	Yes
CGPLLU67	TP53	chr17_7519182-7519182_C_A	HG18	158R>L	0.19%	6943	13	Yes	Yes
CGPLLU164	TP53	chr17_7578450-7578450_C_A	HG19	160M>I	1.86%	5638	105	Yes	No
CGPLLU164	TP53	chr17_7578449-7578449_C_A	HG19	161A>S	1.78%	5734	102	Yes	No
CGPLLU44	TP53	chr17_7519174-7519174_C_T	HG18	161A>T	3.65%	7556	276	Not Assessed	Yes
CGPLLU44	TP53	chr17_7519174-7519174_C_T	HG18	161A>T	5.00%	4627	229	Not Assessed	Yes
CGPLLU198	TP53	chr17_7578445-7578445_A_T	HG19	162I>N	0.87%	5268	46	Yes	No
CGCRC291	TP53	chr17_7578431-7578431_G_A	HG19	167Q>X	22.85%	9599	2193	Yes	Yes
CGCRC303	TP53	chr17_7578413-7578413_C_A	HG19	173V>L	0.08%	6324	5	Yes	Yes

CGCRC336	TP53	chr17_7578406-7578406_C_T	HG19	175R>H	75.26%	7220	5434	Yes	Yes
CGPLLU166	TP53	chr17_7578406-7578406_C_T	HG19	175R>H	0.42%	10271	43	Yes	Yes
CGCRC293	TP53	chr17_7578404-7578404_A_T	HG19	176C>S	0.35%	4244	15	No	No
CGPLLU180	TP53	chr17.fa:7578400-7578400_G_A	HG19	177P>L	0.08%	8874	4	No	Yes
CGPLLU175	TP53	chr17_7578394-7578394_T_C	HG19	179H>R	8.03%	6349	510	Yes	Yes
CGPLBR102	TP53	chr17_7578395-7578395_G_A	HG19	179H>Y	0.16%	5606	9	Not Assessed	Yes
CGPLOV22	TP53	chr17_7578271-7578271_T_G	HG19	193H>P	0.49%	5675	28	Yes	No
CGPLOV20	TP53	chr17_7578265-7578265_A_G	HG19	195I>T	0.21%	3352	7	Yes	Yes
CGCRC306	TP53	chr17_7578263-7578263_G_A	HG19	196R>X	0.12%	4933	6	No	Yes
CGCRC340	TP53	chr17_7578263-7578263_G_A	HG19	196R>X	18.26%	1813	331	Yes	Yes
CGPLLU147	TP53	chr17_7578247-7578247_A_T	HG19	201L>X	0.55%	2930	16	Yes	No

CGPLBR96	TP53	chr17.fa:7578212-7578212_G_A	HG19	213R>X	0.10%	9808	4	No	Yes
CGPLLU1	TP53	chr17_7518937-7518937_G_A	HG18	213R>X	0.29%	4145	12	Yes	Yes
CGPLOV3	TP53	chr17_7518937-7518937_G_A	HG18	213R>X	4.18%	1961	82	Yes	Yes
CGPLLU29	TP53	chr17_7518928-7518928_C_T	HG18	216V>M	0.30%	3039	9	Not Assessed	Yes
CGPLLU9	TP53	chr17_7518900-7518920_ACCTCAGGCGGCTCATAGGGC_	HG18	219PYEPPE>-	0.90%	3842	34	Not Assessed	No
CGPLLU9	TP53	chr17_7518900-7518920_ACCTCAGGCGGCTCATAGGGC_	HG18	219PYEPPE>-	0.18%	5669	10	Not Assessed	No
CGCRC317	TP53	chr17_7578190-7578190_T_C	HG19	220Y>C	0.36%	6753	24	Yes	Yes
CGPLLU144	TP53	chr17_7577559-7577559_G_A	HG19	241S>F	1.95%	5590	109	Yes	Yes
CGPLBR38	TP53	chr17_7577560-7577560_A_G	HG19	241S>P	0.53%	4505	24	Yes	No
CGPLLU19	TP53	chr17_7518282-7518282_A_G	HG18	242C>R	0.53%	2656	14	Yes	No
CGPLLU44	TP53	chr17_7518282-7518282_A_G	HG18	242C>R	0.14%	5584	8	Not Assessed	No

CGPLLU26	TP53	chr17_7518276-7518276_C_A	HG18	244G>C	0.59%	847	5	Yes	Yes
CGCRC316	TP53	chr17_7577548-7577548_C_T	HG19	245G>S	6.52%	7665	500	Yes	Yes
CGCRC334	TP53	chr17_7577548-7577548_C_T	HG19	245G>S	13.44%	3006	404	Yes	Yes
CGPLLU14	TP53	chr17_7518264-7518264_G_C	HG18	248R>G	1.60%	683	11	Not Assessed	No
CGPLLU14	TP53	chr17_7518264-7518264_G_C	HG18	248R>G	0.56%	1070	6	Not Assessed	No
CGPLLU147	TP53	chr17_7577538-7577538_C_T	HG19	248R>Q	0.15%	2644	4	No	Yes
CGPLOV11	TP53	chr17_7577538-7577538_C_T	HG19	248R>Q	0.87%	4580	40	Yes	Yes
CGPLOV17	TP53	chr17_7577538-7577538_C_T	HG19	248R>Q	0.32%	2772	9	Yes	Yes
CGPLLU44	TP53	chr17_7518261-7518261_T_C	HG18	249R>G	0.12%	6072	7	Not Assessed	Yes
CGPLLU208	TP53	chr17_7577532-7577532_G_A	HG19	250P>L	1.33%	4668	62	Yes	Yes
CGCRC294	TP53	chr17_7577525-7577527_GAG_	HG19	252L>-	0.17%	5973	10	Yes	No

CGPLBR92	TP53	chr17_7577511-7577511_A_G	HG19	257L>P	0.20%	5486	11	Yes	No
CGPLBR61	TP53	chr17_7577129-7577129_A_C	HG19	270F>C	0.44%	4573	20	Not Assessed	No
CGCRC291	TP53	chr17_7577124-7577124_C_T	HG19	272V>M	0.10%	5985	6	No	Yes
CGCRC319	TP53	chr17_7577124-7577124_C_T	HG19	272V>M	0.07%	7496	5	No	Yes
CGPLOV2	TP53	chr17_7517846-7517846_G_A	HG18	273R>C	0.14%	4371	6	Yes	Yes
CGCRC305	TP53	chr17_7577120-7577120_C_T	HG19	273R>H	0.19%	6265	12	No	Yes
CGPLBR71	TP53	chr17_7577120-7577120_C_T	HG19	273R>H	0.10%	4834	5	Yes	Yes
CGPLOV19	TP53	chr17_7577120-7577120_C_T	HG19	273R>H	36.83%	5430	2000	Yes	Yes
CGPLLU135	TP53	chr17_7517845-7517845_C_A	HG18	273R>L	0.23%	3976	9	Yes	Yes
CGPLOV21	TP53	chr17_7577114-7577114_C_T	HG19	275C>Y	2.04%	9798	200	Yes	No
CGPLLU164	TP53	chr17_7577106-7577106_G_A	HG19	278P>S	0.10%	4788	5	No	Yes

CGPLOV15	TP53	chr17_7577106-7577106_G_A	HG19	278P>S	3.54%	4629	164	Yes	Yes
CGPLLU146	TP53	chr17_7577093-7577093_C_G	HG19	282R>P	1.30%	5379	70	Yes	No
CGPLLU180	TP53	chr17_7577093-7577093_C_G	HG19	282R>P	1.94%	4546	88	Yes	No
CGPLLU180	TP53	chr17_7577060-7577060_C_A	HG19	293G>V	2.07%	4981	103	Yes	No
CGPLOV10	TP53	chr17_7574003-7574003_G_A	HG19	342R>X	3.14%	2962	93	Yes	Yes
CGPLLU47	TP53	chr17.fa:7514728-7514728_G_A	HG18	342R>X	0.08%	5246	4	Not Assessed	Yes
CGPLLU207	TP53	chr17_7578555-7578555_C_T	HG19	376-1G>A	0.32%	5858	19	Yes	Yes
CGPLLU47	TP53	chr17_7519011-7519019_AGACCTAAG_	HG18	560-5_563delCTTAGGTCT	16.10%	4690	755	Not Assessed	No
CGPLLU47	TP53	chr17_7519011-7519019_AGACCTAAG_	HG18	560-5_563delCTTAGGTCT	18.87%	3392	640	Not Assessed	No
CGPLLU206	TP53	chr17_7578176-7578176_C_T	HG19	672+1G>A	26.13%	6457	1687	Yes	Yes
CGCRC333	TP53	chr17_7577610-7577610_T_C	HG19	673-2A>G	43.03%	2805	1207	Yes	No

CGPLOV23	TP53	chr17_7577498-7577498_C_A	HG19	782+1G>T	1.39%	4523	63	Not Assessed	No
CGPLOV16	TP53	chr17_7579316-7579317_CA_	HG19	C124Hfs*24	1.12%	2668	30	Yes	No
CGCRC337	TP53	chr17_7578376-7578379_CTAT_	HG19	D184Afs*62	57.84%	8975	5191	Yes	No
CGPLOV37	TP53	chr17_7578394-7578394_T_C	HG19	179H>R	0.29%	7173	21	Not Assessed	Yes
CGPLOV38	PTEN	chr10_89624261-89624261_A_T	HG19	12N>I	3.78%	3093	117	Not Assessed	No
CGPLOV38	TP53	chr17_7577539-7577539_G_A	HG19	248R>W	4.89%	4434	217	Not Assessed	Yes
CGPLOV40	APC	chr5_112179623-112179623_G_T	HG19	2778A>S	6.73%	8066	543	Not Assessed	No
CGPLOV40	NRAS	chr1_115256536-115256536_C_T	HG19	59A>T	0.29%	3798	11	Not Assessed	No
CGPLOV40	TP53	chr17_7578406-7578406_C_T	HG19	175R>H	0.63%	9964	63	Not Assessed	Yes
CGPLOV41	TP53	chr17_7578406-7578406_C_T	HG19	175R>H	0.60%	4364	26	Not Assessed	Yes
CGPLOV42	TP53	chr17_7578263-7578263_G_A	HG19	196R>X	1.24%	6793	84	Not Assessed	Yes

CGPLOV44	TP53	chr17_7577022-7577022_G_A	HG19	306R>X	0.37%	4646	17	Not Assessed	Yes
CGPLOV47	ERBB4	chr2_212251595-212251595_C_T	HG19	1155R>Q	3.20%	4467	143	Not Assessed	No
CGPLOV48	RB1	chr13_48951174-48951174_A_G	HG19	1332+4A>G	10.70%	1934	207	Not Assessed	No
CGPLOV48	TP53	chr17_7577120-7577120_C_T	HG19	273R>H	0.13%	3089	4	Not Assessed	Yes
CGPLOV49	TP53	chr17_7577120-7577120_C_T	HG19	273R>H	0.17%	4146	7	Not Assessed	Yes
CGPLOV49	TP53	chr17_7577547-7577547_C_A	HG19	245G>V	2.03%	4184	85	Not Assessed	Yes
CGPLOV40	PTPN11	chr12.fa:112888199-112888199_C_T	HG19	72A>V	0.06%	7095	4	Not Assessed	Yes

*"Hotspot" indicates an alteration occurring at the same nucleotide position in at least 20 other cancer cases in COSMIC. The matching COSMIC cases are indicated in the column labelled "Reported samples with the identical somatic mutation" (for HG19) and "Reported somatic mutations in the same amino acid residue" (for HG18).
#"Alteration Detected in Tumor" indicates mutations that were confirmed in the matching tumor sample of each case. For sample CGCRC307 the corresponding GNAS mutation was not detected in the sample obtained at diagnosis but was identified in other biopsies from the same tumor specimen and from a subsequent metastatic lesion.

Table 3.12. Summary of colorectal cancer patient outcomes.

Patient	Stage at Diagnosis	Maximum Mutant Allele Fraction (TEC-Seq)	Progression-free Survival (0 - Progression-free ; 1 - Progression)	Time to Progression-free Survival (months)	Overall Survival (0 - Alive ; 1 - Dead of disease)	Time to Overall Survival (months)	CEA (ng/ml)	Smoking status
CGCRC291	IV	22.85%	1	21.10	1	25.87	2.10	Non-smoker
CGCRC292	IV	1.41%	0	32.07	0	32.07	8.90	Non-smoker
CGCRC293	IV	0.35%	0	2.67	0	2.67	23.00	Non-smoker
CGCRC294	II	0.17%	0	31.37	0	31.37	2.90	Non-smoker
CGCRC295	IV	2.26%	1	7.43	1	19.00	No Data	Non-smoker
CGCRC296	II	0.00%	0	31.00	0	31.00	3.50	Former-smoker
CGCRC297	III	0.00%	0	30.83	0	30.83	2.70	Former-smoker
CGCRC298	II	0.55%	0	30.80	0	30.80	3.40	Non-smoker
CGCRC299	I	0.00%	0	30.43	0	30.43	4.80	Non-smoker
CGCRC300	I	0.00%	0	30.40	0	30.40	3.50	Non-smoker
CGCRC301	I	0.21%	0	30.37	0	30.37	No Data	Current-smoker
CGCRC302	II	0.12%	0	9.53	0	9.53	2.50	Former-smoker
CGCRC303	III	0.08%	0	30.13	0	30.13	19.00	Non-smoker
CGCRC304	II	0.27%	1	22.73	1	28.90	2.40	Former-smoker
CGCRC305	II	0.19%	0	29.20	0	29.20	1.80	Non-smoker
CGCRC306	II	8.02%	1	11.43	1	11.57	39.00	Non-smoker
CGCRC307	II	0.56%	1	24.17	0	24.17	37.00	Non-smoker
CGCRC308	III	0.11%	0	28.80	0	28.80	116.00	Current-smoker
CGCRC309	III	3.00%	1	7.03	1	7.67	1.90	Non-smoker
CGCRC310	II	0.15%	0	28.57	0	28.57	3.80	Former-smoker
CGCRC311	I	0.00%	0	28.43	0	28.43	1.00	Non-smoker
CGCRC312	III	0.59%	0	28.27	0	28.27	2.60	Current-smoker
CGCRC313	III	0.17%	0	28.00	0	28.00	18.00	Former-smoker
CGCRC314	I	0.38%	0	27.63	0	27.63	4.00	Former-smoker
CGCRC315	III	0.27%	0	27.63	0	27.63	1.50	Non-smoker
CGCRC316	III	6.52%	0	27.17	0	27.17	8.40	Non-smoker

CGCRC317	III	0.36%	0	27.17	0	27.17	6.40	Non-smoker
CGCRC318	I	0.00%	0	26.93	0	26.93	1.90	Non-smoker
CGCRC319	III	0.11%	0	26.83	0	26.83	5.30	Non-smoker
CGCRC320	I	0.64%	0	26.90	0	26.90	3.10	Former-smoker
CGCRC321	I	0.20%	0	26.17	0	26.17	2.40	Non-smoker

CHAPTER 4:

EARLY NONINVASIVE PREDICTION OF RESPONSE TO TARGETED THERAPY IN NON-SMALL CELL LUNG CANCER

METHODS

Patient and sample characteristics

Sixteen patients with metastatic non-small cell lung cancer undergoing treatment with a TKI at UCSD or Johns Hopkins Hospital were included in our study. Clinical and pathological characteristics for all patients are summarized in Tables 4.1, 4.2, 4.3, and 4.4, study design is shown in Figure 4.1 and tumor load dynamics are shown in Figures 4.2, 4.3 and 4.4. Patient enrollment and genomic studies were conducted in accordance with the Declaration of Helsinki, were approved by the Institutional Review Board (IRB) and patients provided written informed consent for sample acquisition for research purposes.

Four patients were initially diagnosed with stage II or III disease and treated with chemotherapy followed by EGFR inhibition upon disease progression. Two patients were diagnosed at stage IV and treated with chemotherapy prior to EGFR inhibition. Six patients were diagnosed at stage IV and treated directly with first-line EGFR inhibition. These 12 patients progressed on first or second line EGFR inhibition and were subsequently treated with osimertinib dosed at 80mg PO daily (Tables 4.1, 4.2 and 4.3). The remaining four patients were diagnosed at stage IV and treated with afatinib.

The response evaluation criteria in solid tumors (RECIST) version 1.1 were used for assessment of response. Of the sixteen patients analyzed, ten achieved a partial response to osimertinib or afatinib while one patient exhibited stable disease after treatment with osimertinib and one patient had unmeasurable disease after treatment with afatinib. Out of these twelve patients, seven eventually experienced disease progression while three continue to derive clinical benefit from

targeted inhibition, one has stable disease and one had unmeasureable disease as of last follow-up. Four patients were considered non-responders and did not exhibit radiographic response by RECIST 1.1 (Tables 4.1, 4.2 and 4.3).

For all patients, serial blood draws were collected over the course of treatment with targeted inhibition for isolation of plasma and extraction of cfDNA for genomic analyses. Time-points were analyzed prior to treatment as well as post treatment and are reported in Supplementary Table 4.4.

Sample preparation and next-generation sequencing of cfDNA

Whole blood was collected in K2 EDTA tubes or Streck tubes and processed immediately or within 2 hours after storage at 4°C to separate plasma and cellular components by centrifugation at 3000 g for 10 minutes at 4°C. Plasma was centrifuged a second time at 18,000 g at room temperature to remove any remaining cellular debris and stored at -80°C until the time of DNA extraction. DNA was isolated from plasma using the Qiagen Circulating Nucleic Acids Kit (Qiagen GmbH, Hilden DE) and eluted in LoBind tubes (Eppendorf AG, Hamburg, DE). Concentration and quality of cfDNA was assessed using the Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA).

TEC-Seq next-generation sequencing cell-free DNA libraries were prepared from 11 to 350 ng of cfDNA. Genomic libraries were prepared as previously described (26). Briefly, the NEBNext DNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA) was used with four main modifications to the manufacturer's guidelines: 1) The library purification steps utilized the on-bead Ampure XP approach, 2) reagent volumes were adjusted accordingly to accommodate

the on-bead strategy, 3) a pool of 8 unique Illumina dual index adapters with 8 bp barcodes were used in the ligation reaction, and 4) cfDNA libraries were amplified with Hotstart Phusion Polymerase. Details of genomic library preparation conditions are provided here6. Concentration and quality of cfDNA genomic libraries were assessed using the Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA).

Targeted capture was performed using the Agilent SureSelect reagents and a custom set of hybridization probes targeting 58 genes (Table 4.5) per the manufacturer's guidelines. The captured library was amplified with HotStart Phusion Polymerase (New England Biolabs). The concentration and quality of captured cfDNA libraries was assessed on the Bioanalyzer 2100 using the DNA 1000 Kit (Agilent Technologies, Santa Clara, CA). TEC-seq libraries were sequenced using 100 bp paired end runs on the Illumina HiSeq 2000/2500 (Illumina, San Diego, CA).

Primary processing of next-generation sequencing data and identification of putative somatic mutations

Primary processing of next-generation sequence data for cfDNA samples was performed as previously described (26) using Illumina CASAVA software (v1.8), including demultiplexing and masking of dual index adapter sequences. Sequence reads were aligned against the human reference genome (hg19) using Novoalign with additional realignment of select regions using the Needleman-Wunsch method (37).

Next, candidate somatic mutations, consisting of point mutations, small insertions, and deletions were identified using VariantDx23 across the targeted regions of interest. VariantDx examined

sequence alignments of cfDNA plasma samples while applying filters to exclude alignment and sequencing artifacts as previously described (26). Specifically, an alignment filter was applied to exclude quality failed reads, unpaired reads, and poorly mapped reads in the plasma. A base quality filter was applied to limit inclusion of bases with reported phred quality score > 30.

Criteria for calling alterations in cfDNA have been previously described (26). Briefly, an alteration was considered a candidate somatic mutation only when: (i) Three distinct paired reads contained the mutation in the plasma and the number of distinct paired reads containing a particular mutation in the plasma was at least 0.1% of the total distinct read pairs; or (ii) Four distinct paired reads contained the mutation in the plasma and the number of distinct paired reads containing a particular mutation in the plasma was at least 0.05% and less than 0.1% of the total distinct read pairs; (iii) the mismatched base was not present in >1% of the reads in a panel of unmatched normal samples as well as not present in a custom database of common germline variants derived from dbSNP; (iv) the altered base did not arise from misplaced genome alignments including paralogous sequences; and (v) the mutation fell within a protein coding region and was classified as a missense, nonsense, frameshift, or splice site alteration.

As previously described (26), candidate alterations were defined as somatic hotspots if the nucleotide change and amino acid change were identical to an alteration observed in ≥ 20 cancer cases reported in the COSMIC database. Alterations that were not hotspots were retained only if either (i) seven or more distinct paired reads contained the mutation in the plasma and the number of distinct paired reads containing a particular mutation in the plasma was at least 0.1% and less than 0.2%, of the total distinct read pairs, or (ii) six or more distinct paired reads contained the mutation in the plasma and the number of distinct paired reads containing a particular mutation in the plasma was at least 0.2% of the total distinct read pairs. In order to track clonal changes over time, any alteration identified in at least one blood draw was followed in the remaining serial

timepoints regardless of whether mutant allele fractions fit the criteria defined to call a single hotspot or non-hotspot mutation.

Candidate mutations were further limited as previously described (26). Briefly, common germline variants were identified and removed if present in ≥ 25% of reads or < 25% of reads if the variant was recurrent and the majority of alterations at that position had a mutant allele fraction ≥ 25%. Variants known to be at a somatic hotspot position, or producing a truncating mutation in a tumor suppressor gene were not excluded as germline changes. Because of the high frequency of mutations in specific genes and the possible confounding between somatic and germline changes, we limited analyses in the APC gene to frameshift or nonsense mutations, and in KRAS, HRAS and NRAS to positions to 12, 13, 61, and 146. Finally, we excluded hematopoietic expansion related variants that have been previously described (39, 41, 50-52), including those in DNMT3A, IDH1, and IDH2 and specific alterations within ATM, GNAS, JAK2, or TP53 (Tables 4.5 and 4.6).

Sequence alterations identified at each timepoint for each patient are reported in Supplementary Table 4.7. At each timepoint, cfTL was assessed as the mutant allele fraction of the most abundant alteration identified among the 80,930 bp analyzed.

RESULTS

As a proof of concept, we evaluated cell-free DNA (cfDNA) from sixteen patients with late-stage non-small cell lung cancer, treated with either afatinib or osimertinib. For each patient, ~5 ml of plasma were collected prior to therapy, at 6-22 days after therapy initiation, and at additional timepoints until disease progression was confirmed by radiographic assessment (Tables 4.1, 4.2 and 4.3). Target lesions were evaluated before therapy with CT/MRI scans and approximately every six weeks until disease progression. Based on response assessment by RECIST 1.1 of the initial scans, eight of sixteen patients experienced partial response, one stable disease, four progressive disease, and one who had unmeasurable disease due to bone-only metastases (Tables 4.1, 4.2 and 4.3). The patient with stable disease achieved a partial response on later scans.

To analyze changes in cfDNA in these patients and capture the clonal heterogeneity of metastatic disease, we used an ultrasensitive approach called targeted error correction sequencing (TEC-Seq) to evaluate 58 well-known cancer driver genes (Figure 4.1 and Tables 4.5, 4.7, and 4.8) (26). This method is based on targeted capture of multiple regions of the genome and deep sequencing (>30,000x) of DNA fragments to provide a high degree of specificity across 80,930 bp of coding gene regions and enable identification of tumor-specific alterations while distinguishing these from amplification and sequencing artifacts, germline changes, or alterations related to blood cell proliferation that may be present in cfDNA (26).

We evaluated ctDNA in all patients at baseline and 6-22 days after the initiation of therapy. In the blood draws that were analyzed, we detected alterations in 14 of 16 cases. At the baseline timepoint, patients had an average of 3.6 tumor-specific somatic mutations, affecting 14 driver genes, ranging from one to 14 alterations per case (Table 4.7). At least one targetable mutation in

either EGFR or ERBB2 was detected in each case analyzed, with levels of ctDNA ranging from mutant allele fractions of 0.10% to 53.71% (Table 4.7). Eight out of fourteen patients had EGFR T790M resistance mutations in the circulation at baseline with ctDNA amounts ranging from mutant allele fractions of 0.17% to 10.09% (Table 4.7). Previously described alterations in genes involved in blood cell proliferation (39, 41, 50-52) were observed in ten patients across all timepoints analyzed, and were removed from further analyses (Table 4.6).

Based on the alterations observed in cfDNA, we developed a new metric, termed cell-free tumor load (cfTL), defined as the mutant allele fraction in ctDNA of the most abundant alteration identified among the 80,930 bp analyzed at any particular timepoint (Figure 4.1 and Table 4.7). This approach has the benefit of providing a comprehensive assessment of tumor-derived alterations that would represent overall tumor burden during the course of disease and selective pressure of therapeutic interventions. All patients with a radiographic response to targeted therapy displayed a rapid suppression of cfTL at 6-22 days after initiation of therapy. Figure 4.2 A depicts a representative patient with metastatic disease (CGPLLU12) who had a rapid decline of cfTL from baseline to day 10. This patient exhibited a progression-free survival of 7.0 months on osimertinib therapy, then subsequently developed resistance in the primary lung lesion. All ten patients with a radiographic response or stable disease had a dramatic reduction in ctDNA levels (Figures 4.2 and 4.4). In these patients, cfTL levels ranged from an average mutant allele fraction of 10.80% at baseline to 0.20%, or >95% reduction in cfTL levels at 6-22 days after treatment (Table 4.7) ($P = 0.016$, Wilcoxon test). In contrast, all four patients that were radiographic non-responders to targeted therapy experienced limited reduction or increases in cfTL ranging from an average of 6.38% at baseline to 5.70% at 6-22 days after treatment, resulting in a range of 72% reduction to 101% increase in cfTL levels (Figures 4.3 and 4.4) ($P = 0.25$, Wilcoxon test).

In addition to changes in cfTL, the number of observed alterations also decreased in responders from 4.0 to 1.2 mutations per patient (P = 0.010, paired t test), while non-responders had no change in the number of mutations observed during therapy (2.5 mutations per patient both before and after therapy). We also observed that all non-responders had at least one mutation with a lower mutant allele fraction, presumably representing tumor subclones, that increased after therapy, while only two of the ten responding patients had such subclonal increases (Figure 4.4). These observations suggest that both ctDNA levels and clonal heterogeneity are dramatically reduced at early timepoints in responding patients due to therapeutic selective pressure, and in non-responding patients the emergence of tumor subclones can be detected earlier than clinical progression.

For a subset of patients, we evaluated multiple follow-up blood draws at extremely early timepoints in therapy. An immediate timepoint at 4-12 hours after the initiation of treatment was available for four patients who experienced a partial radiographic response on the first or second scan (CGPLLU12, CGPLLU14, CGPLLU86, CGPLLU99), one clinical responder with non-measurable disease (CGLU315), and one patient with progressive disease (CGLU294). In these early timepoints after drug administration, we detected a rapid increase in the levels of ctDNA in eight mutations in three patients compared to baseline (cfTL increase of 86%) (Figure 4.1 and Table 4.7). In five of the six patients for whom immediate timepoints were evaluated, increasing ctDNA levels allowed for the identification of seven tumor-derived alterations not previously detected at baseline including the targetable EGFR 746ELREATS>D clone in patient CGPLLU86 detected at a mutant allele fraction of 0.19%. Mutant allele fractions of the newly detected clones ranged from 0.16% to 1.70% with an average of 0.63% indicating that these alterations were likely below the technical limit of detection at baseline and rose above the threshold of sensitivity only due to the increase in ctDNA levels. This dramatic increase is

consistent with studies showing BIM-mediated apoptosis in responsive tumors within 48 hours after exposure to EGFR inhibitors (53, 54). Interestingly, non-responder CGLU294 exhibited an increase in ctDNA levels from baseline to four hours post treatment indicating that the patient may have had some early response to treatment, however ctDNA clones remained high at 7 days post treatment indicating lack of response over time. Patient CGPLLU99 experienced a sustained partial response to osimertinib yet a blood draw taken 8 hours after treatment did not reveal an increase in ctDNA levels compared to any clones identified at baseline. Based on the durability of this patient's response, the half-life of cfDNA in the blood, and the kinetics of osimertinib drug activity we hypothesize that this patient may have exhibited a rapid efflux of ctDNA in response to treatment much earlier than 8 hours post therapy, perhaps on the order of 2-4 hours.

For responders CGPLLU86, CGPLLU89, and CGPLLU99 analyses after 6-22 days showed reduction of ctDNA levels for several weeks after initiation of therapy (Figure 4.4). Patients CGPLLU12 and CGPLLU88, who were treated with osimertinib and experienced partial response at first radiographic assessment, exhibited a rise in the 745KELREA>T EGFR activating clone in subsequent timepoints, while the T790M clone remained controlled and was either undetectable or at low levels (Figure 4.1 A and Figure 4.4). In contrast, non-responders continued to show a rise in ctDNA levels at later timepoints after initiation of therapy, and in all cases these levels were above those observed at baseline (Figure 4.1 B and Figure 4.4).

We evaluated whether the dynamic cfTL changes observed at early timepoints after treatment initiation were associated with differences in clinical outcome. cfTL levels at day 6-22 were bimodal (Figures 4.5 and 4.6), with the lower group clustering at an average percent reduction in cfTL of 99.44% and the higher group having an average percent increase in cfTL of 25.16% (Figure 4.5). We defined molecular responders as those with reduction in cfTL levels within three standard deviations of the lower group average reduction (greater than 96.3%) while non-

responders were above this threshold. Four of seven patients who developed a complete ctDNA response (cfTL reduction of 100%) at day 6-22 experienced a progression-free survival longer than one year while the remaining three patients continue to respond, but have not yet reached one year of follow-up (Figures 4.5 and 4.6). One of the complete molecular responders assessed for cfTL reduction at day 7 and 17 post treatment (CGPLLU86) was classified as having radiographic stable disease with tumor reduction of 15.4% at 38 days post-treatment, but had incremental serial responses with a maximal reduction of 46% by the ninth CT scan, and a progression-free survival of 12.4 months (Figures 4.5 and 4.6). Similarly, another complete molecular responder assessed at day 20 post treatment was classified as radiographically non-measurable yet has a favorable clinical response and remains on treatment as of last follow-up. Overall, we observed a significantly longer median progression-free survival for molecular responders at day 6-22 compared to molecular non-responders (12.0 months vs. 1.5 months, P = 0.0001, Figure 4.7). Importantly, cfTL reduction at day 6-22 was a more accurate predictor of outcome than initial CT imaging performed an average 48 days after initiation of therapy (Figure 4.7).

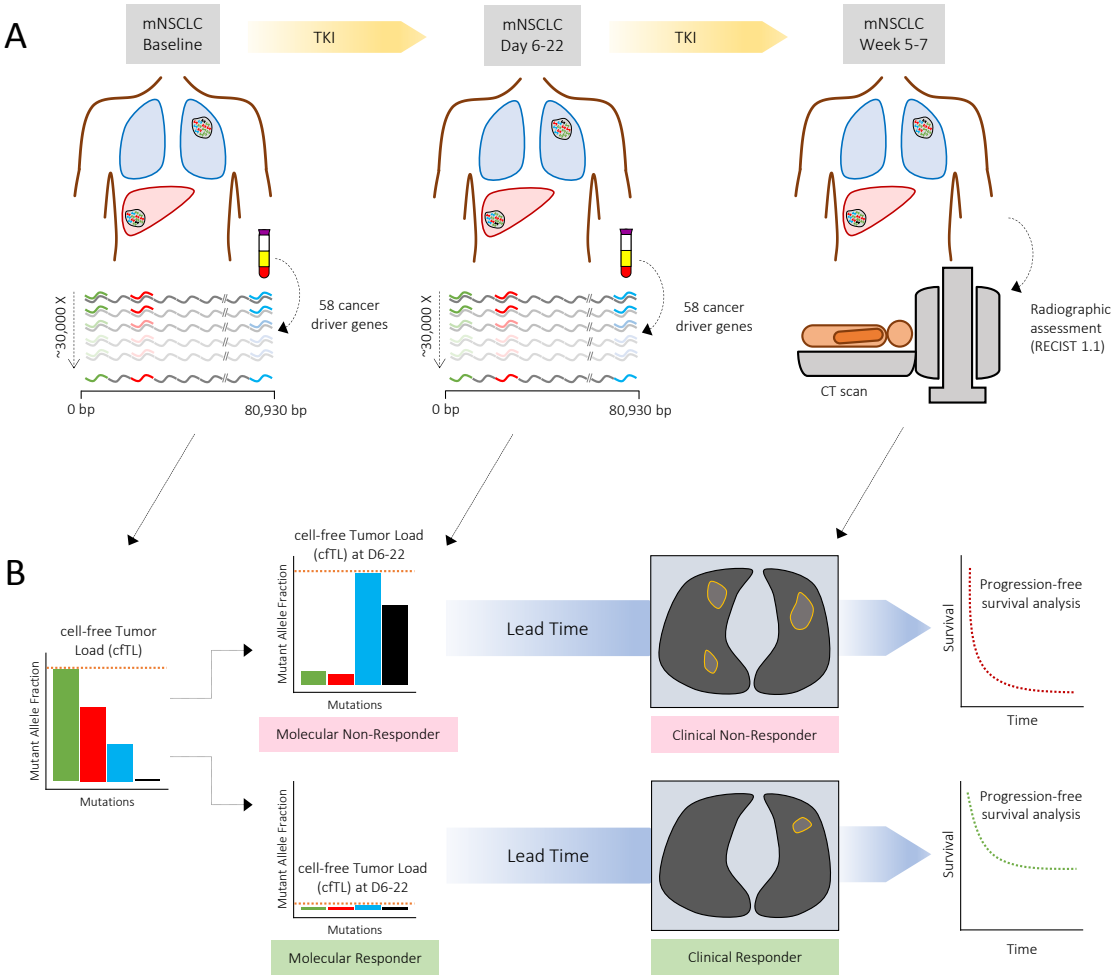

Figure 4.1. Schematic of cfTL determination and prediction of therapeutic response. Patients were analyzed using TEC-Seq liquid biopsy approach at baseline and at day 6-10 after treatment initiation. Cell-free DNA is extracted from blood, captured using probes covering 80,930 bases, and sequenced to ~30,000x coverage. Sequences are analyzed to identify tumor-derived alterations and to distinguish these from hematopoietic and germline changes. cfTL is determined as the highest concentration of ctDNA alterations at any time point. cfTL levels distinguish molecular responders from non-responders and predict clinical response and outcome to targeted therapy.

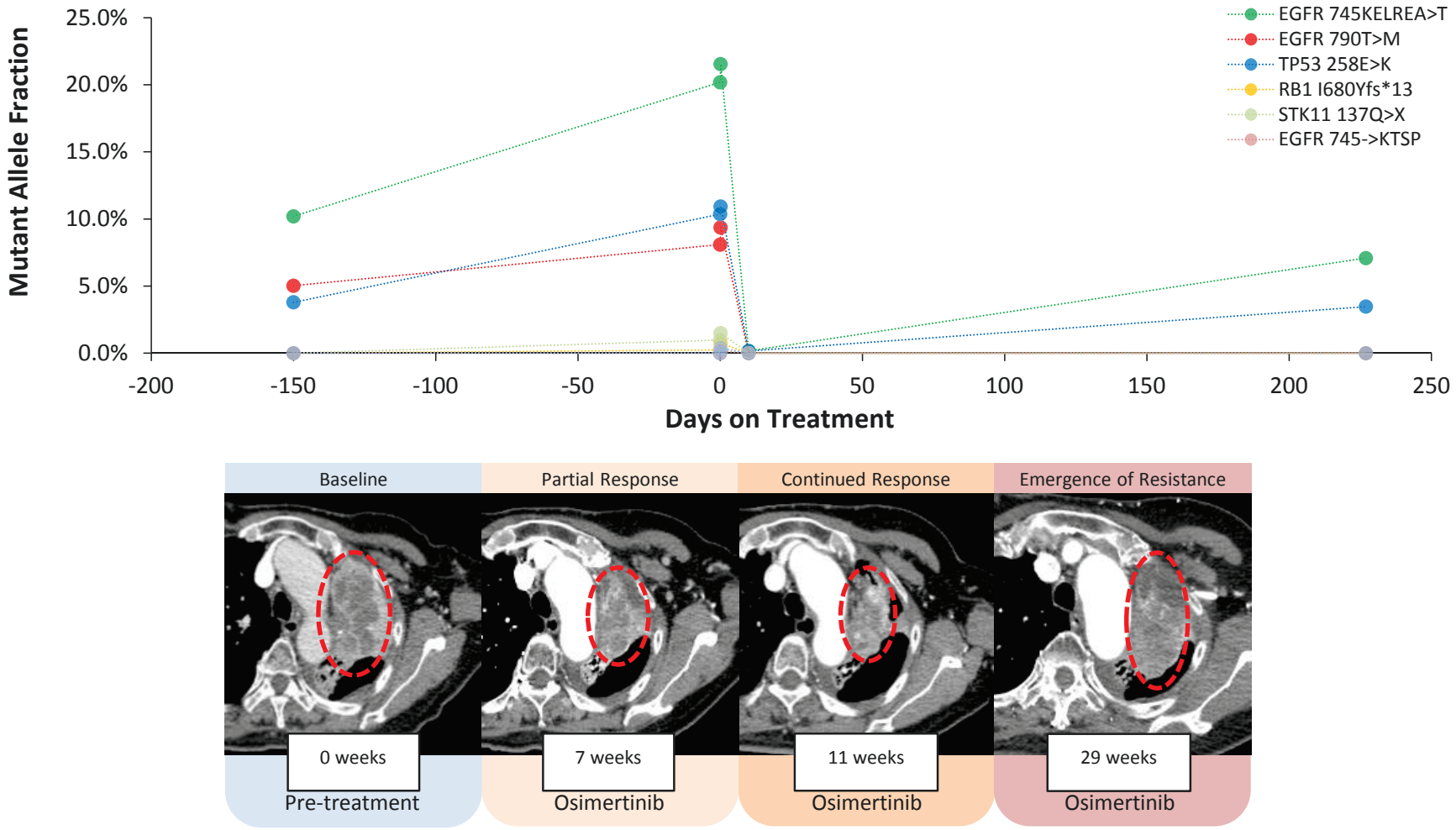

Figure 4.2. Dynamic changes in ctDNA for molecular responder. ctDNA dynamics changes in a molecular responder (CGPLLU12) along with radiographic RECIST 1.1 assessment in the same patient.

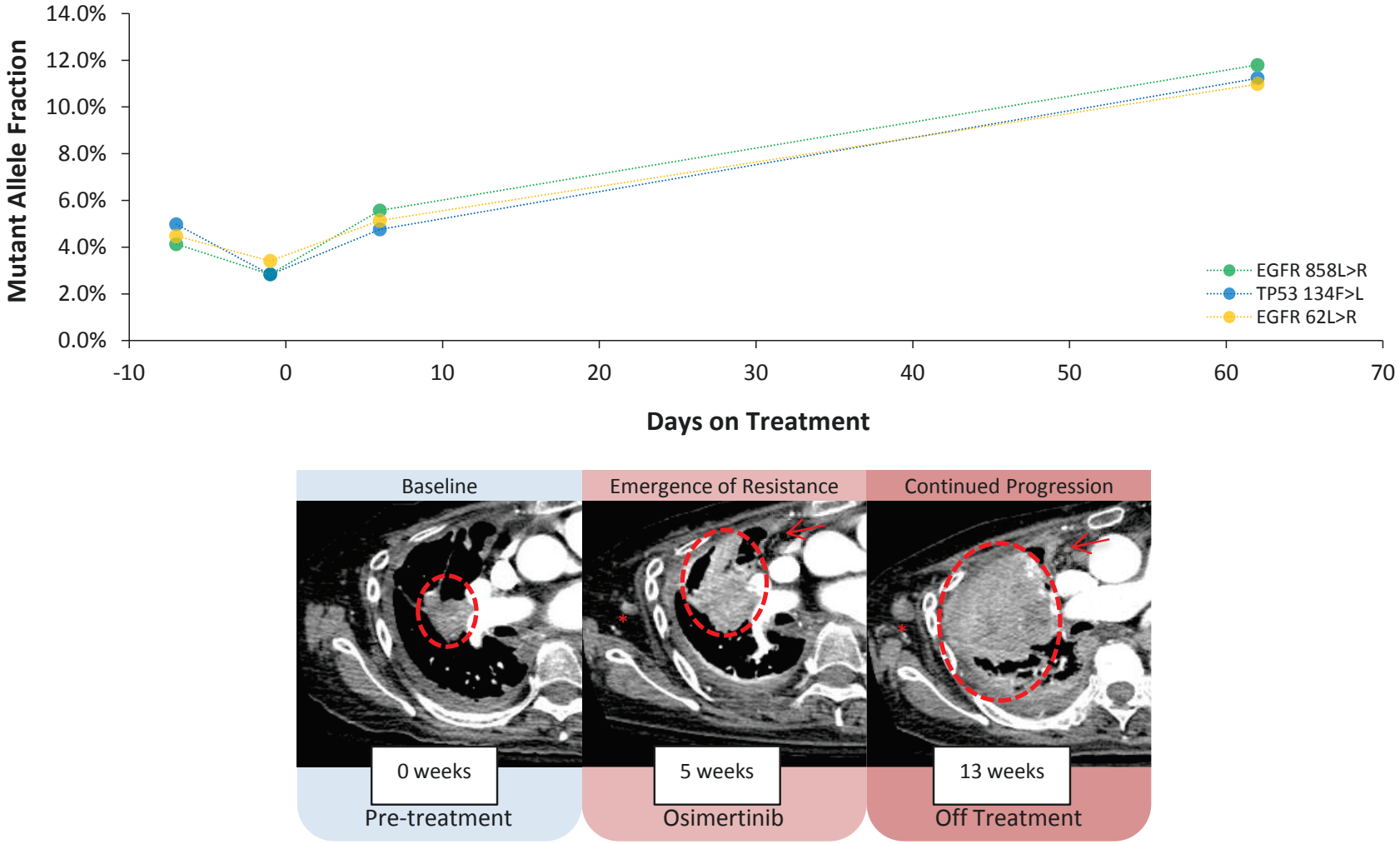

Figure 4.3. Dynamic changes in ctDNA for molecular nonresponder. ctDNA dynamics changes in a molecular non-responder (CGPLLU244) along with radiographic RECIST 1.1 assessment in the same patient.

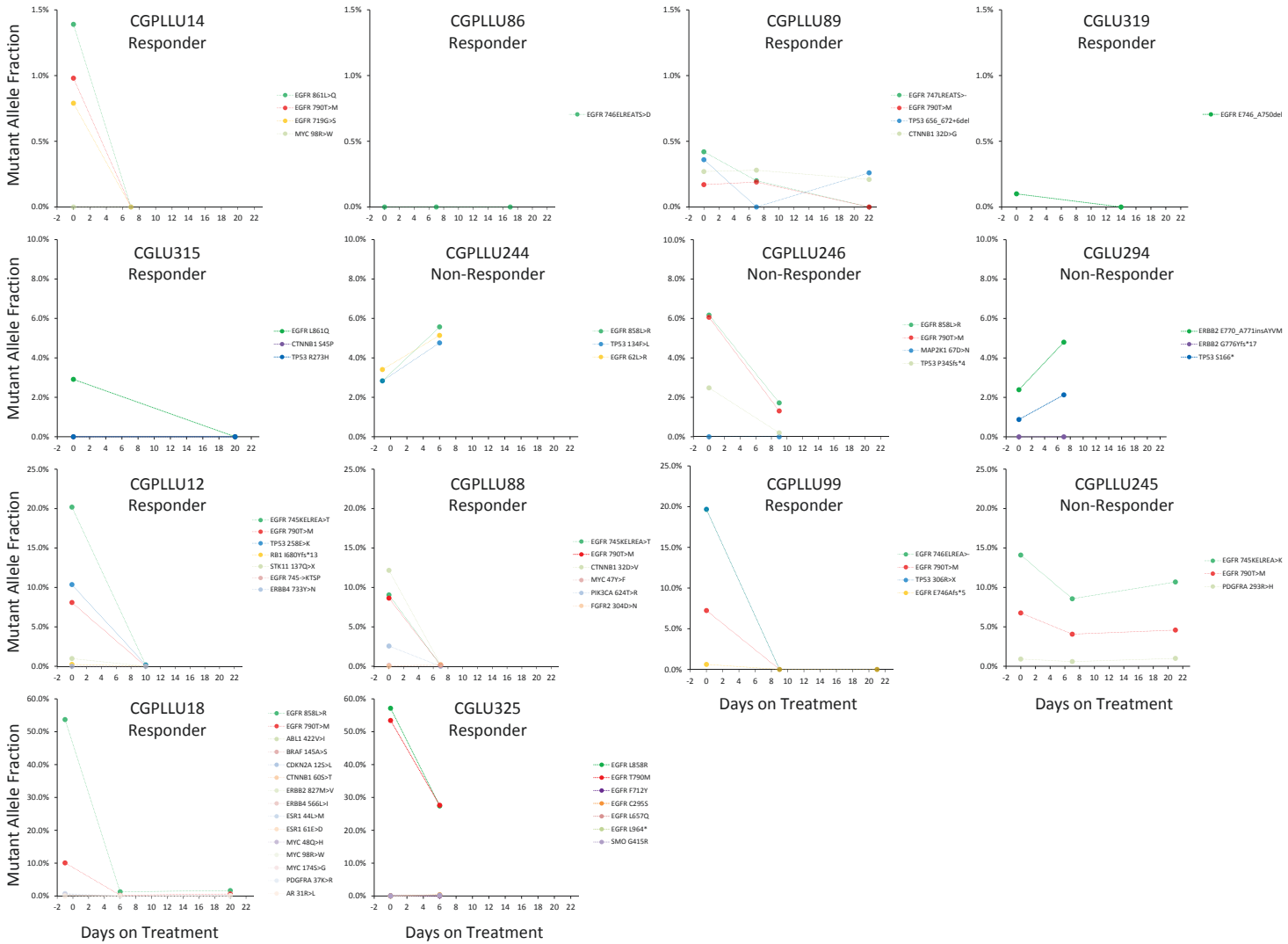

Figure 4.4. Dynamic changes in ctDNA for molecular responders and nonresponders.

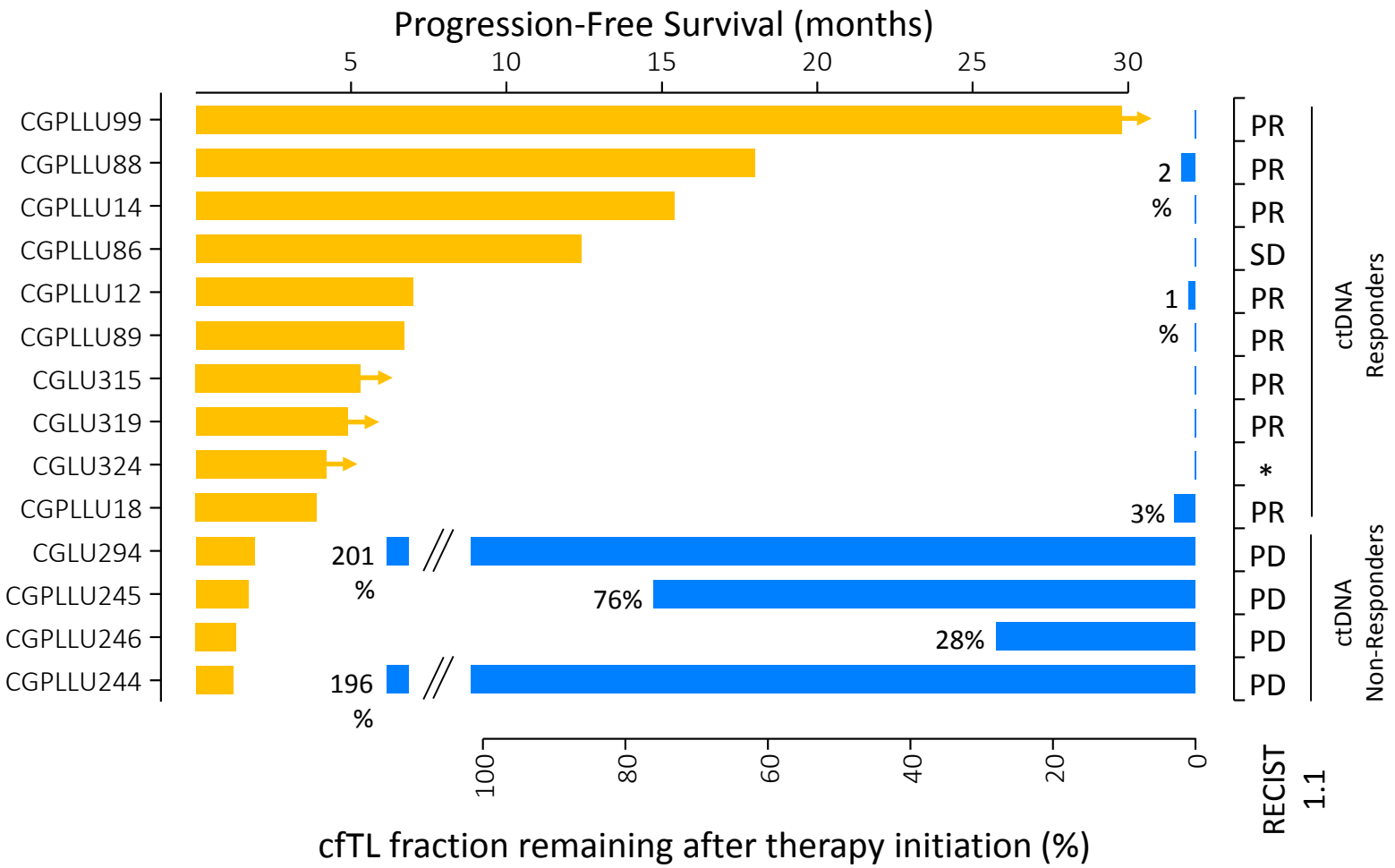

Figure 4.5. Dynamic changes in ctDNA and progression-free survival. cfTL levels at day 6-10 after osimertinib initiation (blue bars) along with corresponding progression-free survival based on imaging (orange bars). RECIST 1.1 responses at initial image assessment (5-7 weeks) are indicated on the right as PR (partial response), SD (stable disease), and PD (progressive disease).

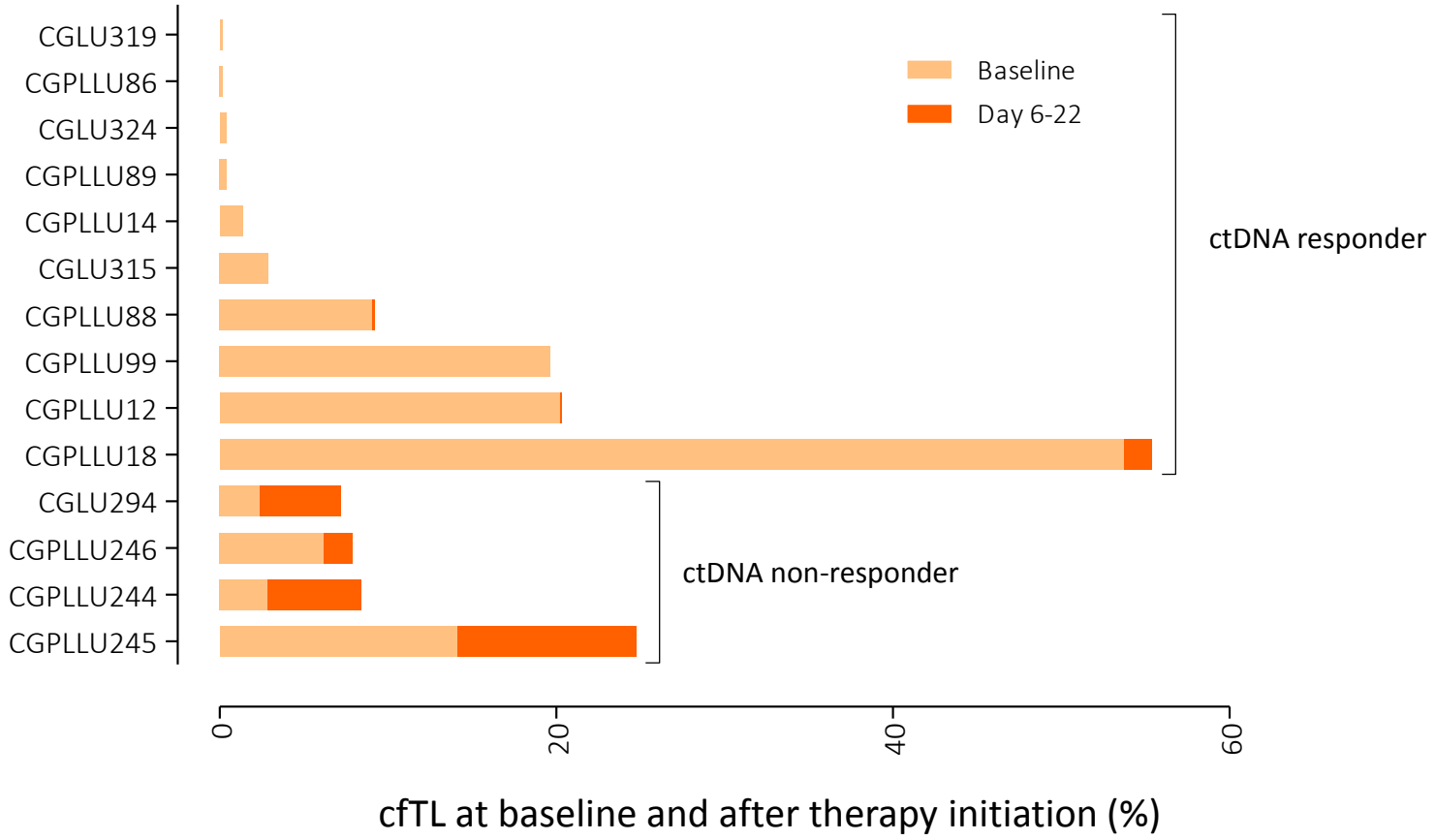

Figure 4.6. Dynamic changes in ctDNA and molecular classification of patients. cfTL levels at baseline and at day 6-10 indicate a bimodal distribution at day 6-10 among molecular responders and non-responders.

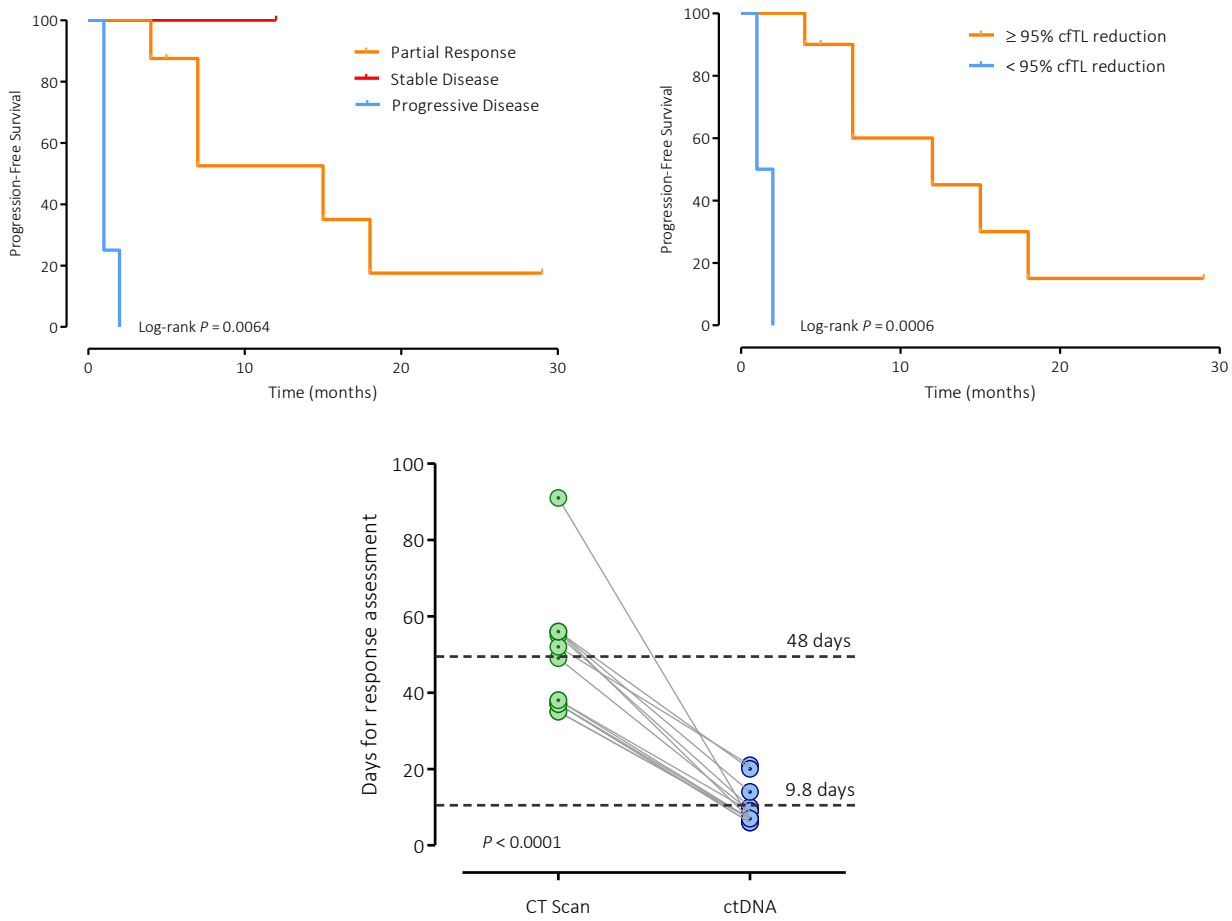

Figure 4.7. ctDNA and prediction of patient outcome. Time to detection of response for CT scans based on RECIST 1.1 responses at week 5-7 (P = 0.0054, Log-rank test, top left panel) and ctDNA (P = 0.0006, Log-rank test, top right panel). Median time to detection of response using CT scans and ctDNA is indicated in the bottom panel (P < 0.0001, Wilcoxon test).

Table 4.1. Clinical characteristics of lung cancer patients analyzed at diagnosis.

Patient	Demographics				Data at Diagnosis						
	Age	Gender	Smoking Status	Pack Years	Histopathological Diagnosis	Stage	TNM	Site of Primary Tumor	Resected Tumor Location	Degree of Differentiation	Location of Metastases
CGPLLU12	67	F	Never	NA	Adenocarcinoma	IV	NA	LUL	NA	NA	Brain, spine, adrenal
CGPLLU14	55	F	Never	NA	Adenocarcinoma	IIA	T1N1M0	RLL	RLL	Moderate	NA
CGPLLU18	50	F	Never	NA	Adenocarcinoma	IIA	T2N1M0	LLL	LLL	Moderate	NA
CGPLLU82	54	M	Never	NA	Adenocarcinoma	IV	NA	RLL	NA	NA	Brain
CGPLLU86	55	M	Never	NA	Adenocarcinoma	IV	NA	LUL	NA	NA	Lung
CGPLLU88	59	M	Former	NA	Adenocarcinoma	IIA	NA	RML	RML	NA	NA
CGPLLU89	54	F	Former	NA	Adenocarcinoma	IV	NA	RUL	NA	NA	Brain, bone, lung
CGPLLU97	57	F	Never	NA	Adenocarcinoma	IV	T3N0M1A	RLL	NA	Poor	Pleura
CGPLLU99	53	M	Never	NA	Adenocarcinoma	IIIA	NA	LUL	LUL,RLL	NA	Lung, bone
CGPLLU244	66	F	Never	NA	Adenocarcinoma	IV	NA	RUL	Liver	Moderate to poor	Liver, rib, brain, pleura

CGPLLU245	49	M	Never	NA	Adenocarcinoma	IV	T2aN2M1B	LUL	LUL	NA	Brain
CGPLLU246	65	F	Never	NA	Adenocarcinoma	IV	NA	RLL	NA	Poor	Pleura
CGLU294	51	M	Never	NA	Adenocarcinoma	IV	T1N2M1	LLL	NA	NA	Lung, lymph node, bone
CGLU315	54	M	Never	NA	Adenocarcinoma	IV	TXN0M1	RLL, extensive	NA	Poor	Lung, bone, adrenal
CGLU319	74	F	Never	NA	Adenocarcinoma	IV	T2N1M1a	RLL	NA	Well	Lung, Hilar Lymph Node, Pleural Effusion
CGLU324	60	F	Former	23	Adenocarcinoma	IV	T4N3M1b	RUL	NA	NA	Lung, Brain, Bone, Thoracic Lymph Node, Pleural Effusion

Table 4.2. Clinical characteristics of lung cancer patients analyzed during prior treatment.

Patient	Prior Treatment		
	Chemotherapy	**Radiation**	**Targeted Inhibitor**
CGPLLU12	None	Radiation	Erlotinib
CGPLLU14	Chemotherapy	Radiation (brain)	Erlotinib
CGPLLU18	Chemotherapy	Radiation (brain)	Erlotinib
CGPLLU82	None	Radiation	Erlotinib
CGPLLU86	None	None	Erlotinib
CGPLLU88	Chemotherapy	None	Erlotinib
CGPLLU89	None	Radiation (brain)	Afatinib
CGPLLU97	Chemotherapy	Radiation	Erlotinib
CGPLLU99	Chemotherapy	None	Erlotinib
CGPLLU244	None	Radiation	PF-06747775

CGPLLU245	Chemotherapy	Radiation	PF-06747775
CGPLLU246	None	Radiation (brain)	PF-06747775
CGLU294	None	None	None
CGLU315	None	None	None
CGLU319	None	None	None
CGLU324	None	Radiation (right ischium)	None

Table 4.3. Clinical characteristics of lung cancer patients analyzed during targeted therapy.

Patient	Targeted Treatment								
	Treatment	Days to initial RECIST Assessment	RECIST Percent Change in Target Lesions	Radiographic Response Type	Days to RECIST Progression	Days on Treatment	Overall response at last follow-up	Status at last follow-up	Location of Metastases
CGPLLU12	Osimertinib	55	-40.0	Partial Response	167	431	Progressive disease	Deceased	Adrenal, liver, renal, spine
CGPLLU14	Osimertinib	35	-33.3	Partial Response	459	713	Progressive disease	Deceased	Brain, sacrum
CGPLLU18	Osimertinib	37	-57.5	Partial Response	NA	104	Continued response	Deceased	Brain, liver, kidney, adrenal, bone
CGPLLU82	Osimertinib	39	-65.2	Partial Response	934	993	Progressive disease		Liver
CGPLLU86	Osimertinib	38	-15.4	Stable Disease	372	986	Progressive disease	Alive	Clivus, liver, other bone
CGPLLU88	Osimertinib	35	-40.7	Partial Response	421	548	Progressive disease	Deceased	Lung, adrenal, bone, pleura
CGPLLU89	Osimertinib	37	-41.2	Partial Response	206	427	Progressive disease	Deceased	Brain, lung, liver, bone
CGPLLU97	Osimertinib	91	-35.2	Partial Response	192	413	Progressive disease		Brain, lung, liver, bone
CGPLLU99	Osimertinib	49	-53.3	Partial Response	NA	Ongoing	Continued response	Alive	Lung, bone
CGPLLU244	Osimertinib	37	39.3	Progressive Disease	37	90	Progressive disease	Deceased	Bone, liver, brain, pleura

CGPLLU245	Osimertinib	52	20.8	Progressive Disease	52	134	Progressive disease	Alive	Bone, liver, pleura
CGPLLU246	Osimertinib	38	33.3	Progressive Disease	38	59	Progressive disease	Deceased	Bone, lung, brain
CGLU294	Afatinib	56	57.0	Progressive Disease	56	57	Progressive disease	Alive	PD in lung, LN and new bone
CGLU315	Afatinib	18*	NA	Non-measurable*	NA	Ongoing	Non-measurable	Alive	Lung improving, bone stable, no remaining adrenal mass
CGLU319	Afatinib		-28.0	Partial response	NA	Ongoing	Continued response	Alive	Same as baseline
CGLU324	Afatinib	69	-33.0	Partial response	NA	Ongoing	Stable disease	Alive	Bone, Lung

Table 4.4. Serial timepoints analyzed.

Patient	Patient Timepoint	Significance of Timepoint	Days since Treatment	Volume of Plasma (ml)	cfDNA Extracted (ng)	cfDNA Input (ng)
CGPLLU12	CGPLLU12P_6	Pre Osimertinib	-150.0	5.00	350.30	350.30
CGPLLU12	CGPLLU12P_22	Pre Osimertinib	0.0	7.00	1254.20	250.00
CGPLLU12	CGPLLU12P_18	Post Osimertinib	0.2	6.80	559.60	250.00
CGPLLU12	CGPLLU12P_20	Post Osimertinib	10.0	6.00	324.30	250.00
CGPLLU12	CGPLLU12P_34	Post Osimertinib	227.0	5.00	191.00	191.00
CGPLLU14	CGPLLU14P_8	Pre Osimertinib	-38.0	4.20	59.50	59.50
CGPLLU14	CGPLLU14P_12	Pre Osimertinib	-16.0	4.20	47.00	47.00
CGPLLU14	CGPLLU14P_15	Pre Osimertinib	-3.0	5.00	72.00	72.00
CGPLLU14	CGPLLU14P_21	Pre Osimertinib	0.0	5.00	16.82	16.82
CGPLLU14	CGPLLU14P_19	Post Osimertinib	0.3	4.60	121.60	121.60
CGPLLU14	CGPLLU14P_23	Post Osimertinib	7.0	3.20	19.26	19.26
CGPLLU18	CGPLLU18P_4	Pre Osimertinib	-1.0	5.00	2196.70	250.00
CGPLLU18	CGPLLU18P_15	Post Osimertinib	6.0	6.00	446.30	250.00
CGPLLU18	CGPLLU18P_11	Post Osimertinib	20.0	3.00	24.00	24.00
CGPLLU244	CGPLLU244P_1	Pre Osimertinib	-7.0	4.50	80.26	80.26
CGPLLU244	CGPLLU244P_2	Pre Osimertinib	-1.0	4.10	132.44	125.00
CGPLLU244	CGPLLU244P_3	Post Osimertinib	6.0	4.70	255.79	125.00
CGPLLU244	CGPLLU244P_4	Post Osimertinib	62.0	2.90	74.95	74.95
CGPLLU245	CGPLLU245P_1	Pre Osimertinib	-32.0	4.70	91.28	91.28
CGPLLU245	CGPLLU245P_2	Pre Osimertinib	0.0	4.30	468.58	125.00
CGPLLU245	CGPLLU245P_3	Post Osimertinib	7.0	3.00	314.40	125.00
CGPLLU245	CGPLLU245P_4	Post Osimertinib	21.0	3.30	161.49	125.00
CGPLLU246	CGPLLU246P_1	Pre Osimertinib	-21.0	5.50	101.78	101.78

CGPLLU246	CGPLLU246P_2	Pre Osimertinib	0.0	5.00	118.00	118.00
CGPLLU246	CGPLLU246P_4	Post Osimertinib	9.0	3.30	43.48	43.48
CGPLLU246	CGPLLU246P_5	Post Osimertinib	42.0	3.40	46.51	46.51
CGPLLU82	CGPLLU82P	Post Osimertinib	0.1	4.70	18.05	18.05
CGPLLU82	CGPLLU82P_1	Post Osimertinib	0.3	4.50	24.69	24.69
CGPLLU82	CGPLLU82P_7	Post Osimertinib	251.0	4.70	41.17	41.17
CGPLLU86	CGPLLU86P_6	Pre Osimertinib	0.0	4.00	31.60	31.60
CGPLLU86	CGPLLU86P_1	Post Osimertinib	0.5	4.00	14.50	14.50
CGPLLU86	CGPLLU86P_8	Post Osimertinib	7.0	4.60	14.50	14.50
CGPLLU86	CGPLLU86P_9	Post Osimertinib	17.0	5.00	19.90	19.90
CGPLLU88	CGPLLU88P_2	Pre Osimertinib	0.0	5.00	138.31	138.31
CGPLLU88	CGPLLU88P	Post Osimertinib	7.0	5.00	32.43	32.43
CGPLLU88	CGPLLU88P_5	Post Osimertinib	297.0	4.00	12.16	12.16
CGPLLU89	CGPLLU89P	Pre Osimertinib	0.0	8.00	67.40	67.40
CGPLLU89	CGPLLU89P_2	Post Osimertinib	7.0	6.50	40.60	40.60
CGPLLU89	CGPLLU89P_5	Post Osimertinib	22.0	6.00	17.90	17.90
CGPLLU97	CGPLLU97P_11	Pre Osimertinib	0.0	6.50	26.10	26.10
CGPLLU97	CGPLLU97P_4	Post Osimertinib	7.0	6.40	22.23	22.23
CGPLLU97	CGPLLU97P_20	Post Osimertinib	273.0	6.00	27.54	27.54
CGPLLU99	CGPLLU99P_8	Pre Osimertinib	0.0	6.00	50.60	50.60
CGPLLU99	CGPLLU99P_10	Post Osimertinib	0.3	5.00	45.99	45.99
CGPLLU99	CGPLLU99P_12	Post Osimertinib	9.0	5.00	24.99	24.99
CGPLLU99	CGPLLU99P_11	Post Osimertinib	21.0	4.00	18.76	18.76
CGLU294	CGLU294P_1	Pre Afatinib	0.0	5.00	17.36	17.36
CGLU294	CGLU294P1_1	Post Afatinib	0.2	5.00	15.52	15.52
CGLU294	CGLU294P2_1	Post Afatinib	3.0	5.00	16.59	16.59
CGLU294	CGLU294P3_2	Post Afatinib	7.0	5.00	10.73	10.73

CGLU294	CGLU294P4_1	Post Afatinib	29.0	5.00	12.22	12.22
CGLU315	CGLU315P_1	Pre Afatinib	0.0	5.00	25.96	25.96
CGLU315	CGLU315P1_1	Post Afatinib	0.2	5.00	22.48	22.48
CGLU315	CGLU315P2_1	Post Afatinib	20.0	5.00	22.62	22.62
CGLU319	CGLU319P_1	Pre Afatinib	0.0	5.00	53.14	53.14
CGLU319	CGLU319P1_1	Post Afatinib	14.0	5.00	48.26	48.26
CGLU324	CGLU324P_1	Post Afatinib	1.0	5.00	32.96	32.96
CGLU324	CGLU324P1_1	Post Afatinib	20.0	5.00	26.71	26.71

Table 4.5. Genes analyzed.

Gene	Region Analyzed	Gene Category*
ABL1	Specific Exons	Cancer Driver Gene
AKT1	Specific Exons	Cancer Driver Gene
ALK	Full Coding Region	Cancer Driver Gene
APC	Specific Exons	Cancer Driver Gene
AR	Full Coding Region	Cancer Driver Gene
ATM	Specific Exons	Cancer Driver and Clonal Hematopoiesis Gene
BRAF	Full Coding Region	Cancer Driver Gene
CDH1	Specific Exons	Cancer Driver Gene
CDK4	Full Coding Region	Cancer Driver Gene
CDK6	Full Coding Region	Cancer Driver Gene
CDKN2A	Specific Exons	Cancer Driver Gene
CSF1R	Specific Exons	Cancer Driver Gene
CTNNB1	Specific Exons	Cancer Driver Gene
DNMT3A	Specific Exons	Cancer Driver and Clonal Hematopoiesis Gene
EGFR	Full Coding Region	Cancer Driver Gene
ERBB2	Specific Exons	Cancer Driver Gene
ERBB4	Full Coding Region	Cancer Driver Gene
ESR1	Full Coding Region	Cancer Driver Gene
EZH2	Specific Exons	Cancer Driver Gene
FBXW7	Specific Exons	Cancer Driver Gene
FGFR1	Specific Exons	Cancer Driver Gene
FGFR2	Specific Exons	Cancer Driver Gene
FGFR3	Specific Exons	Cancer Driver Gene
FLT3	Specific Exons	Cancer Driver Gene
GNA11	Specific Exons	Cancer Driver Gene
GNAQ	Specific Exons	Cancer Driver Gene
GNAS	Specific Exons	Cancer Driver and Clonal Hematopoiesis Gene
HNF1A	Specific Exons	Cancer Driver Gene
HRAS	Full Coding Region	Cancer Driver Gene
IDH1	Specific Exons	Cancer Driver and Clonal Hematopoiesis Gene

IDH2	Specific Exons	Cancer Driver and Clonal Hematopoiesis Gene
JAK2	Full Coding Region	Cancer Driver and Clonal Hematopoiesis Gene
JAK3	Specific Exons	Cancer Driver Gene
KDR	Specific Exons	Cancer Driver Gene
KIT	Full Coding Region	Cancer Driver Gene
KRAS	Full Coding Region	Cancer Driver Gene
MAP2K1	Specific Exons	Cancer Driver Gene
MET	Specific Exons	Cancer Driver Gene
MLH1	Specific Exons	Cancer Driver Gene
MPL	Specific Exons	Cancer Driver Gene
MYC	Specific Exons	Cancer Driver Gene
NPM1	Specific Exons	Cancer Driver Gene
NRAS	Full Coding Region	Cancer Driver Gene
PDGFRA	Full Coding Region	Cancer Driver Gene
PIK3CA	Full Coding Region	Cancer Driver Gene
PIK3R1	Specific Exons	Cancer Driver Gene
PTEN	Full Coding Region	Cancer Driver Gene
PTPN11	Specific Exons	Cancer Driver Gene
RB1	Specific Exons	Cancer Driver Gene
RET	Specific Exons	Cancer Driver Gene
SMAD4	Specific Exons	Cancer Driver Gene
SMARCB1	Specific Exons	Cancer Driver Gene
SMO	Specific Exons	Cancer Driver Gene
SRC	Specific Exons	Cancer Driver Gene
STK11	Full Coding Region	Cancer Driver Gene
TERT	Specific Exons	Cancer Driver Gene
TP53	Full Coding Region	Cancer Driver Gene
VHL	Specific Exons	Cancer Driver Gene

* Analyzed genes inlcuded those that have been commonly implicated in cancer. Specific genes (DNMT3A, IDH1, and IDH2) or certain alterations within ATM, JAK2 and TP53 as described in references (13-17) were considered related to blood cell proliferation *(see Methods).*

Table 4.6. Blood cell proliferation alterations detected in cfDNA.

Patient	Patient Timepoint	Gene Symbol	Nucleotide	Amino Acid (Protein)	Mutant Allele Fraction	Mutant Molecules per ML of Plasma	Total Distinct Coverage	Distinct Mutant Coverage	Hot-spot Alteration
CGPLLU12	CGPLLU12P_18	DNMT3A	chr2_25467464-25467464_T_C	538T>A	0.17%	1.47	5740	10	No
CGPLLU12	CGPLLU12P_34	DNMT3A	chr2_25463508-25463508_C_T	2173+1G>A	0.23%	2.00	4329	10	No
CGPLLU12	CGPLLU12P_6	DNMT3A	chr2_25467464-25467464_T_C	538T>A	0.23%	1.20	2573	6	No
CGPLLU12	CGPLLU12P_20	DNMT3A	chr2_25467464-25467464_T_C	538T>A	0.25%	1.83	4323	11	No
CGPLLU12	CGPLLU12P_34	DNMT3A	chr2_25467464-25467464_T_C	538T>A	0.25%	2.40	4828	12	No
CGPLLU12	CGPLLU12P_22	DNMT3A	chr2_25467464-25467464_T_C	538T>A	0.28%	2.14	5282	15	No
CGPLLU14	CGPLLU14P_19	DNMT3A	chr2_25457173-25457173_A_C	905L>R	0.20%	1.74	4085	8	No
CGPLLU14	CGPLLU14P_15	DNMT3A	chr2_25457173-25457173_A_C	905L>R	0.22%	2.00	4454	10	No
CGPLLU14	CGPLLU14P_12	DNMT3A	chr2_25457173-25457173_A_C	905L>R	0.28%	1.90	2896	8	No
CGPLLU18	CGPLLU18P_4	DNMT3A	chr2_25463168-25463168_T_C	2322+3A>G	0.49%	3.60	3650	18	No
CGPLLU18	CGPLLU18P_15	DNMT3A	chr2_25463168-25463168_T_C	2322+3A>G	0.97%	6.33	3913	38	No

CGPLLU18	CGPLLU18P_11	DNMT3A	chr2_25463168-25463168_T_C	2322+3A>G	1.70%	18.67	3295	56	No
CGPLLU246	CGPLLU246P_4	DNMT3A	chr2_25457243-25457243_G_A	882R>C	0.17%	2.73	5422	9	Yes
CGPLLU246	CGPLLU246P_2	DNMT3A	chr2_25463289-25463289_T_G	735Y>S	0.20%	1.60	3964	8	No
CGPLLU246	CGPLLU246P_2	DNMT3A	chr2_25457243-25457243_G_A	882R>C	0.23%	2.80	5984	14	Yes
CGPLLU246	CGPLLU246P_1	DNMT3A	chr2_25457243-25457243_G_A	882R>C	0.24%	2.91	6640	16	Yes
CGPLLU246	CGPLLU246P_5	DNMT3A	chr2_25463289-25463289_T_G	735Y>S	0.24%	3.24	4666	11	No
CGPLLU246	CGPLLU246P_4	DNMT3A	chr2_25463289-25463289_T_G	735Y>S	0.28%	3.64	4264	12	No
CGPLLU246	CGPLLU246P_5	DNMT3A	chr2_25457243-25457243_G_A	882R>C	0.32%	5.88	6282	20	Yes
CGPLLU246	CGPLLU246P_1	DNMT3A	chr2_25463289-25463289_T_G	735Y>S	0.34%	3.09	4939	17	No
CGPLLU246	CGPLLU246P_1	DNMT3A	chr2_25467468-25467468_G_C	536Y>X	1.25%	15.82	6946	87	No
CGPLLU246	CGPLLU246P_2	DNMT3A	chr2_25467468-25467468_G_C	536Y>X	1.37%	18.60	6783	93	No
CGPLLU246	CGPLLU246P_4	DNMT3A	chr2_25467468-25467468_G_C	536Y>X	1.46%	25.45	5749	84	No
CGPLLU246	CGPLLU246P_5	DNMT3A	chr2_25467468-25467468_G_C	536Y>X	1.65%	29.41	6068	100	No
CGPLLU82	CGPLLU82P_7	DNMT3A	chr2_25463288-25463288_G_C	735Y>X	0.21%	1.70	3897	8	No

CGPLLU86	CGPLLU86P_6	DNMT3A	chr2_25463524-25463524_G_A	720R>C	0.32%	4.50	5575	18	No
CGPLLU86	CGPLLU86P_9	DNMT3A	chr2_25463524-25463524_G_A	720R>C	0.35%	2.00	2857	10	No
CGPLLU86	CGPLLU86P_8	DNMT3A	chr2_25463524-25463524_G_A	720R>C	0.42%	2.61	2888	12	No
CGPLLU86	CGPLLU86P_1	DNMT3A	chr2_25463524-25463524_G_A	720R>C	0.54%	4.00	2962	16	No
CGPLLU88	CGPLLU88P_2	DNMT3A	chr2_25457252-25457252_T_C	879N>D	0.19%	2.60	6691	13	No
CGPLLU88	CGPLLU88P_2	DNMT3A	chr2_25467408-25467408_C_T	1667+1G>A	1.31%	11.60	4440	58	No
CGPLLU88	CGPLLU88P	DNMT3A	chr2_25467408-25467408_C_T	1667+1G>A	1.98%	10.60	2678	53	No
CGPLLU88	CGPLLU88P_5	DNMT3A	chr2_25467408-25467408_C_T	1667+1G>A	2.23%	10.50	1886	42	No
CGPLLU14	CGPLLU14P_8	TP53	chr17_7578461-7578461_C_A	157V>F	1.06%	5.24	2080	22	Yes
CGPLLU14	CGPLLU14P_8	EGFR	chr7_55241707-55241707_G_A	719G>S	0.75%	3.81	2141	16	Yes
CGPLLU14	CGPLLU14P_8	EGFR	chr7_55249071-55249071_C_T	790T>M	0.45%	2.62	2456	11	Yes
CGPLLU14	CGPLLU14P_8	EGFR	chr7_55259524-55259524_T_A	861L>Q	0.89%	5.00	2357	21	Yes
CGPLLU14	CGPLLU14P_12	TP53	chr17_7578461-7578461_C_A	157V>F	0.26%	1.90	3106	8	Yes
CGPLLU14	CGPLLU14P_15	TP53	chr17_7578461-7578461_C_A	157V>F	0.46%	4.80	5178	24	Yes

CGPLLU14	CGPLLU14P_12	EGFR	chr7_55241707-55241707_G_A	719G>S	0.15%	0.95	2722	4	Yes
CGPLLU14	CGPLLU14P_12	EGFR	chr7_55249071-55249071_C_T	790T>M	0.16%	1.19	3196	5	Yes
CGPLLU14	CGPLLU14P_12	EGFR	chr7_55259524-55259524_T_A	861L>Q	0.18%	1.19	2823	5	Yes
CGPLLU14	CGPLLU14P_21	TP53	chr17_7578461-7578461_C_A	157V>F	0.77%	5.00	3254	25	Yes
CGPLLU14	CGPLLU14P_19	TP53	chr17_7578461-7578461_C_A	157V>F	0.86%	9.13	4877	42	Yes
CGPLLU14	CGPLLU14P_15	EGFR	chr7_55241707-55241707_G_A	719G>S	0.49%	3.80	3888	19	Yes
CGPLLU14	CGPLLU14P_15	EGFR	chr7_55249071-55249071_C_T	790T>M	0.48%	4.80	5004	24	Yes
CGPLLU14	CGPLLU14P_15	EGFR	chr7_55259524-55259524_T_A	861L>Q	0.45%	3.80	4233	19	Yes
CGPLLU14	CGPLLU14P_23	TP53	chr17_7578461-7578461_C_A	157V>F	0.77%	7.81	3254	25	Yes
CGPLLU245	CGPLLU245P_2	TP53	chr17_7578191-7578191_A_T	220Y>N	0.10%	1.86	7725	8	No
CGPLLU14	CGPLLU14P_21	EGFR	chr7_55241707-55241707_G_A	719G>S	0.79%	4.40	2801	22	Yes
CGPLLU14	CGPLLU14P_21	EGFR	chr7_55249071-55249071_C_T	790T>M	0.98%	6.40	3281	32	Yes
CGPLLU14	CGPLLU14P_21	EGFR	chr7_55259524-55259524_T_A	861L>Q	1.39%	8.00	2876	40	Yes
CGPLLU14	CGPLLU14P_8	TP53	chr17_7577539-7577539_G_C	248R>G	1.64%	7.38	1890	31	No

CGPLLU14	CGPLLU14P_12	TP53	chr17_7577539-7577539_G_C	248R>G	0.85%	5.24	2576	22	No
CGPLLU14	CGPLLU14P_19	EGFR	chr7_55241707-55241707_G_A	719G>S	1.05%	8.48	3697	39	Yes
CGPLLU14	CGPLLU14P_19	EGFR	chr7_55249071-55249071_C_T	790T>M	0.90%	9.35	4760	43	Yes
CGPLLU14	CGPLLU14P_19	EGFR	chr7_55259524-55259524_T_A	861L>Q	0.96%	9.13	4383	42	Yes
CGPLLU14	CGPLLU14P_19	MYC	chr8_128750755-128750755_C_T	98R>W	0.28%	2.39	3894	11	No
CGPLLU14	CGPLLU14P_15	TP53	chr17_7577539-7577539_G_C	248R>G	0.60%	4.60	3834	23	No
CGPLLU14	CGPLLU14P_21	TP53	chr17_7577539-7577539_G_C	248R>G	1.26%	6.40	2545	32	No
CGPLLU14	CGPLLU14P_19	TP53	chr17_7577539-7577539_G_C	248R>G	0.65%	5.22	3679	24	No
CGPLLU14	CGPLLU14P_23	TP53	chr17_7577539-7577539_G_C	248R>G	1.26%	10.00	2545	32	No
CGPLLU14	CGPLLU14P_23	TP53	chr17_7577539-7577539_G_C	248R>G	0.68%	5.00	2349	16	No
CGPLLU18	CGPLLU18P_4	TP53	chr17_7579376-7579376_T_	Q104Rfs*19	47.18%	279.40	2961	1397	No
CGPLLU99	CGPLLU99P_8	EGFR	chr7_55242466-55242466__GCATCTCC	E746Afs*5	0.62%	6.33	6095	38	No
CGPLLU99	CGPLLU99P_8	EGFR	chr7_55242467-55242481_AATTAAGAGAAGCAA_	746ELREA>-	19.65%	214.00	6533	1284	Yes
CGPLLU99	CGPLLU99P_8	EGFR	chr7_55249071-55249071_C_T	790T>M	7.24%	116.17	9626	697	Yes

CGPLLU18	CGPLLU18P_15	TP53	chr17_7579376-7579376_T_	Q104Rfs*19	0.24%	1.33	3270	8	No
CGPLLU99	CGPLLU99P_10	EGFR	chr7_55242466-55242466__GCATCTCC	E746Afs*5	0.51%	6.40	6246	32	No
CGPLLU99	CGPLLU99P_10	EGFR	chr7_55242467-55242481_AATTAAGAGAAGCAA_	746ELREA>-	15.67%	207.60	6626	1038	Yes
CGPLLU99	CGPLLU99P_10	EGFR	chr7_55249071-55249071_C_T	790T>M	5.76%	100.40	8714	502	Yes
CGPLLU18	CGPLLU18P_11	TP53	chr17_7579376-7579376_T_	Q104Rfs*19	0.43%	4.33	3022	13	No
CGLU294	CGLU294P_1	DNMT3A	chr2_25467482-25467482_C_T	G532S	0.63%	3.8	3026	19	No

Table 4.7. Somatic alterations detected in cfDNA.

Patient	Patient Timepoint	Days since Treatment	Gene Symbol	Nucleotide	Amino Acid (Protein)	Mutant Allele Fraction	Total Distinct Coverage	Distinct Mutant Coverage	Hotspot Alteration *
CGLU294	CGLU294P_1	0	ERBB2	chr17_37880982-37880982__GCATACGTGATG	E770_A771insAYVM	0.02	4098	98	No
CGLU294	CGLU294P_1	0	TP53	chr17_7578433-7578433_G_C	S166*	0.01	3864	34	No
CGLU294	CGLU294P1_1	0.167	ERBB2	chr17_37880982-37880982__GCATACGTGATG	E770_A771insAYVM	0.07	2410	169	No
CGLU294	CGLU294P1_1	0.167	ERBB2	chr17_37880996-37880996__ATACG	G776Yfs*17	0.00	2480	9	No
CGLU294	CGLU294P1_1	0.167	TP53	chr17_7578433-7578433_G_C	S166*	0.02	1909	46	No
CGLU294	CGLU294P2_1	3	ERBB2	chr17_37880982-37880982__GCATACGTGATG	E770_A771insAYVM	0.04	1891	83	No
CGLU294	CGLU294P2_1	3	TP53	chr17_7578433-7578433_G_C	S166*	0.02	1543	26	No

CGLU294	CGLU294P3_2	7	ERBB2	chr17_37880982-37880982__GCATACGTGATG	E770_A771insAYVM	0.05	2227	107	No
CGLU294	CGLU294P3_2	7	TP53	chr17_7578433-7578433_G_C	S166*	0.02	1782	38	No
CGLU294	CGLU294P4_1	29	ERBB2	chr17_37880982-37880982__GCATACGTGATG	E770_A771insAYVM	0.02	490	8	No
CGLU315	CGLU315P_1	0	EGFR	chr7_55259524-55259524_T_A	L861Q	0.03	413	12	Yes
CGLU315	CGLU315P1_1	0.167	CTNNB1	chr3_41266136-41266136_T_C	S45P	0.01	1417	19	Yes
CGLU315	CGLU315P1_1	0.167	EGFR	chr7_55259524-55259524_T_A	L861Q	0.02	1592	31	Yes
CGLU315	CGLU315P1_1	0.167	TP53	chr17_7577120-7577120_C_T	R273H	0.02	1232	21	Yes
CGLU319	CGLU319P_1	0	EGFR	chr7_55242466-55242480_GAATTAAGAGAAGCA_	E746_A750del	0.00		3	Yes

CGLU324	CGLU324P_1	1	EGFR	chr7_55242466-55242480_GAATTAAGAGAAGCA_	E746_A750del	0.00	1325	5	Yes
CGLU324	CGLU324P_1	1	STK11	chr19_1223121-1223121_T_A	L353H	0.01	1137	6	No
CGPLLU12	CGPLLU12P_6	-150	EGFR	chr7_55242465-55242479_GGAATTAAGAGAAGC_	745KELREA>T	0.10	2705	276	Yes
CGPLLU12	CGPLLU12P_6	-150	EGFR	chr7_55249071-55249071_C_T	790T>M	0.05	3182	160	Yes
CGPLLU12	CGPLLU12P_6	-150	TP53	chr17_7577509-7577509_C_T	258E>K	0.04	1928	73	No
CGPLLU12	CGPLLU12P_22	0	EGFR	chr7_55242465-55242479_GGAATTAAGAGAAGC_	745KELREA>T	0.20	5141	1038	Yes
CGPLLU12	CGPLLU12P_22	0	EGFR	chr7_55249071-55249071_C_T	790T>M	0.08	7649	619	Yes
CGPLLU12	CGPLLU12P_22	0	RB1	chr13_49033896-49033896__ATAT	I680Yfs*13	0.00	4183	10	No

CGPLLU12	CGPLLU12P_22	0	STK11	chr19_1219357-1219357_C_T	137Q>X	0.01	1326	13	No
CGPLLU12	CGPLLU12P_22	0	TP53	chr17_7577509-7577509_C_T	258E>K	0.10	3356	348	No
CGPLLU12	CGPLLU12P_18	0.17	EGFR	chr7_55242463-55242463__AAAACATCTCCG	745->KTSP	0.00	5498	9	No
CGPLLU12	CGPLLU12P_18	0.17	EGFR	chr7_55242465-55242479_GGAATTAAGAGAAGC_	745KELREA>T	0.22	5712	1231	Yes
CGPLLU12	CGPLLU12P_18	0.17	EGFR	chr7_55249071-55249071_C_T	790T>M	0.09	8897	834	Yes
CGPLLU12	CGPLLU12P_18	0.17	ERBB4	chr2_212488652-212488652_A_T	733Y>N	0.00	3438	13	No
CGPLLU12	CGPLLU12P_18	0.17	RB1	chr13_49033896-49033896__ATAT	I680Yfs*13	0.01	4480	33	No
CGPLLU12	CGPLLU12P_18	0.17	STK11	chr19_1219357-1219357_C_T	137Q>X	0.01	1410	21	No

CGPLLU12	CGPLLU12P_18	0.17	TP53	chr17_7577509-7577509_C_T	258E>K	0.11	3768	412	No
CGPLLU12	CGPLLU12P_20	10	EGFR	chr7_55242465-55242479_GGAATTAAGAGAAGC_	745KELREA>T	0.00	4095	7	Yes
CGPLLU12	CGPLLU12P_20	10	TP53	chr17_7577509-7577509_C_T	258E>K	0.00	3688	6	No
CGPLLU12	CGPLLU12P_34	227	EGFR	chr7_55242465-55242479_GGAATTAAGAGAAGC_	745KELREA>T	0.07	4189	297	Yes
CGPLLU12	CGPLLU12P_34	227	TP53	chr17_7577509-7577509_C_T	258E>K	0.03	3802	132	No
CGPLLU14	CGPLLU14P_8	-38	EGFR	chr7_55241707-55241707_G_A	719G>S	0.01	2141	16	Yes
CGPLLU14	CGPLLU14P_8	-38	EGFR	chr7_55249071-55249071_C_T	790T>M	0.00	2456	11	Yes
CGPLLU14	CGPLLU14P_8	-38	EGFR	chr7_55259524-55259524_T_A	861L>Q	0.01	2357	21	Yes

CGPLLU14	CGPLLU14P_12	-16	EGFR	chr7_55241707-55241707_G_A	719G>S	0.00	2722	4	Yes
CGPLLU14	CGPLLU14P_12	-16	EGFR	chr7_55249071-55249071_C_T	790T>M	0.00	3196	5	Yes
CGPLLU14	CGPLLU14P_12	-16	EGFR	chr7_55259524-55259524_T_A	861L>Q	0.00	2823	5	Yes
CGPLLU14	CGPLLU14P_15	-3	EGFR	chr7_55241707-55241707_G_A	719G>S	0.00	3888	19	Yes
CGPLLU14	CGPLLU14P_15	-3	EGFR	chr7_55249071-55249071_C_T	790T>M	0.00	5004	24	Yes
CGPLLU14	CGPLLU14P_15	-3	EGFR	chr7_55259524-55259524_T_A	861L>Q	0.00	4233	19	Yes
CGPLLU14	CGPLLU14P_21	0	EGFR	chr7_55241707-55241707_G_A	719G>S	0.01	2801	22	Yes
CGPLLU14	CGPLLU14P_21	0	EGFR	chr7_55249071-55249071_C_T	790T>M	0.01	3281	32	Yes

CGPLLU14	CGPLLU14P_21	0	EGFR	chr7_55259524-55259524_T_A	861L>Q	0.01	2876	40	Yes
CGPLLU14	CGPLLU14P_19	0.33	EGFR	chr7_55241707-55241707_G_A	719G>S	0.01	3697	39	Yes
CGPLLU14	CGPLLU14P_19	0.33	EGFR	chr7_55249071-55249071_C_T	790T>M	0.01	4760	43	Yes
CGPLLU14	CGPLLU14P_19	0.33	EGFR	chr7_55259524-55259524_T_A	861L>Q	0.01	4383	42	Yes
CGPLLU14	CGPLLU14P_19	0.33	MYC	chr8_128750755-128750755_C_T	98R>W	0.00	3894	11	No
CGPLLU18	CGPLLU18P_4	-1	ABL1	chr9_133750433-133750433_G_A	422V>I	0.00	4028	7	No
CGPLLU18	CGPLLU18P_4	-1	BRAF	chr7_140534480-140534480_C_A	145A>S	0.00	3949	8	No
CGPLLU18	CGPLLU18P_4	-1	CDKN2A	chr9_21974792-21974792_G_A	12S>L	0.01	2478	13	No

CGPLLU18	CGPLLU18P_4	-1	CTNNB1	chr3_41266181-41266181_T_A	60S>T	0.00	3251	12	No
CGPLLU18	CGPLLU18P_4	-1	EGFR	chr7_55249071-55249071_C_T	790T>M	0.10	17897	1806	Yes
CGPLLU18	CGPLLU18P_4	-1	EGFR	chr7_55259515-55259515_T_G	858L>R	0.54	14755	7925	Yes
CGPLLU18	CGPLLU18P_4	-1	ERBB2	chr17_37881150-37881150_A_G	827M>V	0.00	5137	14	No
CGPLLU18	CGPLLU18P_4	-1	ERBB4	chr2_212537909-212537909_G_T	566L>I	0.00	3892	10	No
CGPLLU18	CGPLLU18P_4	-1	ESR1	chr6_152129177-152129177_C_A	44L>M	0.01	4528	34	No
CGPLLU18	CGPLLU18P_4	-1	ESR1	chr6_152129230-152129230_G_C	61E>D	0.00	3624	13	No
CGPLLU18	CGPLLU18P_4	-1	MYC	chr8_128750983-128750983_A_G	174S>G	0.00	4935	7	No

CGPLLU18	CGPLLU18P_4	-1	MYC	chr8_128750607-128750607_G_C	48Q>H	0.00	3939	14	No
CGPLLU18	CGPLLU18P_4	-1	MYC	chr8_128750755-128750755_C_T	98R>W	0.00	4728	7	No
CGPLLU18	CGPLLU18P_4	-1	PDGFRA	chr4_55127322-55127322_A_G	37K>R	0.00	4030	8	No
CGPLLU18	CGPLLU18P_15	6	AR	chrX_66765080-66765080_G_T	31R>L	0.00	3995	7	No
CGPLLU18	CGPLLU18P_15	6	EGFR	chr7_55249071-55249071_C_T	790T>M	0.00	6660	17	Yes
CGPLLU18	CGPLLU18P_15	6	EGFR	chr7_55259515-55259515_T_G	858L>R	0.01	5625	73	Yes
CGPLLU18	CGPLLU18P_11	20	AR	chrX_66765080-66765080_G_T	31R>L	0.00	3554	6	No
CGPLLU18	CGPLLU18P_11	20	CDKN2A	chr9_21974792-21974792_G_A	12S>L	0.00	2592	4	No

CGPLLU18	CGPLLU18P_11	20	EGFR	chr7_55249071-55249071_C_T	790T>M	0.01	4423	28	Yes
CGPLLU18	CGPLLU18P_11	20	EGFR	chr7_55259515-55259515_T_G	858L>R	0.0168	4101	69	Yes
CGPLLU244	CGPLLU244P_1	-7	EGFR	chr7_55210075-55210075_T_G	62L>R	0.0448	5898	264	No
CGPLLU244	CGPLLU244P_1	-7	EGFR	chr7_55259515-55259515_T_G	858L>R	0.0413	6681	276	Yes
CGPLLU244	CGPLLU244P_1	-7	TP53	chr17_7578530-7578530_A_G	134F>L	0.0498	7524	375	No
CGPLLU244	CGPLLU244P_2	-1	EGFR	chr7_55210075-55210075_T_G	62L>R	0.0341	5746	196	No
CGPLLU244	CGPLLU244P_2	-1	EGFR	chr7_55259515-55259515_T_G	858L>R	0.0284	7348	209	Yes
CGPLLU244	CGPLLU244P_2	-1	TP53	chr17_7578530-7578530_A_G	134F>L	0.0283	8797	249	No

CGPLLU244	CGPLLU244P_3	6	EGFR	chr7_55210075-55210075_T_G	62L>R	0.0514	6240	321	No
CGPLLU244	CGPLLU244P_3	6	EGFR	chr7_55259515-55259515_T_G	858L>R	0.0557	7526	419	Yes
CGPLLU244	CGPLLU244P_3	6	TP53	chr17_7578530-7578530_A_G	134F>L	0.0476	8721	415	No
CGPLLU244	CGPLLU244P_4	62	EGFR	chr7_55210075-55210075_T_G	62L>R	0.1098	6602	725	No
CGPLLU244	CGPLLU244P_4	62	EGFR	chr7_55259515-55259515_T_G	858L>R	0.118	7043	831	Yes
CGPLLU244	CGPLLU244P_4	62	TP53	chr17_7578530-7578530_A_G	134F>L	0.1123	8098	909	No
CGPLLU245	CGPLLU245P_1	-32	EGFR	chr7_55242466-55242480_GAATTAAGAGAAGCA_	745KELREA>K	0.106	5869	622	Yes
CGPLLU245	CGPLLU245P_1	-32	EGFR	chr7_55249071-55249071_C_T	790T>M	0.0556	9722	541	Yes

CGPLLU245	CGPLLU245P_1	-32	PDGFRA	chr4_55133574-55133574_G_A	293R>H	0.0044	6374	28	No
CGPLLU245	CGPLLU245P_2	0	EGFR	chr7_55242466-55242480_GAATTAAGAGAAGCA_	745KELREA>K	0.141	8086	1140	Yes
CGPLLU245	CGPLLU245P_2	0	EGFR	chr7_55249071-55249071_C_T	790T>M	0.0676	14582	986	Yes
CGPLLU245	CGPLLU245P_2	0	PDGFRA	chr4_55133574-55133574_G_A	293R>H	0.0092	8550	79	No
CGPLLU245	CGPLLU245P_3	7	EGFR	chr7_55242466-55242480_GAATTAAGAGAAGCA_	745KELREA>K	0.0856	6892	590	Yes
CGPLLU245	CGPLLU245P_3	7	EGFR	chr7_55249071-55249071_C_T	790T>M	0.0407	11765	479	Yes
CGPLLU245	CGPLLU245P_3	7	PDGFRA	chr4_55133574-55133574_G_A	293R>H	0.006	7673	46	No
CGPLLU245	CGPLLU245P_4	21	EGFR	chr7_55242466-55242480_GAATTAAGAGAAGCA_	745KELREA>K	0.1069	7287	779	Yes

CGPLLU245	CGPLLU245P_4	21	EGFR	chr7_55249071-55249071_C_T	790T>M	0.0459	13165	604	Yes
CGPLLU245	CGPLLU245P_4	21	PDGFRA	chr4_55133574-55133574_G_A	293R>H	0.01	8020	80	No
CGPLLU246	CGPLLU246P_1	-21	EGFR	chr7_55249071-55249071_C_T	790T>M	0.0049	7780	38	Yes
CGPLLU246	CGPLLU246P_1	-21	EGFR	chr7_55259515-55259515_T_G	858L>R	0.0048	5826	28	Yes
CGPLLU246	CGPLLU246P_1	-21	MAP2K1	chr15_66727483-66727483_G_A	67D>N	0.0018	7080	13	No
CGPLLU246	CGPLLU246P_2	0	EGFR	chr7_55249071-55249071_C_T	790T>M	0.0606	8612	522	Yes
CGPLLU246	CGPLLU246P_2	0	EGFR	chr7_55259515-55259515_T_G	858L>R	0.0617	5933	366	Yes
CGPLLU246	CGPLLU246P_2	0	TP53	chr17_7579574-7579587_TGGGACGGCAAGGG_	P34Sfs*4	0.0248	2181	54	No

CGPLLU246	CGPLLU246P_4	9	EGFR	chr7_55249071-55249071_C_T	790T>M	0.0131	6170	81	Yes
CGPLLU246	CGPLLU246P_4	9	EGFR	chr7_55259515-55259515_T_G	858L>R	0.0172	5051	87	Yes
CGPLLU246	CGPLLU246P_4	9	TP53	chr17_7579574-7579587_TGGGACGGCAAGGG_	P34Sfs*4	0.002	2461	5	No
CGPLLU246	CGPLLU246P_5	42	EGFR	chr7_55249071-55249071_C_T	790T>M	0.043	7229	311	Yes
CGPLLU246	CGPLLU246P_5	42	EGFR	chr7_55259515-55259515_T_G	858L>R	0.0529	5932	314	Yes
CGPLLU246	CGPLLU246P_5	42	MAP2K1	chr15_66727483-66727483_G_A	67D>N	0.0011	6149	7	No
CGPLLU246	CGPLLU246P_5	42	TP53	chr17_7579574-7579587_TGGGACGGCAAGGG_	P34Sfs*4	0.0187	2669	50	No
CGPLLU86	CGPLLU86P_1	0.5	EGFR	chr7_55242469-55242486_TTAAGAGAAGCAACATCT_	746ELREATS>D	0.0019	2626	5	Yes

CGPLLU88	CGPLLU88P_2	0	CTNNB1	chr3_41266098-41266098_A_T	32D>V	0.1217	5474	666	Yes
CGPLLU88	CGPLLU88P_2	0	EGFR	chr7_55242465-55242479_GGAATTAAGAGAAGC_	745KELREA>T	0.0906	6447	584	Yes
CGPLLU88	CGPLLU88P_2	0	EGFR	chr7_55249071-55249071_C_T	790T>M	0.0865	10829	937	Yes
CGPLLU88	CGPLLU88P_2	0	MYC	chr8_128750603-128750603_A_T	47Y>F	0.0011	6119	7	No
CGPLLU88	CGPLLU88P_2	0	PIK3CA	chr3_178937483-178937483_C_G	624T>R	0.0257	3188	82	No
CGPLLU88	CGPLLU88P	7	CTNNB1	chr3_41266098-41266098_A_T	32D>V	0.0022	3623	8	Yes
CGPLLU88	CGPLLU88P	7	EGFR	chr7_55242465-55242479_GGAATTAAGAGAAGC_	745KELREA>T	0.0011	3568	4	Yes
CGPLLU88	CGPLLU88P	7	EGFR	chr7_55249071-55249071_C_T	790T>M	0.0015	4095	6	Yes

CGPLLU88	CGPLLU88P	7	FGFR2	chr10_123279522-123279522_C_T	304D>N	0.0024	3746	9	No
CGPLLU88	CGPLLU88P_5	297	CTNNB1	chr3_41266098-41266098_A_T	32D>V	0.0242	2229	54	Yes
CGPLLU88	CGPLLU88P_5	297	EGFR	chr7_55242465-55242479_GGAATTAAGAGAAGC_	745KELREA>T	0.0093	2155	20	Yes
CGPLLU88	CGPLLU88P_5	297	EGFR	chr7_55249071-55249071_C_T	790T>M	0.0013	2394	3	Yes
CGPLLU89	CGPLLU89P	0	CTNNB1	chr3_41266098-41266098_A_G	32D>G	0.0027	5556	15	Yes
CGPLLU89	CGPLLU89P	0	EGFR	chr7_55242470-55242487_TAAGAGAAGCAACATCTC_	747LREATS>-	0.0042	5434	23	Yes
CGPLLU89	CGPLLU89P	0	EGFR	chr7_55249071-55249071_C_T	790T>M	0.0017	6320	11	Yes
CGPLLU89	CGPLLU89P	0	TP53	chr17_7578171-7578193_CCAGACCTCAGGCGGCTCATAGG_	656_672+6delCCTATGAGCCGCCTGAGGTCTGG	0.0036	5598	20	No

CGPLLU89	CGPLLU89P_2	7	CTNNB1	chr3_41266098-41266098_A_G	32D>G	0.0028	3595	10	Yes
CGPLLU89	CGPLLU89P_2	7	EGFR	chr7_55242470-55242487_TAAGAGAAGCAACATCTC_	747LREATS>-	0.002	3540	7	Yes
CGPLLU89	CGPLLU89P_2	7	EGFR	chr7_55249071-55249071_C_T	790T>M	0.0019	3715	7	Yes
CGPLLU89	CGPLLU89P_5	22	CTNNB1	chr3_41266098-41266098_A_G	32D>G	0.0021	1870	4	Yes
CGPLLU89	CGPLLU89P_5	22	TP53	chr17_7578171-7578193_CCAGACCTCAGGCGGCTCATAGG_	656_672+6delCCTATGAGCCGCCTGAGGTCTGG	0.0026	1929	5	No
CGPLLU99	CGPLLU99P_8	0	EGFR	chr7_55242467-55242481_AATTAAGAGAAGCAA_	746ELREA>-	0.1965	6533	1284	No
CGPLLU99	CGPLLU99P_8	0	EGFR	chr7_55249071-55249071_C_T	790T>M	0.0724	9626	697	Yes
CGPLLU99	CGPLLU99P_8	0	EGFR	chr7_55242466-55242466__GCATCTCC	E746Afs*5	0.0062	6095	38	No

CGPLLU99	CGPLLU99P_8	0	TP53	chr17_7577022-7577022_G_A	306R>X	0.1969	5003	985	Yes
CGPLLU99	CGPLLU99P_10	0.33	EGFR	chr7_55242467-55242481_AATTAAGAGAAGCAA_	746ELREA>-	0.1567	6626	1038	No
CGPLLU99	CGPLLU99P_10	0.33	EGFR	chr7_55249071-55249071_C_T	790T>M	0.0576	8714	502	Yes
CGPLLU99	CGPLLU99P_10	0.33	EGFR	chr7_55242466-55242466__GCATCTCC	E746Afs*5	0.0051	6246	32	No
CGPLLU99	CGPLLU99P_10	0.33	TP53	chr17_7577022-7577022_G_A	306R>X	0.1376	5573	767	Yes

Table 4.8. Summary of lung cancer dynamics genomic analyses.

Patient	Patient Timepoint	Analysis Type	Read Length	Bases in Target Region	Bases Mapped to Genome	Bases Mapped to Target Regions	Percent Mapped to Target Regions	Total Coverage	Distinct Coverage
CGPLLU12	CGPLLU12P_18	TEC-Seq	100	80,930	7,900,133,500	3,863,298,051	49%	45,929	8,508
CGPLLU12	CGPLLU12P_20	TEC-Seq	100	80,930	7,756,705,100	3,517,430,064	45%	40,943	5,816
CGPLLU12	CGPLLU12P_22	TEC-Seq	100	80,930	8,057,252,300	3,802,086,289	47%	45,136	7,805
CGPLLU12	CGPLLU12P_34	TEC-Seq	100	80,930	8,777,269,900	4,524,962,642	52%	52,841	5,766
CGPLLU12	CGPLLU12P_6	TEC-Seq	100	80,930	7,201,712,800	3,608,246,175	50%	44,081	2,867
CGPLLU14	CGPLLU14P_12	TEC-Seq	100	80,930	8,668,655,700	3,980,731,089	46%	48,628	3,148
CGPLLU14	CGPLLU14P_15	TEC-Seq	100	80,930	8,271,043,600	4,105,092,738	50%	50,152	4,497
CGPLLU14	CGPLLU14P_19	TEC-Seq	100	80,930	7,149,809,200	3,405,754,720	48%	40,382	6,170
CGPLLU14	CGPLLU14P_21	TEC-Seq	100	80,930	6,556,332,200	3,289,504,484	50%	39,004	4,081
CGPLLU14	CGPLLU14P_23	TEC-Seq	100	80,930	7,410,378,300	3,464,236,558	47%	41,108	4,259
CGPLLU14	CGPLLU14P_8	TEC-Seq	100	80,930	7,530,190,700	3,752,054,349	50%	45,839	2,469
CGPLLU18	CGPLLU18P_11	TEC-Seq	100	80,930	8,873,158,100	4,118,821,432	46%	48,136	5,160
CGPLLU18	CGPLLU18P_15	TEC-Seq	100	80,930	6,258,355,400	3,199,373,401	51%	37,270	7,926
CGPLLU18	CGPLLU18P_4	TEC-Seq	100	80,930	9,997,401,400	4,940,336,941	49%	57,994	6,805
CGPLLU244	CGPLLU244P_1	TEC-Seq	100	80,930	8,305,560,600	4,182,616,104	50%	50,851	7,569
CGPLLU244	CGPLLU244P_2	TEC-Seq	100	80,930	7,739,951,100	3,788,487,116	49%	45,925	8,552
CGPLLU244	CGPLLU244P_3	TEC-Seq	100	80,930	8,061,928,000	4,225,322,272	52%	51,279	8,646
CGPLLU244	CGPLLU244P_4	TEC-Seq	100	80,930	8,894,936,700	4,437,962,639	50%	53,862	7,361

CGPLLU245	CGPLLU245P_1	TEC-Seq	100	80,930	7,679,235,200	3,935,822,054	51%	47,768	7,266
CGPLLU245	CGPLLU245P_2	TEC-Seq	100	80,930	8,985,252,500	4,824,268,339	54%	58,338	10,394
CGPLLU245	CGPLLU245P_3	TEC-Seq	100	80,930	8,518,229,300	4,480,236,927	53%	54,083	10,125
CGPLLU245	CGPLLU245P_4	TEC-Seq	100	80,930	9,031,131,000	4,824,738,475	53%	58,313	10,598
CGPLLU246	CGPLLU246P_1	TEC-Seq	100	80,930	8,520,360,800	3,509,660,305	41%	42,349	8,086
CGPLLU246	CGPLLU246P_2	TEC-Seq	100	80,930	5,451,467,800	2,828,351,657	52%	34,243	8,256
CGPLLU246	CGPLLU246P_4	TEC-Seq	100	80,930	8,137,616,600	4,135,036,174	51%	50,121	6,466
CGPLLU246	CGPLLU246P_5	TEC-Seq	100	80,930	8,385,724,600	4,413,323,333	53%	53,495	7,303
CGPLLU82	CGPLLU82P	TEC-Seq	100	80,930	7,528,379,600	3,680,247,626	49%	43,812	3,374
CGPLLU82	CGPLLU82P_1	TEC-Seq	100	80,930	6,815,879,400	3,371,577,171	49%	40,210	3,643
CGPLLU82	CGPLLU82P_7	TEC-Seq	100	80,930	8,296,401,700	4,074,081,704	49%	48,653	7,026
CGPLLU86	CGPLLU86P_1	TEC-Seq	100	80,930	8,222,093,400	3,523,035,056	43%	41,165	3,614
CGPLLU86	CGPLLU86P_6	TEC-Seq	100	80,930	8,305,719,500	4,271,264,008	51%	49,508	6,681
CGPLLU86	CGPLLU86P_8	TEC-Seq	100	80,930	6,787,785,300	3,443,658,418	51%	40,192	3,643
CGPLLU86	CGPLLU86P_9	TEC-Seq	100	80,930	6,213,229,400	3,120,325,926	50%	36,413	3,560
CGPLLU88	CGPLLU88P	TEC-Seq	100	80,930	7,679,995,800	4,004,738,253	52%	46,951	6,387
CGPLLU88	CGPLLU88P_2	TEC-Seq	100	80,930	7,252,433,900	3,621,678,746	50%	42,719	8,599
CGPLLU88	CGPLLU88P_5	TEC-Seq	100	80,930	6,509,178,000	3,316,053,733	51%	39,274	2,661
CGPLLU89	CGPLLU89P	TEC-Seq	100	80,930	7,662,496,600	3,781,536,306	49%	44,097	7,909
CGPLLU89	CGPLLU89P_2	TEC-Seq	100	80,930	7,005,599,500	3,339,612,564	48%	38,977	5,034
CGPLLU89	CGPLLU89P_5	TEC-Seq	100	80,930	8,325,998,600	3,094,796,789	37%	36,061	2,822

CGPLLU97	CGPLLU97P_11	TEC-Seq	100	80,930	9,607,549,400	4,714,483,715	49%	55,065	3,275
CGPLLU97	CGPLLU97P_20	TEC-Seq	100	80,930	7,977,402,200	3,981,840,609	50%	46,594	4,592
CGPLLU97	CGPLLU97P_4	TEC-Seq	100	80,930	7,975,580,500	3,458,563,174	43%	40,224	4,228
CGPLLU99	CGPLLU99P_10	TEC-Seq	100	80,930	8,350,456,400	4,112,640,680	49%	48,035	7,434
CGPLLU99	CGPLLU99P_11	TEC-Seq	100	80,930	8,455,525,300	4,248,480,856	50%	49,401	3,388
CGPLLU99	CGPLLU99P_12	TEC-Seq	100	80,930	8,172,750,100	3,937,309,188	48%	46,100	3,983
CGPLLU99	CGPLLU99P_8	TEC-Seq	100	80,930	6,563,589,000	3,167,459,274	48%	36,917	7,020
CGLU294	CGLU294P_1	TEC-Seq	100	80,930	13,391,053,400	6,557,523,556	49%	78,449	4,975
CGLU294	CGLU294P1_1	TEC-Seq	100	80,930	6,123,688,700	2,559,160,575	42%	30,475	2,658
CGLU294	CGLU294P2_1	TEC-Seq	100	80,930	6,826,939,100	2,696,933,517	40%	32,177	2,227
CGLU294	CGLU294P3_2	TEC-Seq	100	80,930	6,537,975,900	2,964,372,304	45%	35,429	2,504
CGLU294	CGLU294P4_1	TEC-Seq	100	80,930	4,382,617,500	2,052,259,803	47%	24,503	764
CGLU315	CGLU315P_1	TEC-Seq	100	80,930	1,511,727,500	772,328,073	51%	9,226	498
CGLU315	CGLU315P1_1	TEC-Seq	100	80,930	3,516,057,800	1,651,476,480	47%	19,709	1,923
CGLU315	CGLU315P2_1	TEC-Seq	100	80,930	2,406,092,800	1,154,037,968	48%	13,766	956
CGLU319	CGLU319P_1	TEC-Seq	100	80,930	6,167,692,000	3,135,436,369	51%	37,285	3,583
CGLU319	CGLU319P1_1	TEC-Seq	100	80,930	5,458,775,500	2,458,429,678	45%	29,096	2,715
CGLU324	CGLU324P_1	TEC-Seq	100	80,930	6,404,352,500	3,124,992,053	49%	37,062	2,071
CGLU324	CGLU324P1_1	TEC-Seq	100	80,930	5,622,041,400	2,679,400,008	48%	31,829	2,574

CHAPTER 5:

DISCUSSION

These studies together show the utility of ctDNA as a biomarker for cancer and the feasibility of clinical translation of liquid biopsies at multiple points during cancer initiation and progression such as early detection and detection of residual or progressive disease.

Analyses of ctDNA in pancreatic cancer highlight the possibility of early detection using digital PCR approaches specific for tumor-derived alterations and detection of residual disease earlier than CT imaging. Although careful measures were taken to increase the sensitivity of detecting genetic changes in the tumors and in the circulation of these patients, some alterations may not have been detected due to low tumor purity, limited plasma amounts and low mutant allele frequency. Despite these limitations, these data add to our growing understanding of pancreatic cancer. We have shown that ctDNA in the circulation of pancreatic cancer patients may provide a marker of early detection of subclinical, residual or recurrent disease. These analyses suggest future efforts to evaluate more intensive therapies for patients with detectable ctDNA after surgical resection.

Intrigued by the potential for early detection of cancer through detection of ctDNA we developed an approach for noninvasive direct detection of patients with early-stage disease across common cancer types. A conceptual benefit of this approach is that detectable alterations in cfDNA are, by definition, clonal and therefore indicate an underlying population of cells with identical somatic mutations. This high degree of specificity is one of the potential benefits of ctDNA detection com- pared to other blood-based biomarkers, which may be increased in other normal tissues in patients without cancer.

Although ctDNA analyses have raised the possibility of direct detection of patients with early-stage disease (4, 55), the de novo identification of somatic alterations has remained a major challenge for the development of early detection approaches. The analytical performance

characteristics of the TEC-Seq method suggest that it may be suitable for such analyses. Other methods have been used for analyses of cf DNA in late-stage cancer patients (4-13), but the specificity and sensitivity of these methods may limit their applicability for detection of early-stage disease. A variety of experimental and bioinformatic aspects may contribute to the high specificity of the TEC-Seq method compared to previous approaches, including deep sequencing (>30,000-fold coverage), use of a small number of adaptors with long prespecified barcodes, and multiple bioinformatic filtering steps comprising error correction, removal of repetitive sequences and mapping artifacts, and identification and removal of germline and hematopoietic sequences. Using the TEC-Seq approach, no tumor-derived alterations were identified in the plasma of the healthy individuals in our study. Although the average age of the healthy cohort was younger than the cancer patients analyzed, this corresponds to an age at which cancer screening may be initiated. Likewise, the concordance between liquid and tumor biopsies was high and suggested that liquid biopsies may have advantages for detection of heterogeneous tumor-specific alterations that may be missed by tissue biopsies. In the colorectal cancer case analyzed through multiple tissue biopsies, we showed that heterogeneous alterations appeared to have lower amounts of ctDNA and may explain the wide range of mutant allele fractions in ctDNA in the same individuals. One concern is that clonal hematopoietic changes may be confounded with heterogeneous tumor-specific mutations (39, 41) and lead to over-diagnoses. Large-scale studies of cell-free alterations in healthy individuals will be important to catalog the frequency and spectrum of these changes in the circulation. The higher fraction of healthy individuals in whom we detected mutations in blood cell proliferation genes compared to previous studies (39-41) will require further investigation to see whether these alterations become clinically relevant over time. Given the different tumors that could potentially be detected, imaging and other diagnostic studies will be needed to complement any positive ctDNA analysis to appropriately identify the tumor of origin. In the future, ctDNA mutations combined with other molecular characteristics

(56) may be helpful to identify the source of occult lesions.

Achieving effective sensitivity in ctDNA analyses has similarly presented a major technical hurdle. The high rate of conversion of cf DNA molecules in TEC-Seq libraries, combined with the use of endogenous as well as a limited number of exogenous barcodes, has increased the number of molecules that can be evaluated through NGS approaches. The parallel analysis of 55 cancer driver genes in this approach has the advantage of detecting a high fraction of tumors without previous knowledge of the genetic makeup of these cancers. The ability to detect multiple alterations in each case can increase sensitivity even when an individual mutation may not be detected. The inclusion of additional genes in larger panels could increase sensitivity, although this would be associated with higher sequencing costs. In some cancer types, we have surpassed the theoretical estimate of cases that could be detected, potentially because of the limited number of cases analyzed or under-estimates of mutation prevalence in existing databases. Overall, sensitivity may be further improved by deeper sequencing, improved error correction methods, larger blood volumes, and repeated testing at regular intervals, but it is likely that biologic characteristics of ctDNA will ultimately determine the ability to detect very small tumors or pre-neoplastic lesions.

Despite these limitations, the ability to detect half to three quarters of patients with early-stage colorectal, ovarian, lung, or breast cancer provides opportunities for early detection and intervention. The survival difference between late- and early-stage disease in these cancers accounts for more than a million lives worldwide each year (17). ctDNA-based cancer detection followed by appropriate intervention at earlier stages in even a fraction of individuals would likely dwarf the current health impact of most late-stage cancer therapies. Additionally, as we observed in colorectal cancer, the amount and type of ctDNA at the time of diagnosis may provide additional insight related to patient prognosis that could inform further clinical

intervention. Although screening for ctDNA will require larger validation studies, the success of cancer screening efforts based on other molecular tests (57) suggests that these approaches could, in principle, be implemented on a broad scale.

To follow-up on our study of direct detection of early stage cancer using ctDNA, we applied the TEC-Seq approach towards charting out ctDNA dynamics after treatment with targeted therapies. Despite the success of targeted therapy for lung cancer, durable responses typically give way to progressive disease due to the evolution of resistant clones. However, the standard method for treatment efficacy assessment is primarily based on tumor dimensions (58), which does not reflect the current knowledge of clonal changes over the treatment. More recently, several studies have addressed the role of ctDNA for disease monitoring, mainly based on technologies that do not allow inferences on the dynamics of multiple clones simultaneously. To our knowledge, this is the first study that addresses the value of measurements of ctDNA across multiple genes within days after treatment initiation.

Here, we demonstrate that dynamic changes in plasma ctDNA immediately after drug exposure may provide insights into clinical efficacy of targeted therapy. Given the heterogeneity of metastatic disease, an ultrasensitive cfTL approach comprehensively assessing a large number of driver genes has the advantage of accurately measuring overall tumor burden of clonal populations during selective pressure of targeted therapies. Faster and predictive determinants of patient response can aid in navigating adaptive therapeutic strategies to reduce toxicity, identify early resistance to targeted therapy, and enable the consideration of combinatorial approaches early in a patient's therapy.

These results suggest a new paradigm in cancer therapeutics and drug development in which cfTL molecular response criteria may be used to provide insight into clinical endpoints including

overall survival and progression-free survival. For patients without molecular response, liquid or tissue biopsies can provide additional information related to mechanisms of resistance and provide a context to consider other therapeutic strategies. cfTL monitoring may provide an early biomarker for proof-of-concept studies of targeted therapies. Combining cfTL response information with early pharmacokinetic data may ultimately provide the biologically effective dose needed for an individual's cancer rather than a maximally tolerated dose. Novel clinical trials could include rapid response assessments using cfTL in basket designs to expedite drug development.

It is clear that the implementation of liquid biopsies in the clinic will be feasible and beneficial. We have observed real-time detection of ctDNA as an indication of the presence of cancer at early stage, residual disease post resection, and progressive disease post treatment. Together these studies highlight noninvasive, sensitive, and specific detection of ctDNA as a biomarker for cancer and show the potential to improve patient diagnosis, management, and outcome.

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CURRICULUM VITAE

JILLIAN PHALLEN

Johns Hopkins University School of Medicine
The Sydney Kimmel Comprehensive Cancer Center
The Koch Cancer Research Building
1550 Orleans Street
CRB2 Room 532
Baltimore, Maryland 21287
P: 410-614-4631
jphalle2@jhmi.edu
Date of Birth: November 3, 1987
Location of Birth: Amherst, New York

Education

2012 – 2017	Ph.D., Graduate Training Program in Cellular and Molecular Medicine, Johns Hopkins University School of Medicine
2005 – 2009	B.A., Biology, Case Western Reserve University

Experience

2010 – 2012	Research Technologist, Johns Hopkins University School of Medicine
2007 – 2009	Research Assistant, Case Western Reserve University School of Medicine

Grants

2016 - 2017	Wolfe Street Competition, The Burroughs-Wellcome Fund and the Maryland-Genetics, Epidemiology and Medicine Training Program (MD-GEM)

Talks

2017	Invited Speaker , 2017 AACR Annual Meeting
2017	Invited Speaker , Personal Genome Diagnostics

Awards

2010	Double Helix Award, Oncology Research Technician Presentation Day, Johns Hopkins University School of Medicine

Memberships

2016 – Present	Associate member, AACR

Peer-reviewed Publications

Labidi-Galy SI, Papp E, Hallberg D, Niknafs N, Adleff V, Noe M, Bhattacharya R, Novak M, Jones S, **Phallen J**, Hruban CA, Hirsch MS, Lin DI, Schwartz L, Maire CL, Tille JC, Bowden M, Ayhan A, Wood LD, Scharpf RB, Kurman R, Wang TL, Shih IM, Karchin R, Drapkin R, Velculescu VE. **High grade serous ovarian carcinomas originate in the fallopian tube.** Nat Commun. 2017 Oct 23;8(1):1093.

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