

Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods 1. Carcinoembryonic Antigen (CEA) Analysis

CEA analysis was performed on a Cobas e601 platform (Roche), according to the manufacturer's recommendations using 500 µL serum. The threshold levels were set to 4.0 µg/L and 6.0 µg/L for non-smokers and smokers, respectively, as recommended by the analysing hospital. A person who had not smoked for 8 weeks before sample collection was considered a former smoker.

eMethods 2. Sample Collection and DNA Extractions

Tumor tissue was collected from all patients, either as fresh frozen (n=102) or as formalin fixed and paraffin embedded tissue (FFPE) (n=27). Four patients presented with synchronous CRCs; from these patients, tissues from both tumors were collected. From three patients with relapse, metastatic tissue was also collected. Constitutional DNA matching all patients was extracted from peripheral blood leukocytes.

Primary fresh frozen or formalin fixed paraffin embedded (FFPE) tissue samples were estimated to have a median pathological tumor cellularity of 50% (range 20-90%). DNA was extracted using the Puregene DNA purification kit (Gentra Systems) or using the QiAamp DNA FFPE tissue kit (Qiagen).

eMethods 3. Whole Exome Sequencing (WES)

WES was performed by the Department of Molecular Medicine on matched tumor DNA (derived from primary fresh frozen and FFPE tissue) and buffy coat DNA (eTable 4) as previously described¹. Libraries of tumor and matching germline DNA were prepared using 100-500 ng DNA and captured by SeqCapEZ MedExomePlusV1_hg19 panel (Roche) with a total (primary and capture) target size of 72 megabases. The MedExomePlusV1_hg19 panel was customized with 1042 SNP sites located throughout the genome. FastQ files were prepared using bcl2fastq2 (v2.20.0.422) and quality checked using FastQC (v0.11.5). Adapters were removed using Trim Galore! (v 0.4.1). The trimmed tumor and germline samples were treated according to the GATK (v3.7) best practices. Reads were mapped to the hg19 reference genome using bwa-mem (v0.7.12) and PCR duplicates were marked for filtering in the downstream analysis using Picard MarkDuplicates (v2.0.1). Local realignment around Indels and recalibration of base quality scores were then performed using GATK IndelRealigner and GATK BaseRecalibrator, respectively. As a quality control for all samples captured by the MedExomePlus panel, tumor and germline alignments were checked using allele counts for 1042 ID SNP sites. Briefly, genotype analysis of the 1042 fingerprint SNP sites served as a control for DNA contamination or sample and/or barcode mix-ups. The analysis was performed as previously described.² Samples were flagged and eliminated in situations where the average minor allele frequency at homozygous sites in a patient-matched normal was observed to be >1%. Samples with more than 55% heterozygous SNP sites were eliminated as such percentages indicate large-scale contamination of DNA from another individual.² SNVs and Indels were called using MuTect2 with information from COSMIC (v84) and dbSNP (v138). Default settings were applied, however, the threshold for maximum alternate alleles in the germline was raised. A custom filter selecting variants only vastly more present in the tumor and in regions with low noise, was subsequently applied as described earlier³. Furthermore, variants identified, but filtered, using MuTect2 were rescued if identified with high confidence using VarScan2 (2.4.1). All variants passing the applied filters were subjected to analysis on the activity of mutational signatures. Variants were initially loaded into a VRanges object and the sequence context was subsequently extracted using the SomaticSignatures R package^{4,5}. De novo extraction of mutation signatures was not applied due to the size of the cohort. The mutational profiles identified in our samples were instead projected onto the known COSMIC signatures using the MutationalPatterns R package^{6,7}. We identified signatures 1, 6 and 10, as previously reported for CRC⁸, but also a strong activity of signature 15 and 18 in a subset of samples.

eMethods 4. Blood Collection and Plasma Isolation

Blood samples were collected in K2-EDTA 10 ml tubes (Becton Dickinson) at Aarhus University Hospital. All samples were processed within 2 hours of collection by double centrifugation of the blood at RT, first for 10 minutes at 3000g, followed by centrifugation of plasma for 10 minutes at 3000g. Plasma was aliquoted into 5 mL cryotubes and stored at -80°C.

eMethods 5. Cell-Free DNA Extraction and Quantification

Up to 10 ml of plasma per case was used for this study (range, 2–10 mL; median 8.5 mL) and cfDNA was extracted using the QIAamp Circulating Nucleic Acid kit (Qiagen) and eluted into 50 µL DNA Suspension Buffer (Sigma). Each cfDNA sample was quantified by Quant-iT High Sensitivity dsDNA Assay Kit (Invitrogen). In 125 patients, cfDNA was isolated from a total of 795 serial plasma samples.

eMethods 6. Plasma DNA Libraries and Plasma Multiplex-PCR NGS Workflow

Up to 66 ng (20,000 genome equivalents) of cfDNA from each plasma sample was used as input into library preparation. The cfDNA was end-repaired, A-tailed, and ligated with custom adapters, as previously described⁹. The purified ligation product was amplified (20 cycles) and purified using Ampure XP beads (Agencourt/Beckman Coulter). Patient-specific somatic variants were identified by analyses of primary tumour and matched normal WES samples for all patients. Clonality of variants was inferred based on the estimated proportion of cancer cells harboring the variant as described in McGranahan et al¹⁰. Note that clonality inference from samples with low tumor cell fraction is limited due to a fairly flat distribution of variant allele frequency. Observed VAF in tissue and sequence context of variants were used to prioritize somatic SNVs and short INDELs identified for each tumour expected to have a low plasma background mutation rate. The Signatera amplicon design pipeline was used to generate PCR primer pairs for the given set of variants. Variant prioritization takes into account several factors such as clonality, allele fractions, and SNV type. Design of the 16-plex PCR assays is based on Natera's optimized set of parameters which includes selecting non-interacting set of primer-pairs. Each variant gets its own PCR primers and amplicon in order to ensure targets aren't sharing reads. For each patient, 16 highly ranked compatible amplicons were selected for the custom patient-specific panel. The PCR primers were ordered from Integrated DNA Technologies. An aliquot of each library was used as input into the associated patient-specific 16-plex PCR reaction. Samples were amplified using the patient-specific assay and barcoded, followed by product pooling. Sequencing was performed on an Illumina HiSeq 2500 Rapid Run with 50 cycles of paired-end reads using the Illumina Paired End v2 kit with an average read depth of >105,000X per amplicon. All paired-end reads were merged using Pear software¹¹. Bases that did not match in forward and reverse reads or that had a low quality score were filtered out to minimize sequencing errors. Merged reads were mapped to the hg19 reference genome with Novoalign version 2.3.4 (<http://www.novocraft.com/>). Amplicons with less than 5000x sequencing coverage were excluded from analyses and samples with less than 8 passing amplicons failed sequencing coverage QC.

eMethods 7. Plasma Variant Calling

A large set of negative control samples (~1000) were pre-processed to build a background error model. For each target variant using mutant and reference alleles depth of read, a confidence score was calculated on the basis of the error model, as described in Abbosh et al. 2017⁹. As previously, a plasma sample with at least 2 variants with a confidence score above a predefined algorithm threshold was defined as ctDNA positive⁸. Signatera technology allows confident detection down to 0.03% VAF and lower VAF levels are also observable in many cases¹².

eMethods 8. Quality Control and Signatera RUO Workflow

For the Signatera RUO plasma workflow, a strictly-controlled semi-automated lab process was performed by trained personnel with signed-off SOPs and witnessing. The process, reagent, and equipment information were captured electronically and uploaded to a database with built-in integrity checks. A total out of 795 plasma samples 793 (99%) passed our sample QC process. In order to track sample integrity, SNP tracers (45 frequent SNPs) were used to measure concordance between a patient's samples. A genotyping concordance score was calculated for all plasma samples by comparing to their matched normal tissue genotypes. Samples were considered to be from different patients if less than 85% of their SNPs showed identical genotype calls. The genotype calls are produced by Natera proprietary algorithm that fits a distribution to observed reads, calculates the probabilities of a particular genotype, and assigns the highest probability genotype to a SNP. A total of 421 sequencing samples including all plasma samples from recurrence patients were tested by SNP tracer to check the concordance between the plasma sample and its corresponding tissue biopsy. All but one plasma sample passed the concordance QC (eFigure 2).

eMethods 9. Time Requirement

The turnaround time for Signatera is broken down into the first blood collection timepoint and all subsequent blood collection timepoints. The first blood collection timepoint entails the following steps: a) tumor tissue and whole blood-based personalized assay design and b) plasma processing, ctDNA analysis, and reporting. The turnaround time for timepoint 1 is less than 4 weeks. All subsequent blood collection timepoints involve only the plasma processing, ctDNA analysis, and reporting, the turnaround time for which is 1-2 weeks. Detailed steps for each of the timepoints are listed below.

Timepoint 1

The clinician will be provided with a blood collection kit (composed of two Streck blood tubes and an EDTA tube) simultaneously with a tissue collection kit. The tissue collection kit and EDTA tube will be used in Step 1. Plasma from the two Streck tubes gets isolated and cfDNA is extracted. cfDNA gets used in Step 2.

Step 1

The clinician is expected to send in the patient's tumor block or FFPE slides from surgery/biopsy along with their blood tubes. It takes approximately 2.5 weeks from the time of tissue block or slides receipt for the following steps: tumor tissue block or slide pathology (sectioning, slide processing), DNA extraction from both tumor FFPE slides and matching whole blood, whole exome sequencing (WES) of tumor and matched normal DNA, WES analysis, variant selection and personalized assay design, primer ordering, primer receipt, and primer pooling. This patient-specific Signatera primer pool/assay is stored and gets accessioned when subsequent blood samples from the same patient are received for ctDNA testing.

Step 2

As soon as the personalized assay has been designed in step 1, the cfDNA can be made into a universal adapter library. An aliquot of the library is combined with the patient-specific multiplex primer pool for the mPCR assay where only the targets of interest are amplified. The mPCR amplicon product is barcoded, pooled, and sequenced. Sequencing data goes through QC followed by the ctDNA calling algorithm. The report is then issued.

Subsequent blood collection timepoints

For all subsequent blood collection timepoints, the clinician is sent only the blood collection kit with the 2 Streck tubes. Plasma from the two Streck tubes gets isolated and cfDNA extracted. This cfDNA then gets used as described above in Step 2 with the patient-specific Signatera assay (originally designed in Step 1)."

eResults 1. Performance Estimates of the Assay Based on Number of Mutations for Assay Design

To demonstrate the effect of the number of mutations tracked by the patient-specific assay on the sensitivity of ctDNA detection, we performed a simulation analysis by using smaller subsets of mutations from the patient-specific assays run in this study, and recalculated the sensitivity of ctDNA detection for pre-surgery samples (n=122) and relapse patients (n=16). Smaller number of mutations (<16) were chosen following the same prioritization and variant selection strategy as employed for the original 16-plex assays. Simulation results for ctDNA detection when using 2,4,8, or 16 of tumor mutations in the customized assays is presented in eTable 2; as expected, the sensitivity of the assay improves with an increase in the number of personalized mutations included in the assay.

eResults 2. Molecular Profiling of Metastases in Low Shedders

For two recurrence patients (ID 20 and 24), longitudinal analysis detected no ctDNA post-op (eFigure 7). We analyzed the same amount of plasma for these two patients, as for the other patients. A possible sample swap can be rejected after SNP tracer analysis, which confirmed that for each patient, the tumor, buffy coat and plasma samples came from the same individual. We performed WES of the metastatic recurrence lesions for the two patients, and confirmed that the mutations selected for plasma profiling were present in the metastases (eTable 8). WES was also performed on the metastases from patient 77. For this patient, the ctDNA longitudinal analysis did not detect ctDNA until after recurrence had been detected by radiological imaging (eFigure 7). Again, WES confirmed that the mutations selected for plasma profiling were present in the metastasis. Accordingly, we conclude that the reason for our negative post-op findings was ctDNA levels below detection level and not that the selected markers were non-informative.

eTable 1. Clinicopathological Patient Characteristics

Patient ID	Age	Gender	Primary tumor site	Primary tumor diameter, mm	UICC stage	TNMV	ACT	First relapse after OP month	Relapse site	Relapse treatment	MSS/MSI status	Perineural invasion	Number of lymph nodes in resected specimen	Microscopic radical resection	Ileus	Anastomotic leakage	Tumor perforation	WHO performance status
1	68	Male	Colon	52	II	T3N0MxV0	No	NA ^a			MSS	No	41	Yes	No	No	No	1
2	70	Male	Colon	54	II	T3N0MxV0	No	NA			MSS	Yes	31	Yes	No	No	No	1
3	67	Female	Colon	32	II	T3N0MxV0	No	NA			MSS	No	24	Yes	No	No	No	1
4	64	Male	Colon	35	III	T3N1MxV0	Yes	NA			MSS	No	39	Yes	N/A ^d	No	No	1
5	77	Male	Colon	70	II	T3N0MxV0	No	NA			MSS	No	34	Yes	No	No	No	1
6	75	Male	Colon	65	II	T3N0MxV0	No	NA			MSS	No	23	Yes	No	No	No	1
7	70	Male	Colon	29	II	T3N0M0V0	No	NA			MSS	No	28	Yes	No	No	No	1
8	65	Female	Colon	42	III	T3N1M0V0	Yes	NA			MSS	No	32	Yes	N/A	No	No	1
9	50	Male	Colon	100	II	T3N0M0V0	Yes	NA			MSS	No	51	Yes	No	N/A ^d	No	1
10	50	Female	Colon	15	III	T3N1M0V0	Yes	NA			MSS	No	27	Yes	N/A	No	No	1
11	70	Male	Colon	40	III	T3N2M0V1	Yes	NA			MSS	Yes	27	Yes	N/A	No	No	1
12	67	Female	Colon	50	II	T3N0M0V0	No	NA			MSS	No	41	Yes	No	No	No	1
13	66	Female	Colon	40	II	T4N0MxV0	Yes	NA			MSS	Yes	27	Yes	No	No	No	1
14	68	Female	Colon	63	II	T3N0MxV0	No	NA			MSI	Yes	28	Yes	No	No	No	1
15	48	Male	Colon	42	III	T3N2MxV0	Yes	NA			MSS	Yes	38	Yes	N/A	No	No	1
16	68	Female	Colon	50	II	T3N0MxV0	No	NA			MSS	Yes	28	Yes	No	No	No	1
18	67	Male	Colon	45	III	T2N2MxV0	Yes	12	Liver	Surgery	MSS	Yes	21	No	N/A	No	No	1
19	69	Female	Colon	58	II	T3N0M0V0	No	NA			MSI	Yes	28	Yes	No	No	No	2
20	73	Female	Rectal	55	III	T3N1M0V2	Yes	12	Lung	Surgery	MSS	Yes	21	Yes	N/A	No	No	1
21	68	Male	Colon	55	III	T3N1M0V0	Yes	NA			MSS	Yes	26	Yes	N/A	No	No	1
22	67	Female	Rectal	60	III	T3N1M0V1	Yes	NA			MSS	Yes	36	Yes	N/A	No	No	1
23	69	Male	Rectal	53	III	T4N1M0V1	Yes	NA			MSS	Yes	41	Yes	N/A	No	No	1
24	82	Male	Colon	77	III	T3N1M0V1	Yes	13	Liver	RFA liver	MSS	Yes	33	Yes	N/A	No	No	1
25	72	Female	Colon	95	II	T3N0M0V0	No	NA			MSI	Yes	62	Yes	No	No	No	1
26	66	Male	Colon	82	III	T4N1M0V0	Yes	NA			MSS	Yes	42	Yes	N/A	No	No	1
27	68	Male	Rectal	37	III	T3N1M0V0	Yes	NA			MSS	Yes	27	Yes	N/A	No	No	1
28	46	Female	Colon	66	III	T4N2M0V1	Yes	31	Multiple	Chemotherapy	MSS	Yes	32	Yes	N/A	No	No	1
29	65	Female	Rectal	37	III	T3N1M0V1	Yes	12	Lung	Chemotherapy	MSS	Yes	15	No	N/A	No	No	1
30	52	Female	Colon	47	III	T3N2M0V2	Yes	9	Bone	Chemotherapy	MSS	Yes	28	No	N/A	No	No	1

eTable 1. Clinicopathological Patient Characteristics																		
Patient ID	Age	Gender	Primary tumor site	Primary tumor diameter , mm	UICC stage	TNMV	ACT	First relapse after OP month	Relapse site	Relapse treatment	MSS/MSI status	Perineural invasion	Number of lymph nodes in resected specimen	Microscopic radical resection	Ileus	Anastomotic leakage	Tumor perforation	WHO performance status
31	54	Male	Colon	25	III	T3N1M0V0	Yes	NA			MSS	Yes	28	Yes	N/A	No	No	1
33	47	Male	Colon	170	III	T3N1M0V1	Yes	NA			MSI	NA	81	Yes	N/A	No	No	1
34	69	Male	Colon	37	II	T3N0M0V1	Yes	31	Liver	Palliative	MSS	Yes	35	Yes	No	No	No	1
35	68	Male	Colon	38	III	T3N1M0V0	Yes	NA			MSS	Yes	21	Yes	N/A	No	No	1
36	70	Male	Colon	47	III	T4N2M0V1	Yes	NA			MSS	Yes	37	Yes	N/A	No	No	1
37	75	Male	Colon	46	II	T3N0M0V0	No	6	Liver	RFA liver	MSS	Yes	30	Yes	No	No	No	1
38	49	Female	Colon	41	II	T3N0M0V0	No	NA			MSI	Yes	27	Yes	No	No	No	1
39	74	Male	Colon	115	III	T4N2M0V1	Yes	NA			MSS	Yes	27	No	N/A	No	No	1
40	69	Male	Colon	85	III	T3N2M0V1	Yes	NA			MSS	Yes	23	No	N/A	No	No	1
41	70	Male	Colon	67	II	T3N0M0V0	No	NA			MSS	Yes	35	Yes	No	No	No	1
42	60	Male	Colon	73	III	T4N2MxV1	Yes	14	Carcinosis	Chemotherapy	MSS	Yes	36	No	N/A	No	No	1
43	48	Male	Rectal	51	II	T3N0MxV0	No	NA			MSS	No	40	Yes	Yes	No	No	1
44	76	Female	Colon	70	II	T4N0M0V0	Yes	NA			MSS	No	19	Yes	No	No	No	1
45	83	Female	Colon	40	II	T3N0MxV0	No	NA			MSI	No	27	Yes	No	No	No	2
46	72	Female	Colon	70	III	T4N2MxV1	Yes	NA			MSI	No	37	No	N/A	No	Yes	2
47	73	Female	Colon	7	II	T3N0MxV0	No	NA			MSS	No	37	Yes	No	No	No	2
48	91	Female	Colon	55	III	T3N1M0V1	No	NA			MSI		32	Yes	N/A	No	No	2
49	80	Female	Colon	20	III	T4N1M0V0	Yes	NA			MSS	Yes	19	No	N/A	No	No	1
50	64	Male	Colon	83	II	T4N0M0V2	Yes	NA			MSS	Yes	40	Yes	No	No	No	1
51	60	Male	Colon	70	II	T3N0M0V0	No	NA			MSS	Yes	40	Yes	No	Yes	No	1
52	81	Male	Colon	30	II	T3N0M0V0	No	NA			MSS	No	17	Yes	No	No	No	2
53	80	Female	Colon	32	III	T3N1M0V0	No	NA			MSS	Yes	18	No	N/A	No	No	2
54	66	Male	Colon	40	III	T3N1M0V0	No	NA			MSS	Yes	17	Yes	N/A	Yes	No	NA
55	78	Male	Colon	40	II	T3N0M0V0	No	NA			MSS	Yes	18	Yes	No	No	No	2
57	50	Male	Colon	45	II	T3N0M0V0	No	NA			MSS	Yes	32	Yes	No	No	No	1
58	77	Male	Colon	52	II	T4N0M0V0	No	NA			MSI	Yes	24	Yes	No	No	No	1
59	51	Female	Colon	20	I	T2N0M0V0	No	NA			MSS	No	16	Yes	N/A	No	No	1
60	70	Female	Colon	32	II	T3N0M0V0	No	NA			MSS	Yes	14	Yes	No	No	No	1
61	67	Male	Colon	24	II	T3N0M0V0	No	NA			MSS	Yes	20	Yes	No	No	No	1
62	55	Male	Colon	30	III	T4N1M0V1	Yes	NA			MSS	No	19	Yes	N/A	No	No	1

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Patient ID	Age	Gender	Primary tumor site	Primary tumor diameter, mm	UICC stage	TNMV	ACT	First relapse after OP month	Relapse site	Relapse treatment	MSS/MSI status	Perineural invasion	Number of lymph nodes in resected specimen	Microscopic radical resection	Ileus	Anastomotic leakage	Tumor perforation	WHO performance status
63	82	Male	Colon	40	III	T3N1MxV0	No	NA			MSI	No	27	Yes	N/A	No	No	1
64	71	Female	Colon	75	III	T3N1MxV1	Yes	NA			MSS	No	19	Yes	N/A	No	No	1
65	72	Male	Colon	55	III	T4N1MxV1	Yes	NA			MSS	Yes	31	No	N/A	No	No	1
66	72	Male	Colon	90	III	T3N2MxV0	Yes	NA			MSS	No	49	Yes	N/A	No	No	1
67	71	Male	Colon	35	III	T4N1MxV0	Yes	NA			MSS	No	31	Yes	N/A	No	No	2
68	83	Female	Colon	75	III	T3N2MxV1	No	13	Carcinosis	Chemotherapy	MSS	No	24	Yes	N/A	No	No	1
69	56	Female	Colon	25	III	T3N1MxV1	Yes	NA			MSS	No	18	Yes	N/A	No	No	2
70	66	Male	Colon	20	III	T3N2MxV0	Yes	NA			MSS	No	37	Yes	N/A	No	No	1
71	77	Male	Colon	90	III	T3N1MxV0	Yes	NA			MSI	No	26	Yes	N/A	No	No	2
72	49	Female	Colon	41	III	T3N1MxV0	Yes	NA			MSS	No	16	Yes	N/A	No	No	1
73	43	Female	Colon	64	III	T4N2MxV1	Yes	NA			MSS	No	30	Yes	N/A	No	No	1
74	72	Male	Colon	18	III	T3N2MxV0	Yes	NA			MSS	No	25	Yes	N/A	No	No	2
75	48	Female	Colon	74	III	T3N1MxV0	Yes	11	Liver	Surgery	MSS	No	48	Yes	N/A	No	No	2
76	75	Male	Colon	73	III	T3N2MxV0	Yes	NA			MSS	No	26	Yes	N/A	No	No	2
77	71	Female	Colon	52	III	T3N1MxV1	Yes	13	Lung	Surgery	MSS	Yes	280	Yes	N/A	No	No	2
78	64	Female	Colon	50	III	T4N2MxV0	Yes	NA			MSS	No	23	Yes	N/A	No	No	1
79	50	Female	Colon	40	III	T3N1MxV0	Yes	15	Liver	Surgery/RFA	MSS	No	20	Yes	N/A	No	No	1
80	73	Female	Colon	82	III	T4N1MxV0	Yes	NA			MSS	No	47	Yes	N/A	No	No	1
81	58	Female	Colon	23	III	T3N2MxV0	Yes	NA			MSS	No	17	Yes	N/A	No	No	1
82	50	Female	Colon	30	III	T3N2M0V1	Yes	11	LN ^b (throat)	Surgery	MSS	Yes	29	Yes	N/A	No	No	NA
83	62	Female	Colon	70	III	T3N2MxV0	Yes	NA			MSS	Yes	23	Yes	N/A	No	No	2
84	65	Female	Colon	100	III	T3N1MxV2	Yes	NA			MSS	No	24	Yes	N/A	No	No	1
85	61	Female	Colon	35	III	T3N2MxV0	Yes	11	Liver & lung	None	MSS	No	27	Yes	N/A	No	No	1
86	79	Male	Colon	90	III	T3N2MxV1	Yes	NA			MSS	No	30	Yes	N/A	No	No	1
87	70	Male	Colon	145	III	T3N1MxV2	Yes	NA			MSS	No	17	Yes	N/A	No	No	3
88	64	Male	Colon	60	III	T3N2MxV1	Yes	NA			MSS	No	47	Yes	N/A	No	No	1
89	75	Male	Colon	40	III	T3N2MxV0	No	17	Lung	Surgery	MSS	No	12	Yes	N/A	No	No	3
90	87	Female	Colon	62	III	T3N1MxV0	No	NA			MSS		21	No	Yes	No	No	3
91	69	Male	Colon	70	III	T3N1MxV0	Yes	NA			MSS	No	15	Yes	N/A	No	No	2
92	73	Female	Colon	100	III	T4N2MxV0	Yes	14	Local	Surgery	MSI	No	18	No	N/A	No	No	2

eTable 1. Clinicopathological Patient Characteristics																		
Patient ID	Age	Gender	Primary tumor site	Primary tumor diameter , mm	UICC stage	TNMV	ACT	First relapse after OP month	Relapse site	Relapse treatment	MSS/MSI status	Perineural invasion	Number of lymph nodes in resected specimen	Microscopic radical resection	Ileus	Anastomotic leakage	Tumor perforation	WHO performance status
93	81	Male	Colon	80	III	T4N2MxV1	Yes	NA			MSS	No	28	Yes	N/A	No	No	2
95	70	Male	Colon	30	I	T2N0MxV0	No	NA			MSS	No	27	Yes	N/A	No	No	1
96	64	Male	Colon	40	III	T4N2M0V1	Yes	NA			MSS	Yes	33	Yes	N/A	No	No	1
97	67	Female	Colon	60	II	T4N0MxV0	Yes	NA			MSS	No	15	Yes	No	No	No	1
98	78	Male	Colon	30	II	T3N0MxV0	No	NA			MSI	No	15	Yes	Yes	No	No	2
99	79	Male	Colon	40	III	T4N2MxV0	No	15	Multiple	Palliative	MSS	No	30	Yes	N/A	No	No	1
100	59	Female	Colon	100	II	T4N0MxV1	No	NA			MSS	Yes	12	Yes	No	No	No	2
101	72	Male	Colon	40	II	T3N0MxV2	No	NA			MSS	No	34	Yes	No	No	No	2
102	69	Male	Colon	35	I	T2N0MxV0	No	NA			MSS	No	13	Yes	N/A	No	No	1
103	70	Male	Colon	100	III	T3N1MxV1	Yes	12	Multiple	None	MSS	No	38	No	N/A	No	No	3
104	50	Male	Colon	30	III	T3N1MxV2	Yes	12	Liver	RFA liver	MSS	No	24	NA	N/A	No	No	NA
105	72	Male	Colon	70	II	T3N0MxV1	No	NA			MSS	No	36	Yes	No	No	No	2
106	70	Female	Colon	25	I	T2N0MxV0	No	NA			MSI	No	21	Yes	N/A	No	No	1
107	75	Male	Colon	30	III	T3N2MxV0	Yes	NA			MSS	No	17	Yes	N/A	No	No	1
108	72	Female	Colon	61	II	T3N0MxV0	No	12	Liver	RFA liver	MSS	No	12	Yes	No	No	No	3
109	74	Male	Colon	50	II	T3N0MxV0	No	NA			MSS	Yes	24	Yes	No	No	No	2
110	67	Male	Colon	35	I	T2N0MxV0	No	NA			MSS	No	20	Yes	N/A	No	No	2
111	52	Male	Colon	45	III	T3N2M0V1	Yes	NA			MSS	Yes	31	No	N/A	No	Yes	1
112	61	Male	Colon	40	III	T3N1MxV1	Yes	NA			MSS	Yes	25	No	N/A	No	No	1
113	78	Male	Colon	70	III	T3N1MxV1	No	NA			MSS	No	22	Yes	N/A	No	No	1
114	79	Male	Colon	60	III	T3N1MxV0	Yes	NA			MSS		33	Yes	N/A	No	No	1
115	78	Male	Colon	35	III	T3N2M0Vx	Yes	NA			MSS	No	24	No	N/A	No	No	2
116	75	Female	Colon	70	III	T4N1MxV0	Yes	NA			MSS	No	31	Yes	N/A	No	No	1
117	84	Female	Colon	56	III	T3N1MxV0	Yes	NA			MSS	No	19	No	N/A	No	No	2
118	69	Male	Colon	80	III	T4N2MxV0	Yes	NA			MSI	No	25	Yes	N/A	No	No	1
119	69	Female	Colon	60	III	T4N1MxV1	Yes	12	Local	HIPEC	MSS	Yes	29	No	N/A	No	Yes	1
120	61	Female	Colon	50	III	T3N2MxV1	Yes	NA			MSI	No	31	Yes	N/A	No	No	1
121	81	Female	Colon	20	III	T3N1MxV0	No	NA			MSS	No	14	Yes	N/A	No	No	2
122	76	Male	Colon	50	III	T3N1MxV1	Yes	NA			MSS	Yes	34	Yes	N/A	No	No	1
123	59	Male	Colon	50	III	T4N1MxV0	Yes	NA			MSS	Yes	24	Yes	N/A	No	No	1

eTable 1. Clinicopathological Patient Characteristics																		
Patient ID	Age	Gender	Primary tumor site	Primary tumor diameter , mm	UICC stage	TNMV	ACT	First relapse after OP month	Relapse site	Relapse treatment	MSS/MSI status	Perineural invasion	Number of lymph nodes in resected specimen	Microscopic radical resection	Ileus	Anastomotic leakage	Tumor perforation	WHO performance status
124	71	Female	Colon	47	III	T3N2MxV2	Yes	12	Multiple	Palliative	MSS	No	41	NA	N/A	No	No	2
125	78	Female	Colon	40	II	T4N0MxV0	No	12	Lung	Palliative	MSS	No	31	Yes	No	No	No	1
126	71	Male	Colon	60	II	T3N0MxV0	No	NA			MSS	No	36	Yes	No	No	No	1
127	67	Male	Colon	30	II	T3N0MxV0	No	NA			MSI	No	23	Yes	No	No	No	1
128	51	Male	Colon	8	III	T4N1M0V0	Yes	NA			MSI	Yes	54	NA	N/A	No	Yes	2
130	66	Male	Colon	60	III	T4N2M0V0	Yes	NA			MSS	Yes	19	Yes	N/A	No	No	1

^aNot applicable
^bLymph node
^cHyperthermic intraperitoneal chemotherapy
^dNot available

eTable 2. Performance Estimates of the Assay Based on Number of Mutations for Assay Design				
	Tracking 2 mutations	Tracking 4 mutations	Tracking 8 mutations	Tracking 16 mutations (as reported in this study)
Relapse prediction (out of n=16 patients)	7 (44%)	13 (81%)	13 (81%)	14 (88%)
Pre-operation ctDNA positive samples (out of n=122)	58 (48%)	82 (67%)	96 (79%)	108 (89%)

eTable 3. Patient Characteristics and Demographics of Eligible Patients		
Patients, n		125
Cancers, n		129 ^a
Age (years), median (range)		69.9 (43.3-91)
Gender, n (%)	Female	52 (41.6)
	Male	73 (58.4)
Imaging follow-up (months), median (range)		12.5, (1.4-38.5)
Location, n (%)	Colon	119 (95.2)
	Rectum	6 (4.8)
Pathological UICC stage, n (%)	I	5 (4)
	II	39 (31.2)
	III	81 (64.8)
Histological type, n (%)	Adenocarcinoma	115 (92)
	Mucinous carcinoma	10 (8.7)
Histological grade, n (%)	Moderately differentiated	96 (76.8)
	Poorly differentiated	19 (15.2)
	ND	10 (8)
Adj. therapy by UICC stage, n (%)	I	0 (0.0)
	II	6 (15.3)
	III	71 (87.7)
	Total	77 (61.6)
Relapse by UICC stage, n (%)	I	0 (0.0)
	II	4 (10.2)
	III	20 (24.7)
	IV	0 (0.0)
	Total	24 (19.2)
Relapse site, n (%) ^b	Distant	23 (95.8)
	Local	1 (4.2)
MSS/MSI status, n (%)	MSS	109 (87.2)
	MSI	20 (16)
Smoking, n (%)	Never	52 (41.6)
	Former	57 (45.6)
	Current	16 (12.8)

^aFour patients with synchronous cancers. Details available in eTable 3

^bDetails available in eTable 1

eTable 4. Summary of Samples and WES Information.

Patient ID	Source of DNA	Sample type ^b	Input, ng	WES coverage	Ti/Tv ratio	Median insert size (bp)	Percentage bases on target	Capture fold enrichment	# of variants
1	Primary tumor	FF	500	100.8	2.2	213	53.6	41.1	261
2	Primary tumor	FF	500	94.3	2.2	233	52.9	40.2	318
3	Primary tumor	FF	500	101.8	2.2	229	59	40	366
4	Primary tumor	FF	500	98.4	2.2	228	58.5	40	462
5	Primary tumor	FF	500	167.8	2.2	237	57.5	39.1	509
6	Primary tumor	FF	500	93	2.2	229	52.9	40.4	388
7	Primary tumor	FF	500	91.2	2.2	244	52.5	39.4	422
8	Primary tumor	FF	500	89.9	2.3	235	60.2	39.6	469
9	Primary tumor	FF	500	91.3	1.8	238	52.3	39.6	11342
10	Primary tumor	FF	500	140.4	2.2	204	62.8	40.5	387
11	Primary tumor	FF	500	104.3	2.3	224	66.6	40.5	448
12	Primary tumor	FF	500	106	2.3	235	58.2	39.6	594
13	Primary tumor	FF	500	47.2	2.3	244	65.6	37.9	593
14	Primary tumor	FF	500	106.6	2.2	239	59.9	39.4	14532
15	Primary tumor	FF	500	105.2	2.2	224	58.7	40.3	366
16	Primary tumor	FF	250	122.7	2.2	208	60.3	41.3	259
18	Primary tumor	FF	500	98	2.3	206	68.2	41.6	448
19	Primary tumor	FF	500	100.3	2.2	237	59.8	39.5	15574
20	Primary tumor	FF	500	112.2	2.2	233	65.4	39.8	428
20	Metastasis	FFPE	500	97.5	2.2	229	63.3	40	435
21	Primary tumor	FF	500	117	2.3	262	59.7	37.2	436
22	Primary tumor	FF	500	102.2	2.2	219	58.9	40.5	423
23	Primary tumor	FF	500	104.9	2.3	212	67.6	41.3	326
24	Primary tumor	FF	500	46.5	2.3	232	67.2	39.8	536
24	Metastasis	FFPE	500	107.4	2.3	162	64.2	45.1	645
25	Primary tumor	FF	500	106.1	2.1	247	59.1	38.8	7116
26	Primary tumor	FF	500	150.9	2.2	218	63.5	40.6	501
27	Primary tumor	FF	500	149.2	2.3	226	63.8	40.2	355
28	Primary tumor	FF	500	129.2	2.3	216	64.2	40.9	492
29	Primary tumor	FF	500	86.1	2.3	219	64.5	40.8	368
30	Primary tumor	FF	500	222	2.2	244	56.4	39.1	589
31	Primary tumor	FFPE	500	123.6	2.2	210	26.3	41.4	380
33 (S1)a	Primary tumor	FF	500	87.7	2.2	226	64.2	40.3	7628
33 (S2)a	Primary tumor	FFPE	500	65.5	2.2	164	42.5	44.4	5881

eTable 4. Summary of Samples and WES Information.

Patient ID	Source of DNA	Sample type ^b	Input, ng	WES coverage	Ti/Tv ratio	Median insert size (bp)	Percentage bases on target	Capture fold enrichment	# of variants
34	Primary tumor	FF	500	90.4	2.3	256	59.1	38.3	261
35	Primary tumor	FF	500	186.3	2.2	245	57.4	38.7	708
36	Primary tumor	FF	500	184.4	2	242	57	39	395
37	Primary tumor	FF	500	87.8	2.3	227	58.9	40	397
38	Primary tumor	FF	500	86.9	2.2	235	58.7	39.5	4657
39	Primary tumor	FF	500	179.4	2.2	242	57.7	39.1	594
40	Primary tumor	FF	500	86.4	2.3	221	63.2	40.5	558
41	Primary tumor	FF	500	88.4	2.3	216	63.6	40.8	347
42	Primary tumor	FF	500	94.9	2.2	200	62.2	41.9	361
43	Primary tumor	FF	500	121.3	2.2	227	53.1	40.5	401
44	Primary tumor	FF	500	103.8	2.3	228	61.4	40.1	434
45	Primary tumor	FF	500	107.6	2.2	228	64.4	40.2	6265
46	Primary tumor	FF	500	99	2.2	254	60.1	38.7	5943
47	Primary tumor	FF	500	94.4	2.3	209	65.3	41.5	551
48 (S1)a	Primary tumor	FF	500	95	2.1	253	60.7	38.8	7014
48 (S2)a	Primary tumor	FFPE	500	108.5	2.1	197	45.6	42.3	6276
49	Primary tumor	FF	200	127.9	2	228	60.1	40.1	308
50	Primary tumor	FF	500	93.1	2.3	225	61.2	40.3	323
51	Primary tumor	FFPE	500	130.7	2.2	219	45.3	41	525
52	Primary tumor	FFPE	500	113	2.3	187	46.2	43.5	393
53	Primary tumor	FF	500	93.7	2.3	226	64.3	40.3	286
54	Primary tumor	FF	500	113.6	2.3	228	66.3	40.1	479
55	Primary tumor	FF	500	161	2.2	258	57	38.1	342
57	Primary tumor	FF	500	85	1.8	227	64.4	40.2	20961
58	Primary tumor	FF	500	79.4	2.2	224	64.4	40.4	5804
59	Primary tumor	FF	250	127.1	2.2	214	59.6	40.9	410
60	Primary tumor	FFPE	500	119.1	2.2	199	45.6	42.4	314
61	Primary tumor	FF	500	93.6	2.2	237	60.6	39.7	423
62	Primary tumor	FFPE	500	120.2	2.3	149	38.8	46	586
63	Primary tumor	FF	100	116.6	2.2	231	61.8	38.9	7314
64	Primary tumor	FF	500	109	2.3	231	65.7	39.9	404
65	Primary tumor	FFPE	500	99.4	2.3	192	42.3	42.8	501
66	Primary tumor	FF	500	105.2	2.3	234	65.8	39.8	477
67	Primary tumor	FF	500	82.1	2.3	220	64.1	40.6	487
68	Primary tumor	FF	500	102	2.3	233	65.8	39.8	373

eTable 4. Summary of Samples and WES Information.

Patient ID	Source of DNA	Sample type ^b	Input, ng	WES coverage	Ti/Tv ratio	Median insert size (bp)	Percentage bases on target	Capture fold enrichment	# of variants
69	Primary tumor	FF	500	97.3	2.3	219	63.2	40.6	413
70	Primary tumor	FFPE	500	150.4	2.2	159	35.1	45.4	483
71	Primary tumor	FF	500	160.4	2.1	310	55.2	35.4	6173
72	Primary tumor	FF	500	126.8	2.3	229	66.1	40.1	309
73	Primary tumor	FF	500	90.3	2.3	222	64.5	40.6	432
74	Primary tumor	FF	500	127.3	2.2	207	61.7	41.3	1076
75	Primary tumor	FF	500	108.8	2.2	239	58.9	39.4	683
76	Primary tumor	FF	250	111.8	2.2	202	59	41.5	533
77	Primary tumor	FF	500	124.5	2.2	233	52.5	40.1	476
77	Metastasis	FFPE	500	118.8	2.3	196	63.8	42.7	744
78	Primary tumor	FF	500	94.2	2.3	219	67.2	41	328
79	Primary tumor	FF	500	101.2	2.3	221	66.9	40.9	263
80	Primary tumor	FF	500	93.8	2.3	243	60	39.2	646
81	Primary tumor	FF	500	101.8	2.3	227	59.4	40.2	295
82	Primary tumor	FF	500	219.9	2.2	237	59.1	39.5	415
83	Primary tumor	FFPE	200	38.6	2.4	136	39.6	46.7	609
84	Primary tumor	FFPE	500	69.2	2.3	182	41.5	43.6	371
85	Primary tumor	FF	500	107.6	2.3	219	59.2	40.6	397
86	Primary tumor	FF	200	116.2	2.2	192	60.2	41.6	630
87	Primary tumor	FF	500	120	2.2	222	52.7	40.8	477
88	Primary tumor	FF	500	192.4	2.2	233	57.5	39.7	571
89	Primary tumor	FF	500	91.7	2.3	235	61.6	39.9	611
90 (S1)a	Primary tumor	FFPE	500	94.6	2.2	187	26.8	43.4	408
90 (S2)a	Primary tumor	FFPE	500	70.8	2.1	171	68.8	44.4	480
91	Primary tumor	FF	500	82.8	2.3	225	64.1	40.4	489
92	Primary tumor	FF	500	132.1	2.1	226	61.5	40.5	6444
93	Primary tumor	FFPE	500	118.4	2.2	209	45.4	41.7	479
95	Primary tumor	FF	100	148.6	2.2	223	60.8	40.5	597
96	Primary tumor	FFPE	500	48.3	2.4	158	64.6	45.3	426
97	Primary tumor	FFPE	500	73.2	2.3	157	25.1	45.4	298
98	Primary tumor	FFPE	500	96.8	2.2	196	26.6	42.4	4659
99	Primary tumor	FFPE	500	125.1	2.2	226	44.6	40.5	503
100	Primary tumor	FF	500	81.5	2.3	199	64.7	41.8	467
101	Primary tumor	FF	500	88.1	2.3	190	64.5	42.3	359
102	Primary tumor	FFPE	500	127.7	2.3	167	44.8	45	432

eTable 4. Summary of Samples and WES Information.

Patient ID	Source of DNA	Sample type ^b	Input, ng	WES coverage	Ti/Tv ratio	Median insert size (bp)	Percentage bases on target	Capture fold enrichment	# of variants
103	Primary tumor	FF	500	169.3	2.2	227	57.8	40	596
104	Primary tumor	FF	500	99.9	2.2	242	52	39.6	247
105	Primary tumor	FF	100	383.4	2.2	222	60.6	40.1	884
106	Primary tumor	FF	500	83.2	2.2	217	64	40.8	4515
107	Primary tumor	FFPE	500	99.5	2.3	183	46.1	43.4	412
108	Primary tumor	FF	500	98.5	2.3	224	63.1	40.4	686
109	Primary tumor	FF	500	168.7	2.2	236	57.6	39.2	285
110	Primary tumor	FF	500	85.8	2.3	210	64.1	41.3	364
111	Primary tumor	FFPE	500	54.5	2.3	135	29.8	47.1	1019
112	Primary tumor	FF	500	86.5	2.3	216	67.7	40.9	315
113	Primary tumor	FFPE	500	92.2	2.3	133	32	47.6	604
114 (S1)a	Primary tumor	FF	500	80.7	2.3	243	60.3	39.2	432
114 (S2)a	Primary tumor	FF	500	98.6	2.3	248	59	38.7	603
115	Primary tumor	FF	500	179.6	2.2	244	57.8	39.1	683
116	Primary tumor	FF	500	97.6	2.3	239	57.8	39.5	436
117	Primary tumor	FF	500	91.5	2.2	228	54.8	40.7	602
118	Primary tumor	FF	500	94.4	2.2	218	66.9	41	6636
119	Primary tumor	FFPE	500	66	2.3	144	41	46.6	642
120	Primary tumor	FF	500	80.4	2.2	204	65.4	41.7	5666
121	Primary tumor	FFPE	500	94.1	2.3	137	35.1	47.7	697
122	Primary tumor	FFPE	500	61.2	2.3	143	44.6	46.4	457
123	Primary tumor	FF	500	176.9	2.2	236	58.1	39.3	525
124	Primary tumor	FF	250	137.2	2.2	220	59.5	40.5	642
125	Primary tumor	FF	500	119.6	2	214	61.7	41.1	492
126	Primary tumor	FF	500	102.6	2.2	225	54	40.7	345
127	Primary tumor	FFPE	500	83.1	2.2	136	32.9	47	7599
128	Primary tumor	FFPE	500	72.3	2.3	155	46.7	45.6	5622
130	Primary tumor	FF	500	88.8	2.3	217	65.2	40.9	452
1	Buffy coat	NA	500	41.9	2.1	247	64.7	37.5	NA ^c
2	Buffy coat	NA	500	36.5	2.3	244	64.9	37.6	NA
3	Buffy coat	NA	500	45.3	2.3	232	67.5	39.9	NA
4	Buffy coat	NA	500	45.5	2.3	240	66.7	39.2	NA
5	Buffy coat	NA	500	40.9	2.3	231	69.1	39.7	NA
6	Buffy coat	NA	500	46.1	2.3	238	67.2	39.4	NA
7	Buffy coat	NA	500	40.8	2.3	243	66.3	38.2	NA

eTable 4. Summary of Samples and WES Information.

Patient ID	Source of DNA	Sample type ^b	Input, ng	WES coverage	Ti/Tv ratio	Median insert size (bp)	Percentage bases on target	Capture fold enrichment	# of variants
8	Buffy coat	NA	500	43.2	2.3	223	68.6	40.5	NA
9	Buffy coat	NA	500	36.1	2.3	248	65.2	37.5	NA
10	Buffy coat	NA	500	41.8	2.3	230	69.6	39.9	NA
11	Buffy coat	NA	500	39.4	2.3	249	66.5	38.6	NA
12	Buffy coat	NA	500	40.8	2.3	246	65.1	37.7	NA
13	Buffy coat	NA	500	97	2.2	229	52.7	40.2	NA
14	Buffy coat	NA	500	61.3	2.3	248	64.9	37.6	NA
15	Buffy coat	NA	500	39.2	2.3	238	67.1	39.3	NA
16	Buffy coat	NA	500	92.2	2.3	267	51.5	36.3	NA
18	Buffy coat	NA	500	49.4	2.3	235	66.9	39.7	NA
19	Buffy coat	NA	500	37	2.3	243	52.8	37.6	NA
20	Buffy coat	NA	500	44.7	2.3	244	64.9	38.6	NA
20	Buffy coat	NA	500	44.7	2.3	244	64.9	38.6	NA
21	Buffy coat	NA	500	56.5	2.3	237	65.9	39.1	NA
22	Buffy coat	NA	500	54.9	2.3	234	66.2	39.4	NA
23	Buffy coat	NA	500	43.1	2.3	236	67.4	39.6	NA
24	Buffy coat	NA	500	104.2	2.3	220	66.6	40.8	NA
24	Buffy coat	NA	500	104.2	2.3	220	66.6	40.8	NA
25	Buffy coat	NA	500	43.6	2.3	253	52.7	37	NA
26	Buffy coat	NA	500	46.5	2.3	239	66.6	39.4	NA
27	Buffy coat	NA	500	54.9	2.3	241	65.7	38.9	NA
28	Buffy coat	NA	500	60.9	2.3	233	66	39.5	NA
29	Buffy coat	NA	500	45.1	2.2	234	67.3	39.7	NA
30	Buffy coat	NA	500	40	2.3	235	69.1	39.5	NA
31	Buffy coat	NA	500	56.8	2.1	233	66.1	39.4	NA
33	Buffy coat	NA	500	55.9	2.3	238	65.7	39.1	NA
34	Buffy coat	NA	500	46.8	2.3	247	52.9	37.3	NA
35	Buffy coat	NA	500	41.6	2.3	235	68.8	39.4	NA
36	Buffy coat	NA	500	36.8	2.3	235	69	39.4	NA
37	Buffy coat	NA	500	50.8	2.3	256	51.9	36.7	NA
38	Buffy coat	NA	500	54.2	2.3	252	52.6	37	NA
39	Buffy coat	NA	500	49.8	2.3	238	68.5	39.3	NA
40	Buffy coat	NA	500	62.7	2.3	280	62.1	36.4	NA
41	Buffy coat	NA	500	60.5	2.3	246	64.5	38.4	NA
42	Buffy coat	NA	500	35.9	2.3	245	65.1	37.6	NA

eTable 4. Summary of Samples and WES Information.

Patient ID	Source of DNA	Sample type ^b	Input, ng	WES coverage	Ti/Tv ratio	Median insert size (bp)	Percentage bases on target	Capture fold enrichment	# of variants
43	Buffy coat	NA	500	35.2	2.3	242	65	37.7	NA
44	Buffy coat	NA	500	45.2	2.3	238	67.2	39.5	NA
45	Buffy coat	NA	500	44.8	2.3	239	67.1	39.4	NA
46	Buffy coat	NA	500	40.6	2.3	232	67.8	39.8	NA
47	Buffy coat	NA	500	37.6	2.3	241	65.3	37.8	NA
48	Buffy coat	NA	500	52.7	2.3	228	67.9	40.1	NA
49	Buffy coat	NA	500	57.7	2.3	233	68.9	39.7	NA
50	Buffy coat	NA	500	43.2	2.3	240	52.9	37.7	NA
51	Buffy coat	NA	500	36.4	2.3	243	52.5	37.4	NA
52	Buffy coat	NA	500	55.4	2.3	244	64.5	38.5	NA
53	Buffy coat	NA	500	56	2.3	234	65.8	39.3	NA
54	Buffy coat	NA	500	79.7	2.3	239	65	38.8	NA
55	Buffy coat	NA	500	69.1	2.3	234	68.7	39.6	NA
57	Buffy coat	NA	500	72.8	2.3	250	64.1	38.2	NA
58	Buffy coat	NA	500	53.7	2.3	237	64.9	38.9	NA
59	Buffy coat	NA	500	58.4	2.3	240	64.7	38.9	NA
60	Buffy coat	NA	500	57.4	2.3	233	65.4	39.3	NA
61	Buffy coat	NA	500	81.9	2.2	236	64.8	39	NA
62	Buffy coat	NA	500	40	2.3	235	68.7	39.4	NA
63	Buffy coat	NA	500	44.8	2.1	240	67.1	39.2	NA
64	Buffy coat	NA	500	41.3	2.3	243	66.8	39.1	NA
65	Buffy coat	NA	500	39.1	2.3	233	67.5	39.7	NA
66	Buffy coat	NA	500	47.7	2.3	231	67.4	39.9	NA
67	Buffy coat	NA	500	50.2	2.3	233	67.2	39.8	NA
68	Buffy coat	NA	500	42	2.3	232	68.1	39.9	NA
69	Buffy coat	NA	500	44.9	2.3	239	67.2	39.4	NA
70	Buffy coat	NA	500	39.8	2.4	234	68.6	39.5	NA
71	Buffy coat	NA	500	39.2	2.3	232	69	39.7	NA
72	Buffy coat	NA	500	57	2.3	232	66.1	39.5	NA
73	Buffy coat	NA	500	55.3	2.1	237	66.1	39.3	NA
74	Buffy coat	NA	500	44.7	2.3	234	67.3	39.7	NA
75	Buffy coat	NA	500	36.5	2.3	239	59.5	38.3	NA
76	Buffy coat	NA	500	55.6	2.3	242	65.4	38.9	NA
77	Buffy coat	NA	500	42.1	2.3	232	67.3	40	NA
77	Buffy coat	NA	500	42.1	2.3	232	67.3	40	NA

eTable 4. Summary of Samples and WES Information.

Patient ID	Source of DNA	Sample type ^b	Input, ng	WES coverage	Ti/Tv ratio	Median insert size (bp)	Percentage bases on target	Capture fold enrichment	# of variants
78	Buffy coat	NA	500	53.5	2.3	236	66.2	39.1	NA
79	Buffy coat	NA	500	54.1	2.3	243	65.6	38.6	NA
80	Buffy coat	NA	500	44.8	2.3	237	59.9	38.6	NA
81	Buffy coat	NA	500	38.3	2.3	248	65.5	37.8	NA
82	Buffy coat	NA	500	69.3	2.3	234	68.8	39.6	NA
83	Buffy coat	NA	500	52.2	2.3	231	66.5	39.3	NA
84	Buffy coat	NA	500	39.6	2.3	244	58.8	37.9	NA
85	Buffy coat	NA	500	63.3	2.3	247	65.6	37.9	NA
86	Buffy coat	NA	500	38.3	2.3	240	59	38.1	NA
87	Buffy coat	NA	500	39.7	2.3	241	59.7	38.3	NA
88	Buffy coat	NA	500	45.4	2.3	235	68.8	39.4	NA
89	Buffy coat	NA	500	56.5	2.3	234	66.5	39.1	NA
90	Buffy coat	NA	500	41.3	2.3	234	59.9	38.8	NA
91	Buffy coat	NA	500	84	2.2	240	65.7	38.7	NA
92	Buffy coat	NA	500	54.1	2.3	235	66.1	39.1	NA
93	Buffy coat	NA	500	41.1	2.3	233	60.1	38.8	NA
95	Buffy coat	NA	500	52.3	2.1	234	66.2	39.1	NA
96	Buffy coat	NA	500	52.9	2.3	235	65.9	39	NA
97	Buffy coat	NA	500	33.1	2.3	230	60.4	38.8	NA
98	Buffy coat	NA	500	41.6	2.3	243	59.4	38.2	NA
99	Buffy coat	NA	500	38.4	2.3	245	65.7	37.9	NA
100	Buffy coat	NA	500	38.4	2.3	246	65.7	38	NA
101	Buffy coat	NA	500	43.3	2.3	247	65.1	37.6	NA
102	Buffy coat	NA	500	32.9	2.2	238	65.4	37.9	NA
103	Buffy coat	NA	500	53.5	2.3	233	68.8	39.6	NA
104	Buffy coat	NA	500	107.4	2.2	236	64	39.1	NA
105	Buffy coat	NA	500	80.5	2.2	250	52.2	37	NA
106	Buffy coat	NA	500	46.9	2.3	258	52.2	36.9	NA
107	Buffy coat	NA	500	45.6	2.3	243	65.7	38	NA
108	Buffy coat	NA	500	57.3	2.3	241	65.8	38.2	NA
109	Buffy coat	NA	500	56.5	2.3	234	68.7	39.5	NA
110	Buffy coat	NA	500	35.6	2.3	241	65	37.8	NA
111	Buffy coat	NA	500	38.8	2.3	235	60.4	38.8	NA
112	Buffy coat	NA	500	54.3	2.3	235	66.2	39.3	NA
113	Buffy coat	NA	500	78.7	2.3	237	64.9	38.9	NA

eTable 4. Summary of Samples and WES Information.									
Patient ID	Source of DNA	Sample type^b	Input, ng	WES coverage	Ti/Tv ratio	Median insert size (bp)	Percentage bases on target	Capture fold enrichment	# of variants
114	Buffy coat	NA	500	100.3	2.2	234	64.3	39.2	NA
115	Buffy coat	NA	500	38.2	2.3	236	68.7	39.4	NA
116	Buffy coat	NA	500	43.2	2.3	252	65.5	37.6	NA
117	Buffy coat	NA	500	74.2	2.3	242	65.6	38.6	NA
118	Buffy coat	NA	500	53	2.3	237	65.8	38.9	NA
119	Buffy coat	NA	500	54.9	2.3	239	65.8	38.8	NA
120	Buffy coat	NA	500	83	2.3	247	65.9	38.2	NA
121	Buffy coat	NA	500	127	2.2	225	65.9	40	NA
122	Buffy coat	NA	500	37.3	2.3	240	66.3	38.3	NA
123	Buffy coat	NA	500	56.5	2.3	234	68.7	39.5	NA
124	Buffy coat	NA	500	93.2	2.3	238	64.8	39.1	NA
125	Buffy coat	NA	500	35	2.3	236	59.9	38.5	NA
126	Buffy coat	NA	500	48.1	2.3	239	65.5	38.7	NA
127	Buffy coat	NA	500	48.5	2.3	239	66.2	38.5	NA
128	Buffy coat	NA	500	105.8	2.3	234	64.5	39.2	NA
130	Buffy coat	NA	500	50.4	2.3	248	52.9	37.2	NA

^aSynchronous CRC were marked with S1 or S2

^bFF=fresh frozen, FFPE=formalin fixed paraffin embedded,

^cNA=Not applicable

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months) ^a	# ctDNA positive targets	Input DNA (ng)	VOF (mean)	VOF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
1	0	1.2	0	24.83	0	NA ^b	8	0
1	0	3.3	0	8.65	0	NA	8.6	0
1	0	5.8	0	35.18	0	NA	8.8	0
1	0	11.5	0	47.61	0	NA	9	0
1	0	15.9	0	7.57	0	NA	7	0
1	0	18.9	0	31.62	0	NA	9	0
1	0	21.7	0	57.27	0	NA	9.1	0
1	0	24.6	0	16.54	0	NA	8.7	0
1	0	28.0	0	33.61	0	NA	9.2	0
1	0	30.8	0	45.2	0	NA	8.1	0
1	0	35.0	0	21.18	0	NA	7.5	0
2	0	-0.1	12	5.56	0.00206	(0.001-0.0031)	7.6	0.52
2	0	1.2	0	28.88	0	NA	7.8	0
2	0	2.8	0	17.26	0	NA	7.7	0
2	0	5.8	0	21.39	0	NA	8.5	0
2	0	8.8	0	29.39	0	NA	8.1	0
2	0	11.3	0	46.8	0	NA	8.5	0
2	0	15.4	0	29.16	0	NA	9.3	0
2	0	18.7	0	27.85	0	NA	9.1	0
2	0	21.4	0	37.65	0	NA	8.5	0
2	0	24.5	0	28.8	0	NA	9	0
2	0	27.6	0	40.32	0	NA	8.8	0
2	0	30.6	0	47.2	0	NA	8	0
2	0	33.3	0	22.53	0	NA	9	0
2	0	35.2	0	31.45	0	NA	7.9	0
3	0	0.0	14	31.16	0.00217	(0.0014-0.0029)	4	5.74
3	0	1.4	0	66.0	0	NA	7.8	0
3	0	4.1	0	66.0	0	NA	8.2	0
3	0	6.2	0	66.0	0	NA	8	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months) ^a	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
3	0	9.5	0	66.0	0	NA	7.5	0
3	0	15.7	0	66.0	0	NA	8.6	0
3	0	18.1	0	50.34	0	NA	8.3	0
3	0	21.2	0	48.96	0	NA	8.9	0
3	0	24.1	0	66.0	0	NA	9.2	0
3	0	28.0	0	41.22	0	NA	7.7	0
3	0	30.9	0	37.6	0	NA	8	0
3	0	34.5	0	34.3	0	NA	8.5	0
3	0	37.8	0	50.41	0	NA	8.5	0
4	0	0.0	10	3.02	0.00170	(0.0011-0.0023)	2	0.93
4	0	1.2	0	45.18	0	NA	8	0
4	0	3.1	0	66.0	0	NA	8.3	0
4	0	5.8	0	66.0	0	NA	7.9	0
4	0	8.8	0	66.0	0	NA	8	0
4	0	11.9	0	62.18	0	NA	8	0
4	0	14.9	0	63.06	0	NA	8.5	0
4	0	17.7	0	60.1	0	NA	9	0
4	0	20.9	0	66.0	0	NA	8.9	0
4	0	24.5	0	56.46	0	NA	8.7	0
4	0	26.9	0	53.75	0	NA	8.7	0
4	0	30.1	0	54.0	0	NA	8.3	0
4	0	32.7	0	31.52	0	NA	8.7	0
4	0	35.6	0	37.34	0	NA	8	0
5	0	-0.1	16	66.0	0.01055	(0.0075-0.0136)	7.5	65.30
5	0	1.4	0	45.52	0	NA	7.2	0
5	0	3.3	0	27.32	0	NA	7.5	0
5	0	6.1	0	22.22	0	NA	8	0
5	0	9.2	0	33.08	0	NA	8	0
5	0	12.1	0	39.36	0	NA	8	0
5	0	14.8	0	26.77	0	NA	8	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months) ^a	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
5	0	17.7	0	26.32	0	NA	9.3	0
5	0	20.5	0	33.84	0	NA	8.5	0
5	0	23.8	0	33.26	0	NA	8	0
6	0	-0.1	15	63.09	0.00164	(0.0013-0.002)	8.5	4.14
6	0	1.9	0	54.04	0	NA	8	0
6	0	3.3	0	46.39	0	NA	8	0
6	0	6.0	0	53.2	0	NA	8	0
6	0	9.2	0	32.63	0	NA	9	0
6	0	11.4	0	36.96	0	NA	8.6	0
6	0	14.6	0	66.0	0	NA	9.5	0
6	0	18.0	0	62.07	0	NA	9.3	0
6	0	21.4	0	41.71	0	NA	9.2	0
6	0	24.2	0	53.2	0	NA	9.2	0
6	0	27.5	0	29.13	0	NA	8	0
6	0	31.3	0	50.38	0	NA	8.5	0
6	0	34.6	0	47.53	0	NA	8.6	0
7	0	-0.1	0	63.8	0	NA	8.2	0
7	0	1.2	0	51.85	0	NA	8.5	0
7	0	3.3	0	43.2	0	NA	9	0
7	0	6.2	0	22.92	0	NA	9.5	0
7	0	9.0	0	45.66	0	NA	8.5	0
7	0	12.0	0	51.24	0	NA	9.5	0
7	0	14.9	0	41.52	0	NA	8.8	0
7	0	17.9	0	35.2	0	NA	8.6	0
7	0	21.0	0	36.0	0	NA	8.2	0
7	0	23.9	0	33.02	0	NA	8.9	0
7	0	27.2	0	59.72	0	NA	8.1	0
7	0	30.2	0	29.19	0	NA	9	0
7	0	33.1	0	33.45	0	NA	9	0
8	0	0.9	0	1.22	0	NA	8	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months) ^a	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
8	0	3.0	0	66.0	0	NA	8.5	0
8	0	4.4	0	39.88	0	NA	9	0
8	0	7.6	0	29.39	0	NA	8.5	0
8	0	12.0	0	26.0	0	NA	9	0
8	0	14.7	0	34.54	0	NA	9	0
8	0	17.7	0	16.0	0	NA	9	0
8	0	20.8	0	19.16	0	NA	8.5	0
8	0	23.8	0	29.95	0	NA	7.5	0
8	0	27.1	0	34.8	0	NA	8	0
8	0	30.2	0	12.85	0	NA	8	0
8	0	33.4	0	43.6	0	NA	8.5	0
9	0	0.0	14	30.2	0.01623	(0.0106-0.0219)	8.5	19.83
9	0	0.9	0	66.0	0	NA	9	0
9	0	2.6	0	66.0	0	NA	9	0
9	0	5.2	0	66.0	0	NA	9	0
9	0	7.9	0	38.99	0	NA	8.5	0
9	0	11.5	0	66.0	0	NA	9.2	0
9	0	15.8	0	59.32	0	NA	10	0
9	0	19.2	0	42.8	0	NA	9	0
9	0	23.3	0	33.0	0	NA	8.5	0
9	0	26.9	0	47.5	0	NA	9	0
9	0	29.2	0	66.0	0	NA	8.5	0
9	0	32.7	0	66.0	0	NA	9	0
10	0	0.0	0	13.32	0	NA	8	0
10	0	2.8	0	66.0	0	NA	10	0
10	0	6.7	0	66.0	0	NA	9	0
10	0	10.2	0	66.0	0	NA	9.7	0
10	0	12.7	0	66.0	0	NA	8.7	0
10	0	16.2	0	66.0	0	NA	8.8	0
10	0	18.7	0	66.0	0	NA	8.9	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months) ^a	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
10	0	21.9	0	66.0	0	NA	9.3	0
10	0	24.7	0	63.6	0	NA	9	0
10	0	28.1	0	66.0	0	NA	9.4	0
11	0	-0.1	0	14.17	0	NA	8	0
11	0	1.4	0	21.98	0	NA	8	0
11	0	6.1	0	66.0	0	NA	9.3	0
11	0	8.6	0	66.0	0	NA	9.3	0
11	0	12.0	0	66.0	0	NA	8.5	0
11	0	14.8	0	66.0	0	NA	9.2	0
11	0	18.2	0	66.0	0	NA	8.9	0
11	0	21.2	0	66.0	0	NA	8.7	0
11	0	24.2	0	66.0	0	NA	8.5	0
11	0	26.9	0	66.0	0	NA	8.5	0
11	0	30.5	0	66.0	0	NA	9.1	0
12	0	0.0	5	42.63	0.00033	(0.0002-0.0004)	8.3	0.53
12	0	1.5	0	28.45	0	NA	9	0
12	0	3.1	0	35.5	0	NA	9.3	0
12	0	6.1	0	26.39	0	NA	8.3	0
12	0	9.3	0	23.67	0	NA	8.7	0
12	0	12.1	0	21.54	0	NA	9.2	0
13	0	0.0	9	33.8	0.00036	(0.0002-0.0005)	8.8	0.47
13	0	1.9	0	29.68	0	NA	9	0
13	0	5.8	0	64.89	0	NA	8.6	0
13	0	8.8	0	56.76	0	NA	8.2	0
13	0	12.7	0	66.0	0	NA	8.2	0
13	0	14.8	0	28.94	0	NA	9.5	0
13	0	18.0	0	42.6	0	NA	9	0
13	0	21.4	0	66.0	0	NA	8.6	0
13	0	24.4	0	48.69	0	NA	8.3	0
13	0	27.3	0	52.46	0	NA	8.7	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months) ^a	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
13	0	30.2	0	35.6	0	NA	8.7	0
14	0	0.0	14	30.07	0.00132	(0.0009-0.0017)	8	1.7
14	0	1.1	0	38.96	0	NA	8.6	0
14	0	3.6	0	31.47	0	NA	8.6	0
14	0	6.0	0	28.58	0	NA	9	0
14	0	8.9	0	25.51	0	NA	8.5	0
14	0	11.5	0	27.84	0	NA	7.3	0
14	0	15.0	0	41.57	0	NA	8.5	0
14	0	22.1	0	46.73	0	NA	8	0
14	0	25.3	0	57.21	0	NA	8.4	0
15	0	0.0	2	1.61	0.00761	(0.0015-0.0121)	8	0.47
15	0	1.5	0	3.62	0	NA	8	0
15	0	3.6	0	37.94	0	NA	8.4	0
15	0	5.8	0	25.76	0	NA	8	0
15	0	8.9	0	32.14	0	NA	8.9	0
15	0	12.0	0	21.03	0	NA	9	0
15	0	15.2	0	41.67	0	NA	8.7	0
15	0	18.7	0	43.74	0	NA	9	0
15	0	25.4	0	37.41	0	NA	8.6	0
15	0	29.7	0	35.74	0	NA	8.5	0
16	0	0.0	10	32.47	0.00042	(0.0003-0.0005)	9	0.46
16	0	2.1	0	31.13	0	NA	9	0
16	0	5.7	0	31.97	0	NA	9.4	0
16	0	8.5	0	36.34	0	NA	8.6	0
16	0	11.7	0	47.03	0	NA	8.6	0
16	0	15.4	0	37.2	0	NA	9	0
16	0	18.9	0	37.07	0	NA	7.5	0
16	0	21.8	0	28.0	0	NA	8.5	0
16	0	24.8	0	36.24	0	NA	8.2	0
16	0	27.8	0	43.67	0	NA	7.5	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months) ^a	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
16	0	30.8	0	42.21	0	NA	8.5	0
18	1	0.0	16	44.0	0.00259	(0.0013-0.0039)	8	4.9
18	1	1.4	0	36.15	0	NA	8	0
18	1	3.4	0	66.0	0	NA	9	0
18	1	7.3	8	66.0	0.00034	(0.0002-0.0005)	8	1.4
18	1	10.6	5	66.0	0.00037	(0.0001-0.0006)	9	1.4
18	1	13.7	0	66.0	0	NA	8.5	0
18	1	16.4	0	66.0	0	NA	9	0
18	1	19.6	0	65.73	0	NA	9	0
18	1	21.9	0	59.13	0	NA	8.5	0
18	1	27.4	0	66.0	0	NA	8.5	0
18	1	31.1	0	66.0	0	NA	8.5	0
19	0	0.0	14	66.0	0.00197	(0.0014-0.0026)	8.8	42
19	0	1.0	0	66.0	0	NA	8	0
19	0	2.7	0	66.0	0	NA	8.5	0
19	0	6.6	0	59.7	0	NA	9.5	0
19	0	8.8	0	66.0	0	NA	9	0
19	0	11.9	0	31.27	0	NA	7.5	0
19	0	15.4	0	66.0	0	NA	9.4	0
19	0	18.2	0	66.0	0	NA	8.1	0
19	0	21.4	0	66.0	0	NA	8.2	0
19	0	24.1	0	51.56	0	NA	8.3	0
19	0	26.8	0	58.0	0	NA	8.6	0
20	1	0.0	5	26.8	0.00026	(0.0002-0.0003)	8	0.29
20	1	0.8	0	21.31	0	NA	8	0
20	1	2.7	0	30.45	0	NA	8.4	0
20	1	6.4	0	36.27	0	NA	9.5	0
20	1	8.7	0	26.81	0	NA	8.7	0
20	1	11.7	0	28.52	0	NA	9	0
20	1	15.1	0	39.12	0	NA	8.3	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months) ^a	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
20	1	18.5	0	32.92	0	NA	8.5	0
20	1	21.1	0	66.0	0	NA	9	0
20	1	26.7	0	45.2	0	NA	9	0
21	0	0.0	13	17.12	0.00133	(0.0008-0.0019)	8	0.97
21	0	2.7	0	36.3	0	NA	8.8	0
21	0	6.1	0	60.19	0	NA	9.3	0
21	0	11.5	0	25.8	0	NA	8.2	0
21	0	15.6	0	37.12	0	NA	9.5	0
21	0	19.7	0	44.55	0	NA	9	0
21	0	24.0	0	33.05	0	NA	8.8	0
21	0	26.8	0	35.25	0	NA	8.8	0
22	0	0.0	2	1.51	0.00138	(0.0013-0.0015)	8	0.09
22	0	1.3	0	18.81	0	NA	8	0
22	0	2.2	0	14.65	0	NA	8	0
22	0	4.8	0	45.27	0	NA	7.8	0
22	0	8.2	0	59.92	0	NA	8.7	0
22	0	11.9	0	31.34	0	NA	9.4	0
22	0	14.4	0	29.39	0	NA	9.5	0
22	0	17.9	0	27.59	0	NA	8.9	0
22	0	20.6	0	31.2	0	NA	8.2	0
22	0	23.7	0	14.82	0	NA	8.4	0
22	0	26.7	0	22.68	0	NA	8.5	0
23	0	0.0	6	8.84	0.00141	(0.0005-0.0023)	8	0.53
23	0	1.1	0	7.93	0	NA	8	0
23	0	2.6	0	66.0	0	NA	9.2	0
23	0	5.8	0	53.91	0	NA	8.7	0
23	0	8.4	0	29.68	0	NA	8.9	0
23	0	11.4	0	28.8	0	NA	9.2	0
23	0	14.4	0	22.1	0	NA	9.1	0
23	0	17.2	0	43.42	0	NA	8.5	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months) ^a	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
23	0	20.1	0	22.22	0	NA	8.5	0
23	0	23.4	0	27.81	0	NA	8.4	0
23	0	26.6	0	39.14	0	NA	8	0
24	1	0.0	11	12.4	0.00088	(0.0006-0.0012)	8	0.47
24	1	1.0	0	5.96	0	NA	8	0
24	1	3.1	0	31.4	0	NA	9	0
24	1	5.7	0	26.97	0	NA	9.3	0
24	1	8.9	0	22.3	0	NA	9	0
24	1	12.3	0	22.44	0	NA	8.6	0
24	1	14.8	0	26.4	0	NA	9.2	0
24	1	17.8	0	15.81	0	NA	8.5	0
24	1	21.7	0	19.12	0	NA	8.3	0
24	1	25.8	0	25.91	0	NA	9	0
25	0	0.0	16	45.73	0.00657	(0.0044-0.0088)	8	13
25	0	1.2	0	57.58	0	NA	8.5	0
25	0	3.1	0	30.05	0	NA	9.5	0
25	0	6.1	0	30.67	0	NA	9.5	0
25	0	8.8	0	35.19	0	NA	9.6	0
25	0	10.5	0	38.06	0	NA	9	0
25	0	14.8	0	50.93	0	NA	9	0
25	0	17.8	0	29.92	0	NA	9	0
25	0	21.2	0	50.66	0	NA	8.8	0
25	0	24.8	0	42.39	0	NA	8.4	0
25	0	28.1	0	37.63	0	NA	8.7	0
26	0	0.0	16	66.0	0.00943	(0.0072-0.0117)	8	33
26	0	1.2	0	25.77	0	NA	8	0
26	0	3.6	0	48.66	0	NA	8.9	0
26	0	6.0	0	45.85	0	NA	9	0
26	0	9.0	0	66.0	0	NA	8.7	0
26	0	12.4	0	60.83	0	NA	8.4	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months) ^a	# ctDNA positive targets	Input DNA (ng)	VOF (mean)	VOF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
26	0	14.8	0	66.0	0	NA	9.5	0
26	0	18.2	0	66.0	0	NA	9	0
26	0	21.4	0	66.0	0	NA	8	0
26	0	24.1	0	66.0	0	NA	9	0
27	0	-0.1	14	18.4	0.00369	(0.002-0.0049)	8	2.7
27	0	1.0	0	21.71	0	NA	8	0
27	0	3.1	0	36.36	0	NA	9.2	0
27	0	6.0	0	26.0	0	NA	8.9	0
27	0	9.9	0	23.27	0	NA	9.5	0
27	0	12.0	0	23.02	0	NA	9.4	0
27	0	15.0	0	28.11	0	NA	8	0
27	0	18.0	0	29.88	0	NA	8.7	0
27	0	22.1	0	20.92	0	NA	8	0
27	0	25.3	0	30.0	0	NA	8.6	0
28	1	0.0	7	7.25	0.00069	(0.0005-0.001)	8	0.22
28	1	0.9	0	10.73	0	NA	8	0
28	1	2.8	0	66.0	0	NA	9.6	0
28	1	6.2	0	66.0	0	NA	8.9	0
28	1	9.7	0	66.0	0	NA	8.5	0
28	1	12.3	0	49.6	0	NA	8.3	0
28	1	15.2	13	22.1	0.00066	(0.0004-0.001)	8.5	0.62
28	1	18.0	16	44.93	0.00182	(0.0013-0.0024)	9.1	3.1
28	1	21.8	16	52.92	0.00777	(0.006-0.0095)	8.5	16
28	1	25.3	16	66.0	0.01076	(0.0086-0.0129)	8.3	43
29	1	0.0	0	6.74	0	NA	8	0
29	1	1.9	0	12.91	0	NA	8	0
29	1	3.4	0	39.36	0	NA	8.5	0
29	1	6.3	0	26.46	0	NA	9.3	0
29	1	10.9	11	33.2	0.00067	(0.0004-0.0009)	8.8	0.85
29	1	15.5	6	22.29	0.00041	(0.0002-0.0006)	8.4	0.38

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months) ^a	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
29	1	17.9	13	25.51	0.00095	(0.0007-0.0012)	8.3	1.0
29	1	22.1	4	33.52	0.00042	(0.0002-0.0006)	3.3	1.4
30	1	0.0	15	14.11	0.04070	(0.0315-0.05)	8	24
30	1	1.2	15	14.15	0.07946	(0.0645-0.0947)	8	47
30	1	3.0	5	66.0	0.00013	(0.0001-0.0002)	8.5	3.2
30	1	6.0	0	66.0	0	NA	9	0
30	1	9.4	14	66.0	0.00343	(0.0026-0.0043)	9	15
30	1	13.5	15	66.0	0.33035	(0.2524-0.4076)	9.3	2743
30	1	15.6	15	66.0	0.00118	(0.0009-0.0015)	9	4.5
30	1	18.6	15	66.0	0.04747	(0.0343-0.0608)	8.7	181
31	0	0.0	4	24.63	0.00035	(0.0003-0.0004)	8	0.36
31	0	1.1	0	19.17	0	NA	8	0
31	0	3.2	0	66.0	0	NA	9.2	0
31	0	6.0	0	66.0	0	NA	8.3	0
31	0	9.3	0	66.0	0	NA	9.1	0
31	0	12.1	0	66.0	0	NA	8.8	0
31	0	15.1	0	66.0	0	NA	9.1	0
31	0	18.2	0	66.0	0	NA	8.3	0
31	0	21.3	0	49.92	0	NA	8.2	0
31	0	25.0	0	43.5	0	NA	8.2	0
33	0	0.0	9	12.73	0.00992	(0.0025-0.0174)	6	7.2
33	0	1.1	7	16.8	0.00718	(0.0024-0.01)	8	4.4
33	0	2.8	0	58.2	0	NA	8	0
33	0	5.6	0	54.16	0	NA	9	0
33	0	8.7	0	39.02	0	NA	9	0
33	0	9.9	0	51.56	0	NA	8	0
33	0	11.7	0	47.92	0	NA	9.5	0
33	0	15.3	0	40.8	0	NA	8.4	0
33	0	18.3	0	32.01	0	NA	8.5	0
33	0	21.9	0	29.17	0	NA	8.7	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months) ^a	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
34	1	0.0	12	34.4	0.00049	(0.0004-0.0006)	9.3	0.62
34	1	1.2	0	31.75	0	NA	9.2	0
34	1	2.6	0	41.44	0	NA	8.5	0
34	1	5.3	0	34.79	0	NA	8.5	0
34	1	8.5	0	35.36	0	NA	9	0
34	1	11.2	0	66.0	0	NA	8.9	0
34	1	14.0	7	29.6	0.00051	(0.0004-0.0007)	8.4	0.61
34	1	17.1	14	31.76	0.00185	(0.0015-0.0022)	8.5	2.4
34	1	20.5	14	42.63	0.08891	(0.0768-0.1009)	8.5	152
35	0	0.0	12	66.0	0.00042	(0.0003-0.0005)	9.4	1.5
35	0	0.9	0	57.65	0	NA	8.1	0
35	0	2.0	0	37.32	0	NA	8.7	0
35	0	6.1	0	66.0	0	NA	9	0
35	0	9.0	0	45.58	0	NA	9	0
35	0	11.9	0	40.29	0	NA	8.5	0
35	0	15.1	0	55.17	0	NA	8.8	0
35	0	18.3	0	56.49	0	NA	9	0
36	0	0.0	15	62.06	0.00098	(0.0006-0.0013)	8.9	2.3
36	0	1.0	0	66.0	0	NA	9.5	0
36	0	3.0	0	66.0	0	NA	9.5	0
36	0	6.3	0	66.0	0	NA	9.2	0
36	0	8.9	0	65.38	0	NA	9	0
36	0	11.8	0	51.1	0	NA	8.7	0
36	0	14.7	0	41.29	0	NA	8.9	0
36	0	18.0	0	66.0	0	NA	9	0
37	1	0.0	16	49.2	0.00582	(0.0043-0.0073)	8.8	11
37	1	1.7	16	36.8	0.00440	(0.0035-0.0053)	9.5	5.8
37	1	3.0	16	14.44	0.01216	(0.0091-0.0154)	9.2	6.53
37	1	5.8	16	55.16	0.00609	(0.0044-0.0078)	8.4	14
37	1	9.0	0	32.66	0	NA	9	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months) ^a	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
37	1	12.2	0	29.99	0	NA	8.6	0
37	1	15.2	0	29.62	0	NA	8.5	0
37	1	18.5	0	31.6	0	NA	8.6	0
38	0	0.0	7	4.29	0.00147	(0.0007-0.0022)	8.8	0.25
38	0	1.0	0	30.25	0	NA	9	0
38	0	4.5	0	27.15	0	NA	8.5	0
38	0	6.5	0	36.41	0	NA	9	0
38	0	9.4	0	29.05	0	NA	8.9	0
38	0	12.1	0	20.8	0	NA	8.5	0
38	0	16.8	0	16.79	0	NA	8.3	0
39	0	0.0	16	40.4	0.00508	(0.0041-0.0061)	9.6	7.3
39	0	1.0	0	66.0	0	NA	8.9	0
39	0	3.9	0	49.73	0	NA	9.5	0
39	0	6.5	0	66.0	0	NA	9.1	0
39	0	9.7	0	49.02	0	NA	8.5	0
39	0	12.3	0	50.25	0	NA	9	0
39	0	15.9	0	39.6	0	NA	8.5	0
39	0	18.7	0	36.58	0	NA	7.9	0
40	0	0.0	10	41.6	0.00027	(0.0002-0.0004)	9.2	0.40
40	0	0.9	0	48.23	0	NA	9	0
40	0	3.3	0	66.0	0	NA	9.2	0
40	0	6.2	0	66.0	0	NA	9.2	0
40	0	9.4	0	66.0	0	NA	9	0
40	0	11.8	0	66.0	0	NA	9.3	0
41	0	0.0	16	35.1	0.00819	(0.0061-0.0103)	9	11
41	0	0.5	0	54.27	0	NA	9	0
41	0	3.0	0	54.62	0	NA	9	0
41	0	6.2	0	28.8	0	NA	8.5	0
41	0	11.8	0	28.23	0	NA	8.8	0
42	1	0.0	10	27.29	0.00060	(0.0005-0.0007)	7.3	0.76

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months) ^a	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
42	1	2.5	0	66.0	0	NA	7.8	0
43	0	-0.1	16	27.63	0.00226	(0.0016-0.003)	6.8	3.1
43	0	2.6	0	33.39	0	NA	7.5	0
44	0	0.0	2	39.8	0.00026	NA	4	0.78
44	0	1.8	0	37.67	0	NA	8	0
45	0	0.0	0	1.0	0	NA	8	0
45	0	2.3	0	44.8	0	NA	9	0
46	0	-0.1	4	8.4	0.00182	(0.0008-0.0025)	4	1.2
46	0	1.7	0	36.56	0	NA	9.4	0
47	0	0.0	16	66.0	0.00815	(0.0061-0.0102)	9.1	23
47	0	3.4	0	45.97	0	NA	7.3	0
48	0	0.0	0	6.8	0	NA	8	0
48	0	0.4	0	4.64	0	NA	8	0
49	0	0.0	2	42.4	0.00019	(0.0002-0.0002)	8	0.33
49	0	0.4	0	66.0	0	NA	7.5	0
50	0	0.0	12	41.96	0.00150	(0.0008-0.0022)	8.8	2.4
50	0	0.5	0	66.0	0	NA	9.1	0
51	0	0.0	16	28.4	0.01376	(0.0101-0.0175)	8	17
51	0	0.5	0	66.0	0	NA	8.2	0
52	0	-0.3	15	66.0	0.00112	(0.0008-0.0014)	8.5	4.7
52	0	0.6	0	45.2	0	NA	8	0
53	0	0.0	15	62.4	0.00561	(0.0044-0.0069)	9.2	13
53	0	0.2	0	66.0	0	NA	8.5	0
54	0	0.0	16	66.0	0.00155	(0.0012-0.002)	9.6	19
54	0	0.5	0	66.0	0	NA	8.8	0
55	0	0.0	5	65.26	0.00059	(0.0001-0.0011)	8.8	1.5
55	0	0.4	0	66.0	0	NA	9	0
57	0	0.0	7	46.2	0.00095	(0.0004-0.0015)	9	1.7
57	0	0.9	0	66.0	0	NA	9.5	0
58	0	0.0	8	61.6	0.00025	(0.0002-0.0003)	8.5	0.63

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months) ^a	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
58	0	1.0	0	66.0	0	NA	8.2	0
59	0	0.0	0	10.8	0	NA	9	0
59	0	0.9	0	66.0	0	NA	9.3	0
60	0	0.0	12	50.8	0.00043	(0.0003-0.0006)	9.4	0.79
60	0	0.9	0	66.0	0	NA	9.4	0
61	0	0.0	10	33.46	0.00090	(0.0006-0.0012)	8.5	1.2
61	0	0.6	0	65.6	0	NA	8.8	0
62	0	-0.3	8	2.37	0.00168	(0.0005-0.0029)	8	0.17
62	0	0.7	5	9.77	0.00063	(0.0004-0.0008)	8	0.26
62	0	2.8	0	66.0	0	NA	8.6	0
62	0	6.0	0	43.39	0	NA	8.5	0
62	0	9.0	0	28.95	0	NA	8.7	0
62	0	12.5	0	31.6	0	NA	7	0
62	0	15.1	0	21.27	0	NA	8.6	0
62	0	17.7	0	27.62	0	NA	8.2	0
62	0	21.1	0	41.97	0	NA	8.6	0
62	0	23.7	0	50.25	0	NA	9	0
62	0	26.7	0	31.62	0	NA	8.5	0
62	0	29.7	0	37.6	0	NA	8.5	0
63	0	-0.2	12	13.09	0.00926	(0.0052-0.0134)	8	5.2
63	0	0.9	0	66.0	0	NA	8	0
63	0	3.5	0	55.57	0	NA	9	0
63	0	5.6	0	47.87	0	NA	9.5	0
63	0	9.2	0	43.09	0	NA	8.8	0
63	0	12.4	0	61.6	0	NA	8.7	0
63	0	15.0	0	31.29	0	NA	8.7	0
63	0	18.2	0	43.22	0	NA	8.2	0
63	0	21.0	0	51.11	0	NA	8.8	0
63	0	23.5	0	66.0	0	NA	8.5	0
63	0	26.6	0	45.19	0	NA	7.8	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months) ^a	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
63	0	30.1	0	42.4	0	NA	8.2	0
64	0	-0.2	3	2.94	0.00133	(0.0009-0.0017)	8	0.16
64	0	0.7	0	8.26	0	NA	8	0
64	0	3.1	0	66.0	0	NA	8.4	0
64	0	6.0	0	66.0	0	NA	9.5	0
64	0	9.6	0	43.65	0	NA	8.5	0
64	0	11.8	0	45.8	0	NA	8.5	0
64	0	15.5	0	28.37	0	NA	8.3	0
64	0	18.0	0	52.38	0	NA	8.7	0
64	0	21.3	0	59.28	0	NA	8	0
64	0	24.0	0	56.33	0	NA	8.4	0
64	0	26.6	0	41.2	0	NA	8	0
64	0	30.0	0	30.33	0	NA	8	0
65	0	-0.3	8	10.4	0.00135	(0.0007-0.002)	8	0.60
65	0	0.7	0	12.86	0	NA	8	0
65	0	2.9	0	66.0	0	NA	9	0
65	0	6.0	0	66.0	0	NA	9	0
65	0	8.8	0	66.0	0	NA	9	0
65	0	11.6	0	41.77	0	NA	8.7	0
66	0	-0.2	3	3.49	0.00318	(0-0.008)	8	0.54
66	0	0.6	0	14.88	0	NA	8	0
66	0	3.0	0	66.0	0	NA	8.8	0
66	0	6.0	0	43.8	0	NA	8.7	0
66	0	9.0	0	42.25	0	NA	9	0
66	0	11.8	0	27.01	0	NA	9.1	0
66	0	15.2	0	32.46	0	NA	8.5	0
66	0	18.2	0	38.0	0	NA	8	0
66	0	21.0	0	24.79	0	NA	8.5	0
66	0	24.0	0	27.48	0	NA	8.8	0
66	0	27.4	0	35.25	0	NA	8	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months) ^a	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
66	0	30.2	0	51.16	0	NA	8.4	0
67	0	-0.3	10	23.24	0.00064	(0.0004-0.0008)	8	0.63
67	0	0.9	0	23.2	0	NA	8	0
67	0	2.8	0	66.0	0	NA	8.6	0
67	0	5.7	0	66.0	0	NA	9	0
67	0	8.7	0	66.0	0	NA	8.5	0
67	0	11.5	0	55.68	0	NA	8.5	0
67	0	15.0	0	66.0	0	NA	8.3	0
67	0	18.0	0	66.0	0	NA	8	0
67	0	20.8	0	66.0	0	NA	8.5	0
67	0	24.0	0	66.0	0	NA	8.6	0
67	0	27.6	0	66.0	0	NA	8	0
67	0	30.0	0	66.0	0	NA	8.5	0
68	1	-0.2	14	15.9	0.00142	(0.0008-0.002)	8	0.96
68	1	1.0	0	13.93	0	NA	8	0
68	1	3.1	6	19.33	0.00044	(0.0003-0.0006)	8.9	0.32
68	1	6.1	2	31.81	0.00030	NA	8.5	0.34
68	1	9.4	11	31.08	0.00075	(0.0005-0.0009)	9	0.86
68	1	12.0	16	40.71	0.00218	(0.0017-0.0026)	8.8	3.4
68	1	15.0	16	35.09	0.01345	(0.0106-0.0164)	8.8	18
68	1	18.4	16	25.56	0.00420	(0.0029-0.0055)	8.5	4.3
68	1	21.6	16	8.02	0.00516	(0.0037-0.0065)	8.5	1.7
69	0	-0.2	8	12.35	0.00065	(0.0004-0.0008)	8	0.31
69	0	0.9	0	5.37	0	NA	8	0
69	0	3.0	0	66.0	0	NA	8.2	0
69	0	6.0	0	66.0	0	NA	8.7	0
69	0	8.9	0	40.17	0	NA	8.6	0
69	0	12.0	0	33.33	0	NA	8.5	0
69	0	15.2	0	34.23	0	NA	8.5	0
69	0	18.0	0	30.15	0	NA	8.3	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months) ^a	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
69	0	21.0	0	50.25	0	NA	8.5	0
69	0	23.9	0	43.27	0	NA	8	0
69	0	27.1	0	50.93	0	NA	8	0
70	0	-0.3	3	19.69	0.00048	(0.0001-0.0009)	8	0.41
70	0	0.9	0	36.35	0	NA	8	0
70	0	3.0	0	66.0	0	NA	8.5	0
70	0	6.1	0	66.0	0	NA	8.7	0
70	0	8.9	0	42.8	0	NA	8.5	0
70	0	12.0	0	26.12	0	NA	8.4	0
70	0	15.0	0	34.62	0	NA	8.5	0
70	0	18.1	0	37.67	0	NA	8.5	0
70	0	20.9	0	42.63	0	NA	8.4	0
71	0	-0.2	2	1.65	0.01366	(0-0.0294)	8	0.78
71	0	0.7	0	12.28	0	NA	8	0
71	0	3.3	0	66.0	0	NA	8.6	0
71	0	6.2	0	66.0	0	NA	8.4	0
71	0	9.2	0	52.1	0	NA	9.5	0
71	0	12.1	0	63.2	0	NA	9	0
71	0	14.7	0	66.0	0	NA	8.8	0
71	0	18.4	0	48.83	0	NA	8	0
71	0	21.0	0	40.4	0	NA	8.5	0
71	0	23.9	0	47.73	0	NA	8	0
71	0	26.4	0	66.0	0	NA	8.3	0
72	0	-0.4	16	26.13	0.00127	(0.001-0.0015)	9	1.2
72	0	0.8	0	1.64	0	NA	8	0
72	0	3.3	0	22.71	0	NA	9	0
72	0	6.0	0	35.07	0	NA	8.9	0
72	0	9.0	0	29.06	0	NA	9	0
72	0	12.1	0	23.14	0	NA	8.3	0
72	0	15.1	0	26.86	0	NA	8.9	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months) ^a	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
72	0	18.0	0	28.95	0	NA	8.7	0
72	0	21.0	0	40.03	0	NA	8.5	0
72	0	24.0	0	30.8	0	NA	9	0
73	0	-0.3	0	12.68	0	NA	8	0
73	0	0.8	0	15.2	0	NA	8	0
73	0	2.9	0	66.0	0	NA	9.4	0
73	0	5.9	0	66.0	0	NA	8.4	0
73	0	8.4	0	66.0	0	NA	8.8	0
73	0	12.1	0	66.0	0	NA	8.5	0
73	0	15.3	0	62.03	0	NA	8.6	0
73	0	18.1	0	66.0	0	NA	8	0
73	0	21.4	0	66.0	0	NA	8.5	0
73	0	24.1	0	61.87	0	NA	8.7	0
74	0	-0.2	2	30.66	0.00053	(0-0.0011)	8	0.69
74	0	0.5	0	20.8	0	NA	8	0
74	0	3.7	0	60.49	0	NA	8.7	0
74	0	6.2	0	52.95	0	NA	8	0
74	0	9.5	0	63.08	0	NA	8.5	0
74	0	12.2	0	66.0	0	NA	8.3	0
74	0	14.8	0	59.77	0	NA	8.2	0
74	0	18.0	0	66.0	0	NA	8	0
74	0	21.4	0	56.84	0	NA	8.5	0
75	1	-0.2	14	15.81	0.02285	(0.008-0.0379)	8	16
75	1	0.5	0	66.0	0	NA	8	0
75	1	3.2	15	26.0	0.10138	(0.0395-0.1637)	4	225
75	1	5.8	15	66.0	0.11723	(0.0519-0.1829)	8.7	311
75	1	8.6	15	66.0	0.20991	(0.122-0.2986)	8	857
75	1	11.7	15	66.0	0.42629	(0.3125-0.5401)	8	8240
75	1	14.7	0	34.88	0	NA	6.5	0
75	1	18.2	0	32.87	0	NA	8.2	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months) ^a	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
75	1	20.3	2	27.53	0.00036	(0.0003-0.0004)	8	0.43
75	1	24.4	0	60.4	0	NA	8	0
76	0	-0.2	0	1.0	0	NA	8	0
76	0	0.9	0	36.5	0	NA	8	0
76	0	3.0	0	66.0	0	NA	8	0
76	0	5.9	0	66.0	0	NA	8	0
76	0	8.2	0	66.0	0	NA	8.8	0
76	0	12.2	0	66.0	0	NA	9	0
76	0	14.9	0	66.0	0	NA	8.4	0
76	0	17.7	0	66.0	0	NA	8.5	0
76	0	20.5	0	66.0	0	NA	8.5	0
76	0	23.3	0	66.0	0	NA	8.4	0
77	1	-0.2	2	26.27	0.00065	(0.0003-0.001)	8	0.73
77	1	0.7	0	56.18	0	NA	8	0
77	1	3.2	0	66.0	0	NA	8.6	0
77	1	5.7	0	66.0	0	NA	8.9	0
77	1	8.9	0	43.65	0	NA	8.5	0
77	1	12.2	0	61.7	0	NA	9.1	0
77	1	14.8	0	66.0	0	NA	7.5	0
77	1	17.7	2	66.0	0.00014	(0.0001-0.0002)	9	2.00
77	1	20.5	0	66.0	0	NA	8	0
78	0	-0.2	9	66.0	0.00034	(0.0002-0.0005)	8.6	1.6
78	0	0.8	0	66.0	0	NA	8.3	0
78	0	2.6	0	66.0	0	NA	8.6	0
78	0	6.0	0	47.6	0	NA	8.6	0
78	0	9.1	0	40.4	0	NA	8.5	0
78	0	12.7	0	43.6	0	NA	8.2	0
78	0	15.9	0	33.6	0	NA	8.8	0
78	0	18.5	0	40.4	0	NA	8.3	0
79	1	-0.1	15	10.4	0.00870	(0.0062-0.0112)	8.2	3.8

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months) ^a	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
79	1	0.6	15	25.6	0.00299	(0.002-0.0039)	8.5	3.1
79	1	3.4	13	31.6	0.00124	(0.0008-0.0017)	8.2	1.6
79	1	6.4	15	40.0	0.00330	(0.0025-0.0042)	8.5	5.3
79	1	9.2	15	24.8	0.03044	(0.0217-0.0393)	8.9	29
79	1	12.2	15	30.8	0.04347	(0.0306-0.0565)	8	57
79	1	15.7	15	25.6	0.14889	(0.1118-0.1863)	7.8	167
79	1	17.8	6	30.8	0.00051	(0.0001-0.0009)	8	0.68
80	0	-0.2	16	56.49	0.00112	(0.0008-0.0015)	9	2.4
80	0	0.9	0	66.0	0	NA	9	0
80	0	3.1	0	66.0	0	NA	8.9	0
80	0	5.7	0	66.0	0	NA	8.8	0
80	0	9.0	2	66.0	0.00024	(0.0002-0.0003)	9	0.75
80	0	12.0	0	45.17	0	NA	8.9	0
80	0	15.2	0	48.37	0	NA	8.5	0
80	0	18.0	0	62.02	0	NA	8.5	0
81	0	-0.2	5	66.0	0.00023	(0.0001-0.0003)	8.7	0.82
81	0	0.6	0	66.0	0	NA	8.4	0
81	0	3.1	0	66.0	0	NA	8.1	0
81	0	5.9	0	66.0	0	NA	8.7	0
81	0	8.9	0	66.0	0	NA	7.8	0
81	0	12.0	0	66.0	0	NA	8.9	0
81	0	14.7	0	66.0	0	NA	8.4	0
81	0	17.8	0	66.0	0	NA	9.5	0
82	1	-0.3	16	13.6	0.00880	(0.0066-0.011)	8	5.1
82	1	0.4	13	25.55	0.00291	(0.0022-0.0036)	9	2.8
82	1	3.4	14	45.41	0.00104	(0.0008-0.0013)	8.9	1.8
82	1	7.3	15	26.58	0.00804	(0.0063-0.0098)	8.5	8.5
82	1	11.3	15	21.6	0.03572	(0.0277-0.0437)	8.6	31
83	0	-0.2	15	44.4	0.00050	(0.0003-0.0007)	8	0.93
83	0	0.5	0	47.6	0	NA	8.5	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months) ^a	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
83	0	2.8	0	66.0	0	NA	9	0
83	0	6.0	0	66.0	0	NA	8.7	0
83	0	9.2	0	56.8	0	NA	8.5	0
83	0	12.6	0	66.0	0	NA	8.5	0
83	0	14.8	0	50.8	0	NA	8.2	0
84	0	-0.2	15	66.0	0.00423	(0.0031-0.0054)	8	39
84	0	0.9	0	66.0	0	NA	8.5	0
84	0	2.9	0	53.2	0	NA	8.7	0
84	0	6.4	0	40.0	0	NA	8.5	0
84	0	8.9	0	50.0	0	NA	8	0
84	0	15.0	0	37.6	0	NA	8	0
85	1	-0.2	12	27.2	0.00512	(0.0034-0.0069)	8.2	5.8
85	1	0.4	11	66.0	0.00635	(0.0048-0.0079)	8.6	18
85	1	3.2	11	60.08	0.01166	(0.009-0.0144)	7.3	33
85	1	6.6	11	66.0	0.09014	(0.073-0.1078)	8	729
85	1	8.7	11	66.0	0.39998	(0.3217-0.4803)	8	4347
85	1	12.2	11	66.0	0.53680	(0.4215-0.6526)	9.5	70790
86	0	-0.2	16	52.0	0.02194	(0.0175-0.0265)	9.1	43
86	0	0.3	0	66.0	0	NA	10	0
87	0	-0.2	16	35.85	0.00378	(0.0033-0.0043)	8.4	5.5
87	0	0.5	0	49.6	0	NA	8.5	0
88	0	-0.2	8	13.52	0.00207	(0.0012-0.0029)	9	1.1
88	0	0.5	0	31.41	0	NA	9	0
89	1	-0.2	0	66.0	0	NA	8.6	0
89	1	0.5	0	66.0	0	NA	9.3	0
90	0	-0.1	2	62.0	0.00210	(0-0.006)	8.3	0.26
90	0	0.3	0	66.0	0	NA	9.1	0
91	0	-0.4	16	66.0	0.00173	(0.0012-0.0023)	8.5	6.4
91	0	0.3	0	66.0	0	NA	8.5	0
92	1	-0.3	13	66.0	0.00896	(0.0061-0.0119)	9.4	22

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months) ^a	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
92	1	0.4	0	66.0	0	NA	8.8	0
93	0	-0.2	14	29.6	0.00110	(0.0008-0.0014)	8.5	1.3
93	0	0.5	0	49.2	0	NA	9	0
95	0	-0.2	10	39.18	0.00064	(0.0004-0.0009)	8.9	0.86
95	0	0.3	0	66.0	0	NA	8.5	0
96	0	-0.2	14	50.4	0.00057	(0.0004-0.0007)	9	1.1
96	0	0.4	0	66.0	0	NA	9	0
97	0	-0.2	10	20.8	0.00096	(0.0006-0.0011)	8	0.78
97	0	0.4	0	66.0	0	NA	8	0
98	0	-0.2	0	51.2	0	NA	9	0
98	0	0.4	0	66.0	0	NA	8.2	0
99	1	-0.2	13	66.0	0.00065	(0.0002-0.0011)	8.4	2.2
99	1	0.3	0	66.0	0	NA	9.1	0
100	0	-0.2	7	23.6	0.00046	(0.0003-0.0007)	8.4	0.45
100	0	0.3	0	66.0	0	NA	8.7	0
101	0	-0.2	16	66.0	0.00292	(0.0021-0.0038)	8.5	9.7
101	0	0.4	0	66.0	0	NA	8.2	0
102	0	-0.3	13	44.0	0.00044	(0.0003-0.0006)	8.4	0.78
102	0	0.4	0	66.0	0	NA	8.6	0
103	1	-0.1	16	33.58	0.03621	(0.0219-0.0505)	8.5	49
103	1	0.3	0	66.0	0	NA	8.8	0
104	1	-0.1	16	16.29	0.00220	(0.0017-0.0027)	8.5	1.5
104	1	0.4	4	28.15	0.00043	(0.0003-0.0006)	8.8	0.47
105	0	-0.2	16	34.0	0.00861	(0.0059-0.0114)	8.5	12
105	0	0.4	0	66.0	0	NA	8.7	0
106	0	-0.2	0	33.2	0	NA	8.5	0
106	0	0.5	0	66.0	0	NA	9	0
107	0	-0.3	5	35.2	0.00045	(0.0002-0.0007)	8.5	0.65
107	0	0.5	0	66.0	0	NA	8.7	0
108	1	-0.4	3	66.0	0.00041	(0.0001-0.0007)	9	1.5

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months) ^a	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
108	1	0.3	0	66.0	0	NA	8.8	0
109	0	-0.1	6	36.04	0.00068	(0.0003-0.0011)	9	0.93
109	0	0.3	0	66.0	0	NA	8.9	0
110	0	-0.2	0	62.8	0	NA	8.7	0
110	0	0.6	0	63.6	0	NA	8.8	0
111	0	0.0	16	49.47	0.00542	(0.0023-0.0086)	8	12
111	0	3.1	0	66.0	0	NA	8	0
111	0	5.8	0	66.0	0	NA	8	0
111	0	9.3	0	41.78	0	NA	9	0
111	0	11.8	0	41.17	0	NA	8.5	0
111	0	15.5	0	53.71	0	NA	8.2	0
111	0	18.3	0	51.93	0	NA	8.8	0
111	0	21.0	0	39.2	0	NA	8	0
111	0	27.5	0	21.96	0	NA	8.5	0
112	0	0.0	3	25.32	0.00045	(0.0003-0.0006)	8	0.47
112	0	0.9	0	14.55	0	NA	8	0
112	0	3.1	0	65.59	0	NA	8	0
112	0	6.3	0	66.0	0	NA	8	0
112	0	11.2	0	46.05	0	NA	8.5	0
112	0	15.7	0	66.0	0	NA	7.9	0
112	0	18.2	0	34.65	0	NA	8	0
112	0	21.6	0	25.46	0	NA	7.8	0
112	0	27.2	0	42.97	0	NA	8	0
113	0	0.0	16	40.69	0.00320	(0.0022-0.0043)	8	5.6
113	0	1.2	0	9.51	0	NA	8	0
113	0	3.9	0	37.2	0	NA	8.8	0
113	0	6.7	0	52.0	0	NA	8.5	0
113	0	9.5	0	56.4	0	NA	8.3	0
113	0	11.6	0	51.2	0	NA	8.3	0
113	0	15.0	0	60.8	0	NA	7.8	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months) ^a	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
113	0	18.2	0	43.6	0	NA	8	0
113	0	21.3	0	52.8	0	NA	9	0
114	0	-0.2	4	13.59	0.00513	NA	8	2.6
114	0	0.7	0	19.37	0	NA	8	0
114	0	3.2	0	66.0	0	NA	8.8	0
114	0	6.0	0	54.4	0	NA	7.8	0
114	0	9.0	0	52.24	0	NA	8	0
114	0	12.7	0	65.62	0	NA	8.6	0
114	0	15.2	0	66.0	0	NA	8.5	0
114	0	18.0	0	57.84	0	NA	6.5	0
115	0	0.0	12	66.0	0.00067	(0.0003-0.001)	8.8	1.9
115	0	0.7	0	56.82	0	NA	8.5	0
115	0	2.9	0	49.39	0	NA	7.8	0
115	0	5.7	0	60.52	0	NA	8	0
115	0	8.5	0	54.15	0	NA	7.7	0
115	0	11.9	0	59.2	0	NA	8.5	0
115	0	15.1	0	66.0	0	NA	8.3	0
115	0	18.1	0	66.0	0	NA	8.5	0
116	0	-0.1	10	55.23	0.00076	(0.0006-0.0009)	9	1.6
116	0	0.5	0	66.0	0	NA	8.5	0
116	0	3.5	0	50.35	0	NA	7.5	0
116	0	6.5	0	66.0	0	NA	8	0
116	0	9.3	0	66.0	0	NA	9	0
116	0	12.0	0	66.0	0	NA	8.5	0
116	0	15.0	0	59.45	0	NA	7.5	0
116	0	18.2	0	65.2	0	NA	9	0
117	0	-0.1	6	30.74	0.00064	(0.0002-0.0011)	7.5	0.89
117	0	0.7	0	66.0	0	NA	4.3	0
118	0	0.0	14	27.2	0.00142	(0.0007-0.0021)	8.6	1.51
118	0	0.9	0	63.6	0	NA	8.5	0

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months) ^a	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
118	0	3.0	0	66.0	0	NA	8.4	0
118	0	5.7	0	66.0	0	NA	8.8	0
118	0	9.0	0	66.0	0	NA	6.5	0
118	0	12.4	0	61.2	0	NA	7	0
118	0	15.1	0	66.0	0	NA	8.2	0
119	1	-0.1	15	59.6	0.00118	(0.0009-0.0015)	8.5	2.8
119	1	0.9	12	66.0	0.00054	(0.0003-0.0007)	8.5	4.3
119	1	3.5	6	50.4	0.00026	(0.0002-0.0004)	8.2	0.53
119	1	6.2	0	66.0	0	NA	8.4	0
119	1	11.1	8	66.0	0.00022	(0.0002-0.0003)	8.7	0.61
119	1	13.4	13	60.8	0.00075	(0.0005-0.001)	9	1.7
120	0	0.0	14	17.6	0.00654	(0.0044-0.0087)	8	4.9
120	0	0.5	0	14.0	0	NA	8.1	0
120	0	3.4	0	66.0	0	NA	7	0
120	0	6.4	0	66.0	0	NA	8.2	0
120	0	9.0	0	19.6	0	NA	8.5	0
121	0	0.0	0	66.0	0	NA	9.2	0
121	0	0.8	0	66.0	0	NA	7.1	0
122	0	0.0	11	66.0	0.00060	(0.0004-0.0008)	8.4	7.7
122	0	0.7	0	66.0	0	NA	8.5	0
123	0	0.0	5	42.13	0.00054	(0.0001-0.001)	8.5	0.53
123	0	0.4	0	66.0	0	NA	8.5	0
123	0	3.0	0	66.0	0	NA	8.1	0
123	0	6.2	0	66.0	0	NA	8.5	0
123	0	9.2	0	35.58	0	NA	7.8	0
123	0	11.7	0	48.02	0	NA	8.5	0
124	1	0.0	16	66.0	0.04668	(0.0396-0.0542)	9	375
124	1	0.5	13	48.87	0.00074	(0.0005-0.0009)	8	1.5
124	1	3.1	7	43.62	0.00054	(0.0003-0.0007)	8.4	0.91
124	1	5.8	13	39.78	0.00063	(0.0005-0.0008)	9	0.96

eTable 5. ctDNA Results for All 795 Plasma Samples

Patient ID	Recurrence	Time post surgery (months) ^a	# ctDNA positive targets	Input DNA (ng)	VAF (mean)	VAF CI 95%	Plasma (mL)	ctDNA copies per mL plasma
124	1	8.8	16	51.85	0.03123	(0.026-0.0367)	8.5	65
124	1	12.0	16	44.17	0.23820	(0.2037-0.2736)	9	398
125	1	-0.2	15	32.16	0.00140	(0.0011-0.0017)	8.5	1.8
125	1	0.7	0	38.8	0	NA	7.5	0
126	0	-0.2	13	51.65	0.00772	(0.0028-0.0131)	8.2	17
126	0	0.5	0	44.72	0	NA	7.6	0
127	0	0.0	15	66.0	0.00136	(0.0008-0.002)	9.1	12
127	0	0.6	0	66.0	0	NA	8.3	0
128	0	21.3	0	62.4	0	NA	8.5	0
128	0	24.0	0	42.4	0	NA	8.5	0
128	0	27.0	0	62.4	0	NA	8.3	0
128	0	30.0	0	44.8	0	NA	8.4	0
128	0	33.2	0	57.2	0	NA	8.4	0
128	0	36.6	0	46.0	0	NA	8.1	0
130	0	0.0	14	50.48	0.00099	(0.0006-0.0014)	9.6	1.9
130	0	0.4	2	66.0	0.00019	(0-0.0005)	9.8	0.98

^aA negative value indicates that the blood was drawn on a day prior to surgery. A null value indicates that the sampling timepoint is on the same day as surgery, but prior to surgery.

^bNot applicable

eTable 6. Recurrence-Free Survival Analysis by Clinicopathological Variables and Post-op ctDNA Status at Day 30							
Variable	Univariate analysis			Multivariate analysis			
	HR	(95% CI)	P-value	HR	(95% CI)	P-value	Schoenfeld P-value
All patients with a day 30 postoperative sample (n = 94)							
Age							
< mean versus ≥ Mean	1.4	(0.52-3.8)	.51				
Stage							
Stage II versus stage III	5.3	(1.2-23.0)	.028	2.4	(0.48-12.2)	.29	
Tumor site							
Right versus left	1.9	(0.72-5.3)	.19				
Lymphovascular invasion							
No versus yes	2.7	(1.0-7.0)	.044	1.9	(0.63-5.6)	.26	
MMR-status							
Deficient versus proficient	0.45	(0.059-3.4)	.43				
Radical resection (micro)							
Yes versus no	2.3	(0.72-7.2)	.16				
Histology							
Adeno- versus mucinouscarcinoma	1.6	(0.35-7.2)	.53				
Tumor differentiation							
Medium/well versus poor	0.86	(0.19-3.8)	.85				
Gender							
Female versus male	0.24	(0.079-0.74)	.013^a	.21	(0.06-0.69)	.010	
ctDNA status up to 6 weeks after OP (no ACT)							
ctDNA- versus ctDNA+	7.2	(2.7-19.0)	<.001	4.5	(1.6-12.8)	.004	
Global goodness-of fit test for Cox proportional hazards models							.22
^a The present consecutive cohort unexpectedly has an extreme high proportion of relapse events among women 28.8% (15/52) and an a low proportion among men 12.3% (9/73). In the Danish population the overall relapse rates in women and men, in the period from 2001 to 2011, were 22% (2233/9958) and 25% (2803/11194), respectively. ¹³ Hence, the association between female sex and relapse observed in the present cohort is likely false.							

eTable 7. Recurrence-Free Survival Analysis by Clinicopathological Variables, Post-op ctDNA, and Post-op CEA Status at First Timepoint Post-ACT

Variable	Univariate analysis			Multivariate analysis			
	HR	(95% CI)	P-value	HR	(95% CI)	P-value	Schoenfeld P-value
All patients with longitudinally collected plasma and ACT (n=58)							
Age							
< mean versus ≥ Mean	1.3	(0.44-3.8)	.61				
Stage							
Stage II versus stage III	1.3	(0.17-10.0)	.79				
Tumor site							
Right versus left	1.8	(0.63-5.3)	.27				
Lymphovascular invasion							
No versus yes	3.0	(0.93-9.5)	.066				
MMR-status							
Deficient versus proficient	1.1	(0.0-Inf)	>.99				
Radical resection (micro)							
Yes versus no	2.6	(0.77-9)	.13				
Histology							
Adeno- versus mucinouscarcinoma	1.1	(.14-8.3)	.94				
Tumor differentiation							
Medium/well versus poor	1.2	(0.27-5.6)	.78				
Tumor perforation							
No versus yes	0.91	(0.12-7)	.93				
Gender							
Female versus male	0.2	(0.055-0.71)	.013^a	0.37	(0.09-1.5)	.16	
CEA							
CEA- versus CEA+	2.4	(0.74-7.9)	.14				
ctDNA							
ctDNA- versus ctDNA+	17.5	(5.4-56.5)	<.001	11.8	(3.4-40.8)	<.001	
Global goodness-of fit test for Cox proportional hazards models							.80

^aThe present consecutive cohort unexpectedly has an extreme high proportion of relapse events among women 28.8% (15/52) and an a low proportion among men 12.3% (9/73). In the Danish population the overall relapse rates in women and men, in the period from 2001 to 2011, were 22% (2233/9958) and 25% (2803/11194), respectively.¹³ Hence, the association between female sex and relapse observed in the present cohort is likely false.

eTable 8. Matched Tumor and Metastatic WES			
	Patient ID		
	20 ^a	24 ^a	77 ^b
Mutations present in tumor also present in the metastasis (%)	62.6	50.0	79.4
Mutations screened in plasma also present in the metastasis (%)	100.0	87.5	93.8

^aNo ctDNA detected

^bctDNA detected after relapse

eTable 9. Recurrence-Free Survival Analysis by Clinicopathological Variables and Post-op ctDNA and CEA Status in Surveillance Samples

Variable	Univariate analysis			Multivariate analysis			Schoenfeld P-value
	HR	(95% CI)	P-value	HR	(95% CI)	P-value	
All patients with longitudinally collected plasma (n=75)							
Age							
< mean versus ≥ Mean	1.0	(0.39-2.8)	.95				
Stage							
Stage II versus stage III	3.3	(0.74-15)	.12				
Tumor site							
Right versus left	1.2	(0.45-3.2)	.72				
Lymphovascular invasion							
No versus yes	4.1	(1.4-12)	.010	1.1	(0.35-3.4)	.87	
MMR-status							
Deficient versus proficient	1.2	(0.0-Inf)	>.99				
Radical resection (micro)							
Yes versus no	2.9	(0.88-9.3)	.080				
Histology							
Adeno- versus mucinous carcinoma	0.84	(.84-6.4)	.87				
Tumor differentiation							
Medium/well versus poor	0.92	(0.21-4.1)	.91				
Tumor perforation							
No versus yes	1.1	(0.15-8.7)	.89				
Gender							
Female versus male	0.25	(0.08-0.8)	.018 ^a	0.94	(0.28-3.2)	.92	
CEA							
CEA- versus CEA+	2.8	(0.95-8.0)	.061				
ctDNA							
ctDNA- versus ctDNA+	43.5	(9.8-193.5)	<.001	39.9	(7.5-211.0)	<.001	
Global goodness-of fit test for Cox proportional hazards models							.84

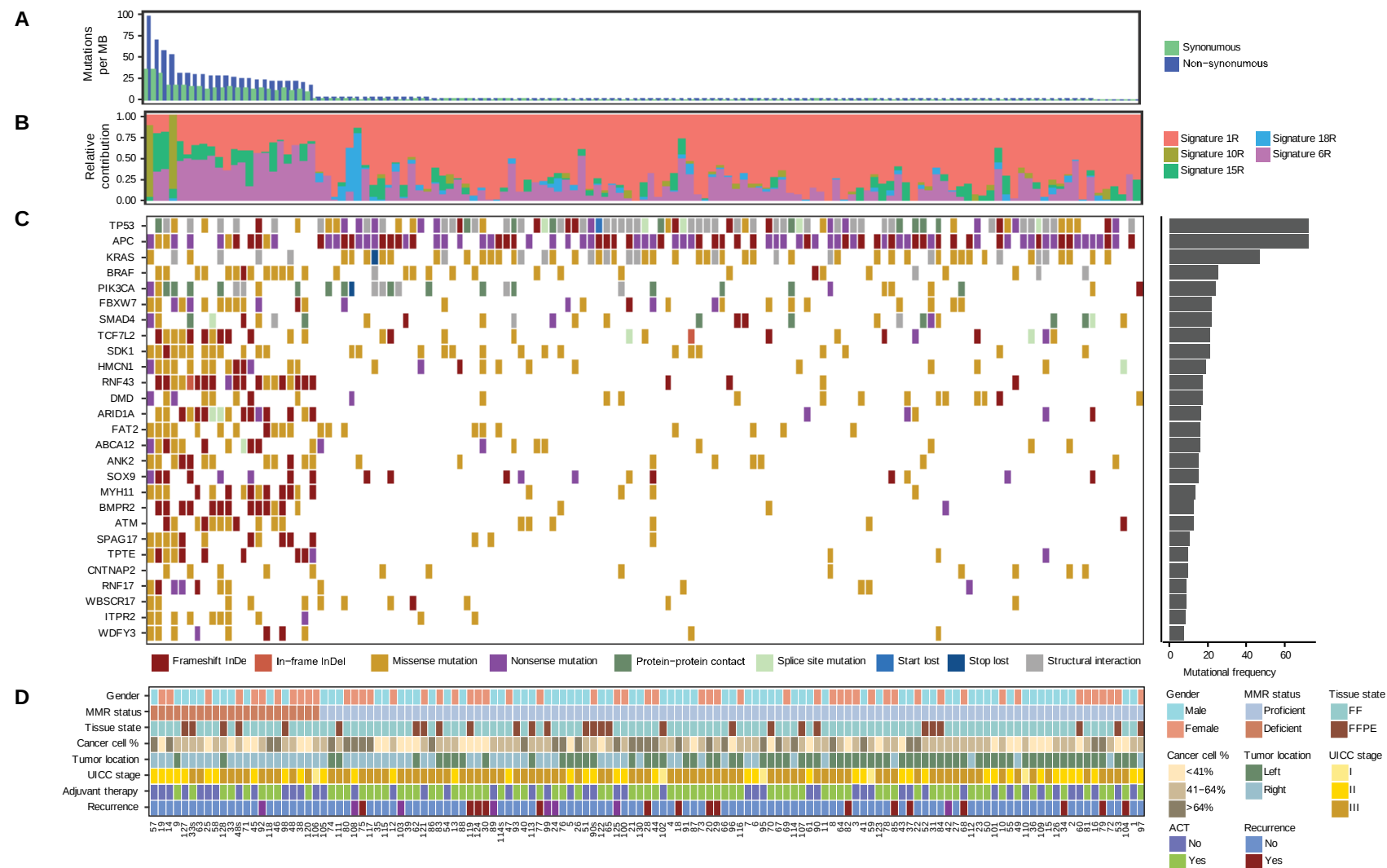
^aThe present consecutive cohort unexpectedly has an extreme high proportion of relapse events among women 28.8% (15/52) and an a low proportion among men 12.3% (9/73). In the Danish population the overall relapse rates in women and men, in the period from 2001 to 2011, were 22% (2233/9958) and 25% (2803/11194), respectively.¹³ Hence, the association between female sex and relapse observed in the present cohort is likely false.

eTable 10. Patients With Actionable Mutations Detected in the Primary Tumor

Pt. ID	Chr	Gene	POS (Hg19)	SNP ID	Nucleotide	Alt. nucleotide	POS change	Aminoacid change	Cancer type	Drug	Database	ctDNA+ longitudinal plasma
42	chr14	<i>AKT1</i>	105246551	rs121434592	C	T	c.49G>A	p.Glu17Lys	Solid Tumors	AZD5363	https://www.mycancergenome.org/	
42	chr7	<i>BRAF</i>	140453136	rs113488022	A	T	c.1799T>A	p.Val600Glu	CRC	RTK-Inhibitor+Trametinib	http://oncokb.org/#/actionableGenes	
18	chr12	<i>KRAS</i>	25398284	rs121913529	C	T	c.35G>A	p.Gly12Asp	All Tumors	GDC-0994, KO-947, LY3214996	http://oncokb.org/#/actionableGenes	Yes
20	chr12	<i>KRAS</i>	25398284	rs121913529	C	T	c.35G>A	p.Gly12Asp	All Tumors	GDC-0994, KO-947, LY3214996	http://oncokb.org/#/actionableGenes	
20	chr10	<i>PTEN</i>	89692981	.	T	TG	c.988dupG	p.Glu330fs	CRC	GSK2636771 & AZD8186	http://oncokb.org/#/actionableGenes	
68	chr3	<i>PIK3CA</i>	178936091	rs104886003	G	A	c.1633G>A	p.E545K	All Tumors	AZD8186	https://www.mycancergenome.org/	Yes
68	chr12	<i>KRAS</i>	25398284	rs121913529	C	T	c.35G>A	p.Gly12Asp	All Tumors	GDC-0994, KO-947, LY3214996	http://oncokb.org/#/actionableGenes	Yes
24	chr12	<i>KRAS</i>	25398284	rs121913529	C	A	c.35G>T	p.Gly12Val	CRC	GDC-0994, KO-947, LY3214996	http://oncokb.org/#/actionableGenes	
24	chr18	<i>SMAD4</i>	48604706	.	G	T	c.1528G>T	p.Gly510*	All Tumors		https://www.mycancergenome.org/	
29	chr12	<i>KRAS</i>	25398281	rs112445441	C	T	c.38G>A	p.Gly13Asp	CRC	GDC-0994, KO-947, LY3214996	http://oncokb.org/#/actionableGenes	Yes
30	chr3	<i>PIK3CA</i>	178936082	.	G	A	c.1624G>A	E542K	CRC/Breast cancer	Aspirin/PI3K inhibitors	https://www.mycancergenome.org/	Yes
30	chr12	<i>KRAS</i>	25398284	rs121913529	C	A	c.35G>T	p.Gly12Val	CRC/Breast cancer	GDC-0994, KO-947, LY3214996	http://oncokb.org/#/actionableGenes	Yes
30	chr18	<i>SMAD4</i>	48591928	rs377767350	T	G	c.1091T>G	p.Leu364Trp	CRC		https://www.mycancergenome.org/	Yes
75	chr17	<i>ERBB2</i>	37880261	.	G	T	c.2305G>T	p.Asp769Tyr	All Tumors	Neratinib/afatinib	https://civcdb.org	Yes
75	chr17	<i>ERBB2</i>	37881000	rs121913471	G	T	c.2329G>T	p.Val777Leu	All Tumors	Neratinib/afatinib	https://civcdb.org	Yes
77	chr7	<i>BRAF</i>	140453136	rs113488022	A	T	c.1799T>A	p.Val600Glu	CRC	RTK-Inhibitor+Trametinib	http://oncokb.org/#/actionableGenes	Yes
37	chr12	<i>KRAS</i>	25398285	rs121913530	C	T	c.34G>A	p.Gly12Ser	CRC	GDC-0994, KO-947, LY3214996	http://oncokb.org/#/actionableGenes	Yes
89	chr12	<i>KRAS</i>	25398285	rs121913530	C	A	c.34G>T	p.Gly12Cys	CRC	GDC-0994, KO-947, LY3214996	http://oncokb.org/#/actionableGenes	
125	chr12	<i>KRAS</i>	25398284	rs121913529	C	A	c.35G>T	p.Gly12Val	CRC	GDC-0994, KO-947, LY3214996	http://oncokb.org/#/actionableGenes	
85	chr3	<i>PIK3CA</i>	178938934	.	G	A	c.2176G>A	p.Glu726Lys	CRC	Aspirin/PI3K inhibitors	https://www.mycancergenome.org/	Yes
85	chr12	<i>KRAS</i>	25398285	rs121913530	C	A	c.34G>T	p.Gly12Cys	All Tumors	GDC-0994, KO-947, LY3214996	http://oncokb.org/#/actionableGenes	Yes
119	chr12	<i>KRAS</i>	25398285	rs121913530	C	T	c.34G>A	p.Gly12Ser	CRC	GDC-0994, KO-947, LY3214996	http://oncokb.org/#/actionableGenes	Yes

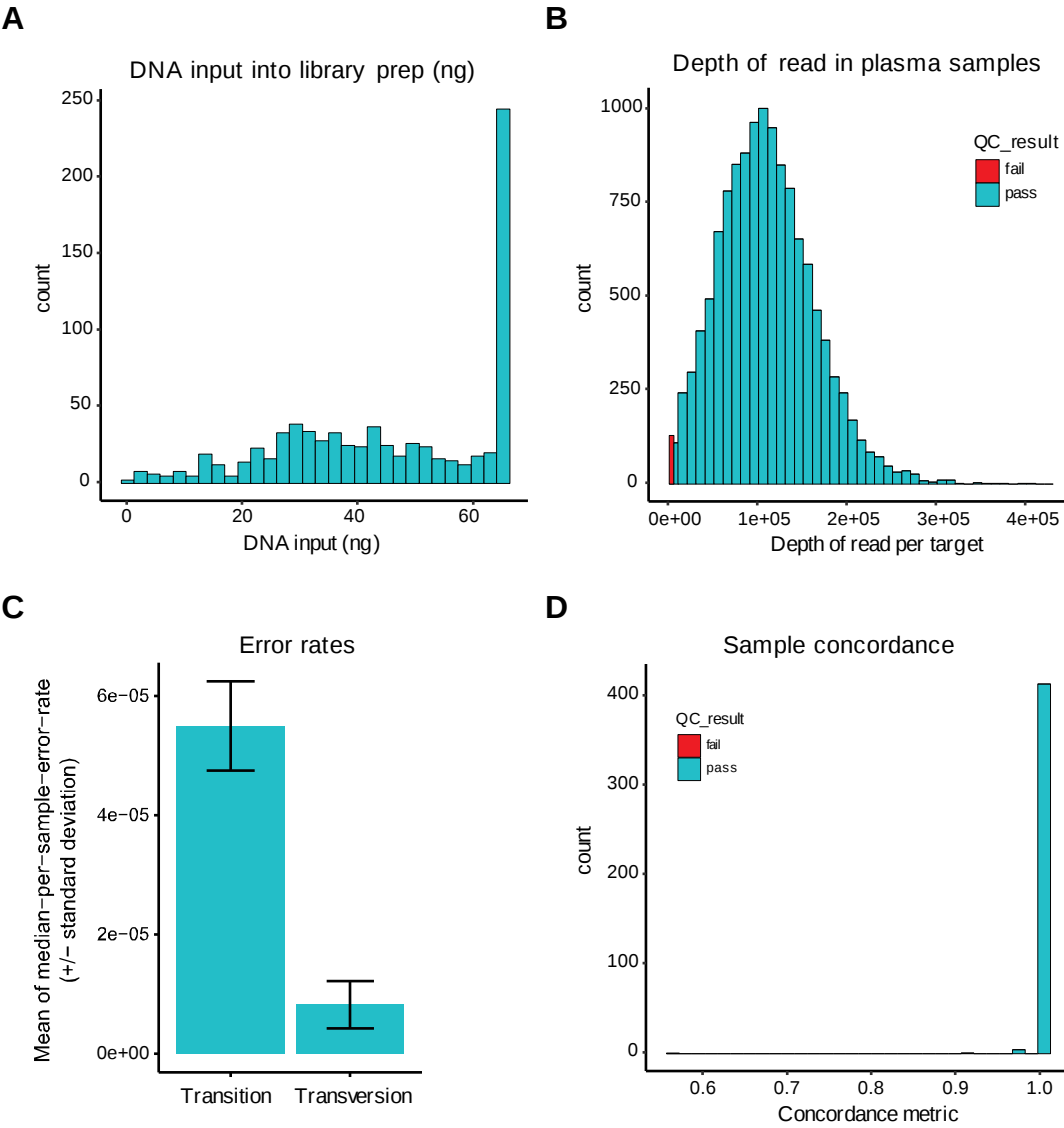
99	chr12	KRAS	25380278	.	A	T	c.180T>A	p.Gly60Gly	CRC	GDC-0994, KO-947, LY3214997	http://oncokb.org/#!/actionableGenes	
99	chr12	KRAS	25380277	rs121913238	G	T	c.181C>A	p.Gln61Lys	CRC	GDC-0994, KO-947, LY3214998	http://oncokb.org/#!/actionableGenes	
104	chr18	SMAD4	48604701	.	G	C	c.1523G>C	p.Gly508Ala	CRC		https://www.mycancergenome.org/	Yes
124	chr7	BRAF	140453136	rs113488022	A	T	c.1799T>A	p.Val600Glu	CRC	RTK- Inhibitor+Trametinib	http://oncokb.org/#!/actionableGenes	Yes
108	chr3	PIK3CA	178936094	rs121913286	C	A	c.1636C>A	Q546K	CRC	Aspirin/PI3K inhibitors	https://www.mycancergenome.org/	
108	chr12	KRAS	25398285	rs121913530	C	A	c.34G>T	p.Gly12Cys	CRC	GDC-0994, KO-947, LY3214996	http://oncokb.org/#!/actionableGenes	

eFigure 1. Summary of Clinical, Histopathological, and Molecular Parameters for All 125 Patients. A) Rate of synonymous and non-synonymous mutations called from WES. B) The relative contribution of the five most prevalent colorectal cancer associated mutational signatures. C) Mutations in frequently mutated genes in colorectal cancer.¹¹ D) Clinical and histopathological characteristics.

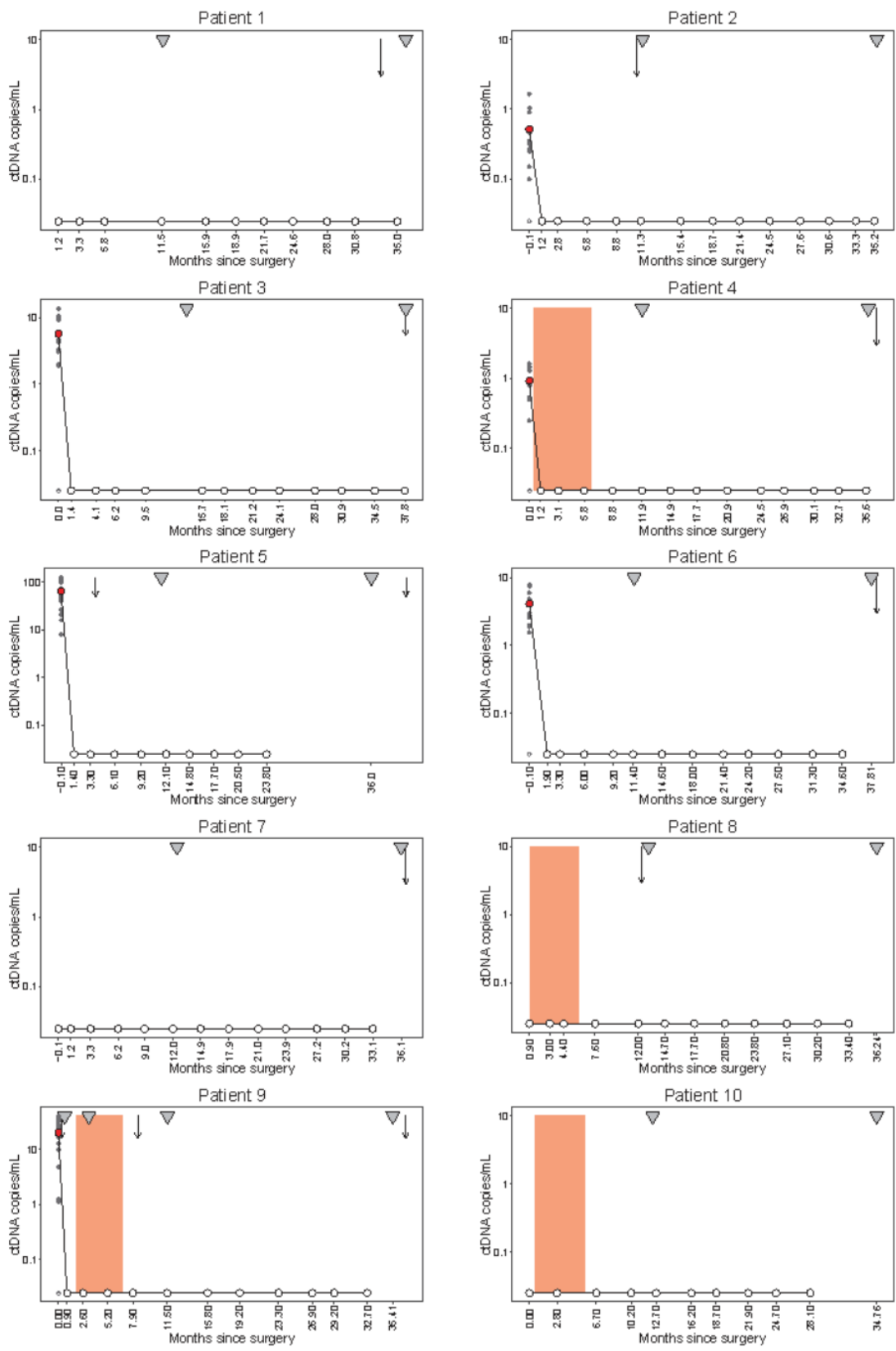


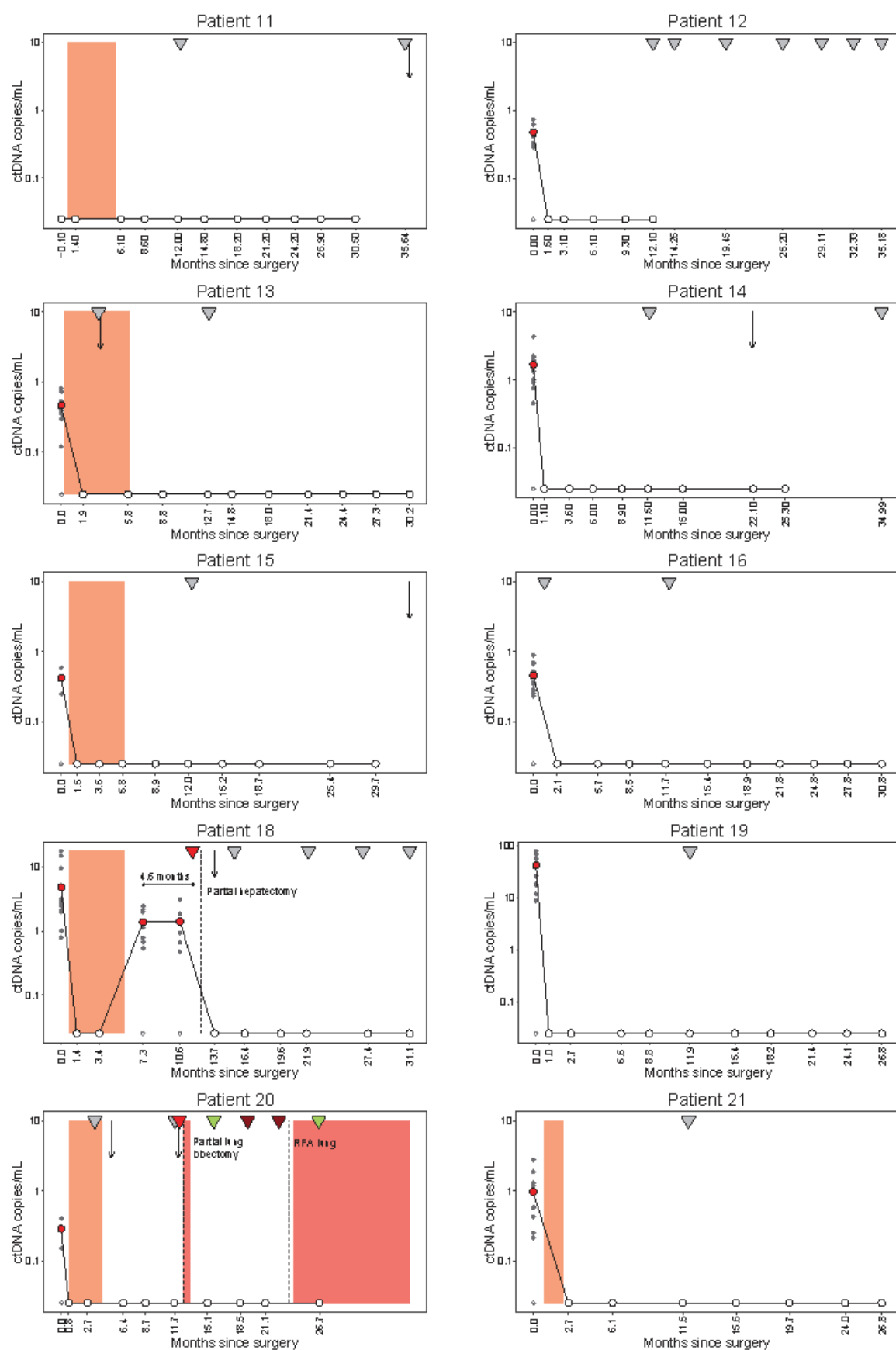
eFigure 2. Quality Control Metrics for cfDNA Sequencing Using Multiplex PCR NGS.

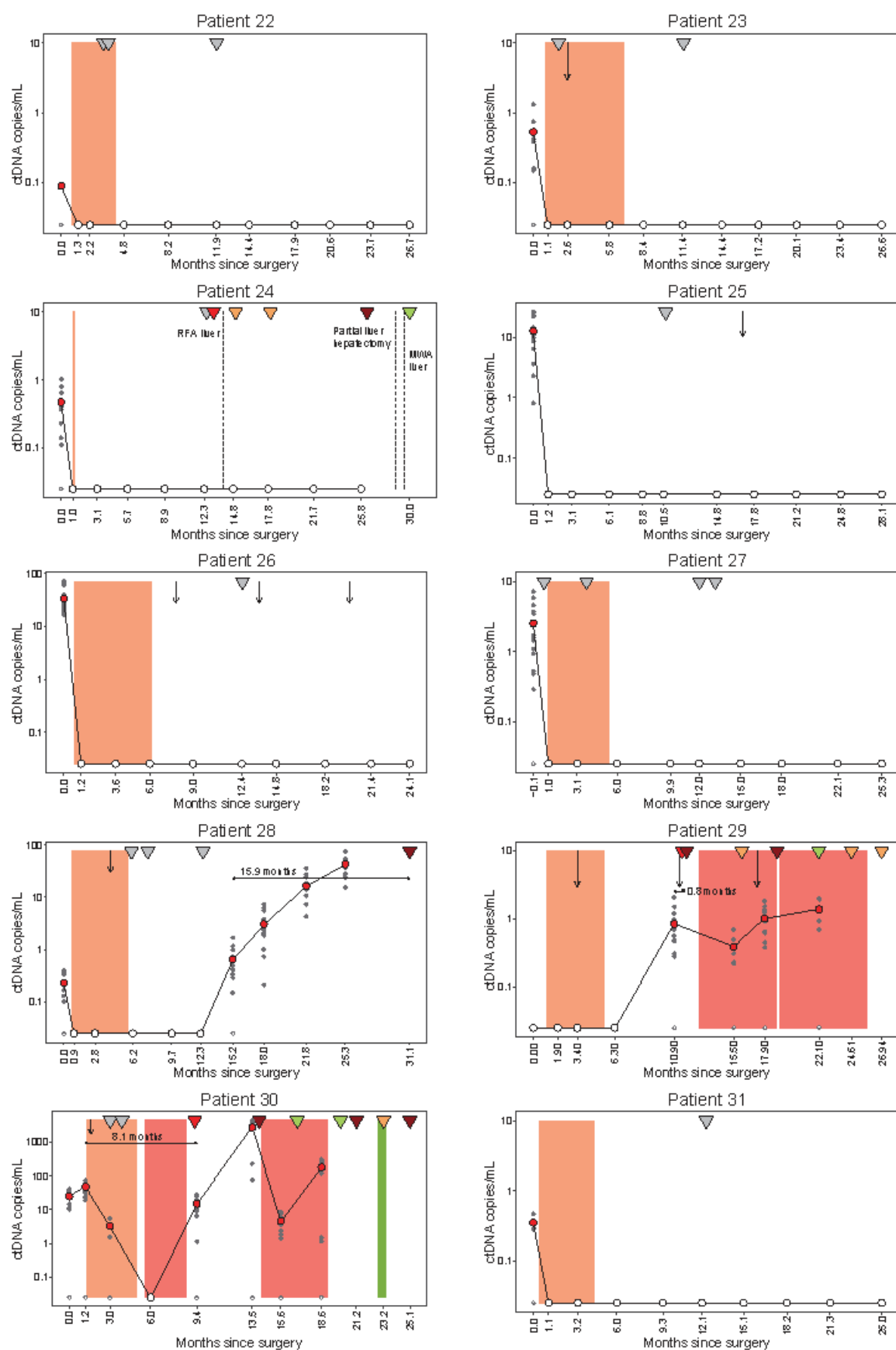
A) DNA input into NGS libraries. A maximum of 66ng was used for library preparation (approximately 20,000 genome equivalents). B) read depth of amplicons. Amplicons with coverage less than 5000x were excluded from analyses and samples with less than 8 passing amplicons (out of 16) failed sequencing coverage QC. C) mean error rates. D) Sequencing sample concordance between plasma samples from the same patient as well as the corresponding tissue biopsy sample. All plasma samples from the relapse patients were tested and no mixup was identified.

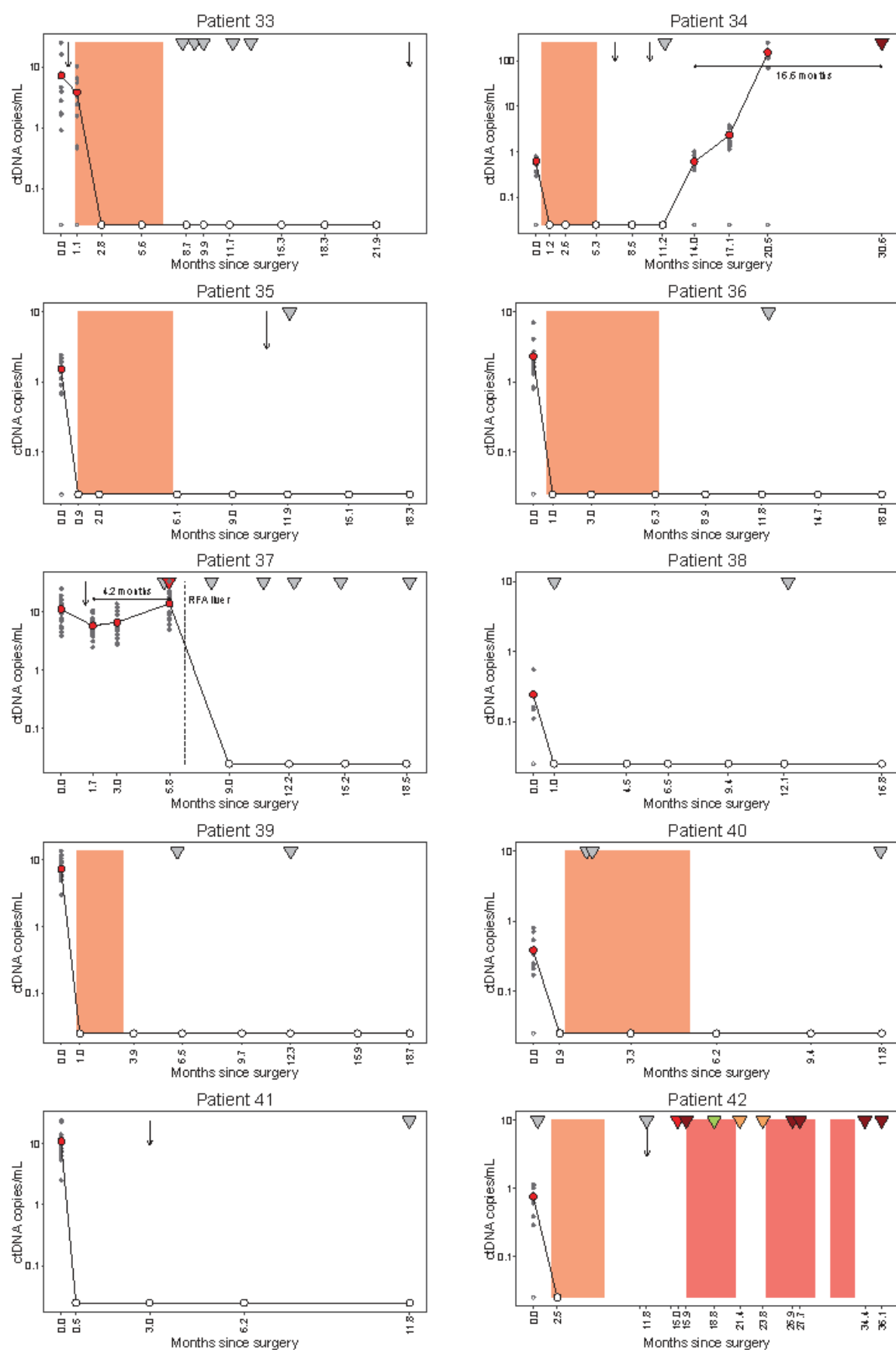


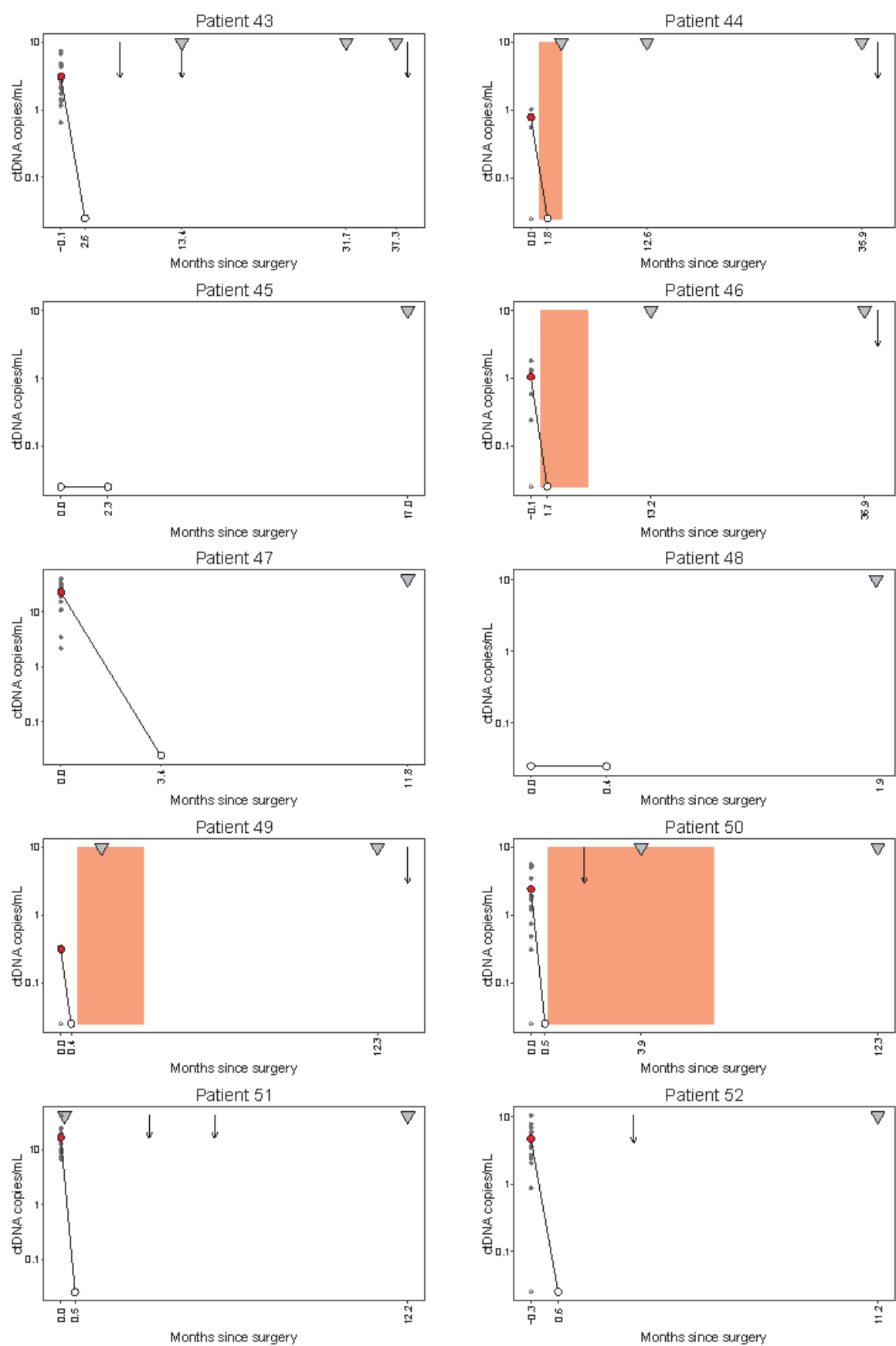
eFigure 3. Detailed ctDNA Results and Disease Course Information. Representation of detailed disease courses, applied treatments and longitudinal ctDNA analyses for all 125 patients. ctDNA is represented as copies/mL plasma for each mutation individually and as a mean of all detected mutations.

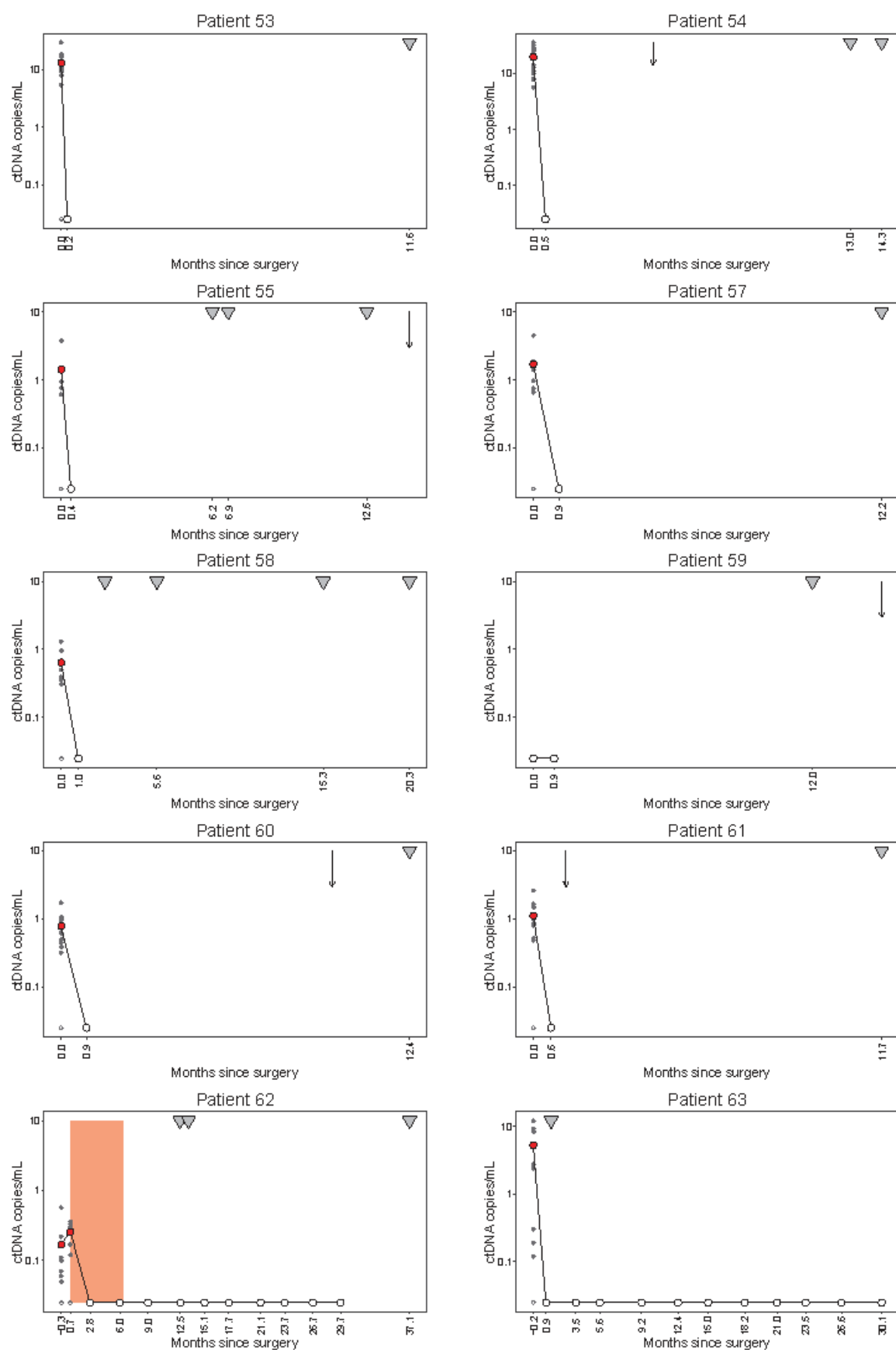


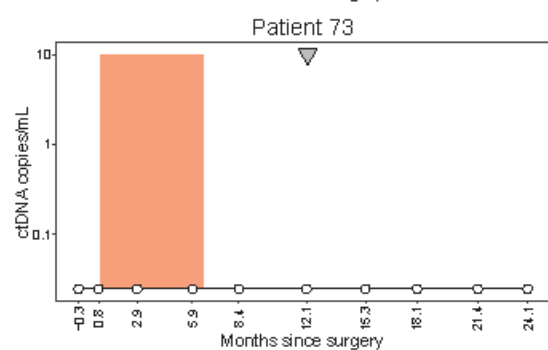
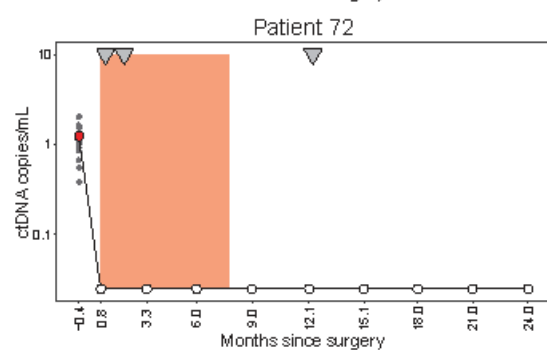
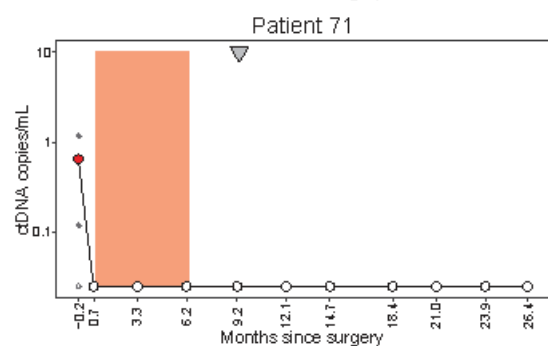
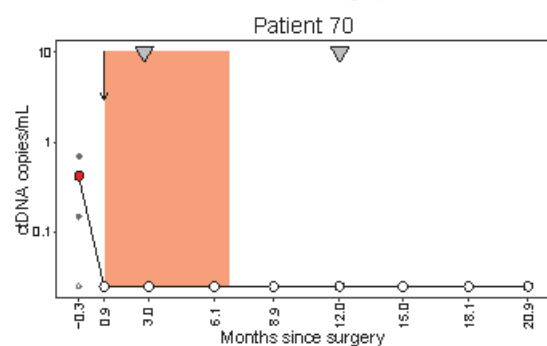
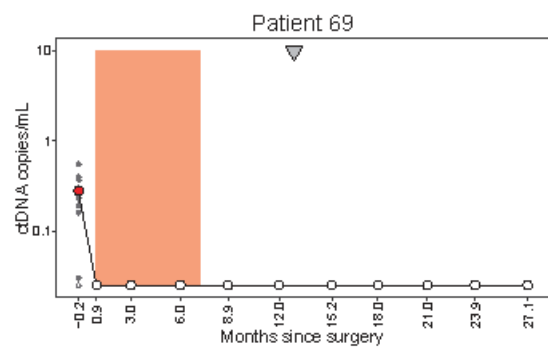
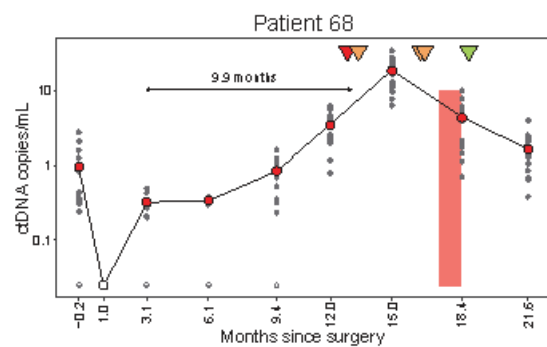
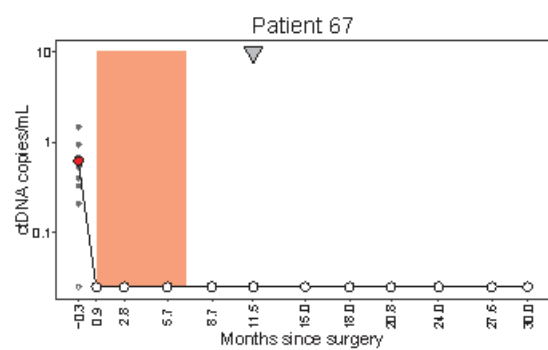
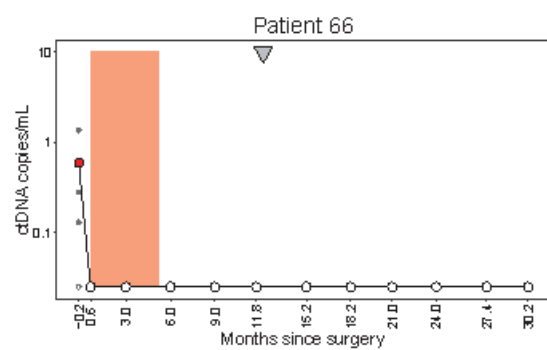
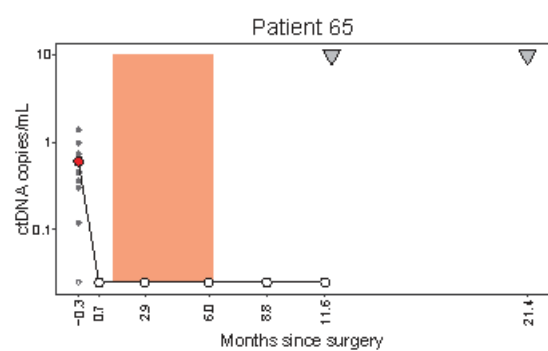
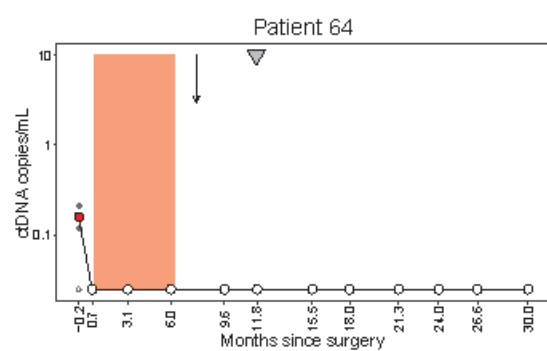


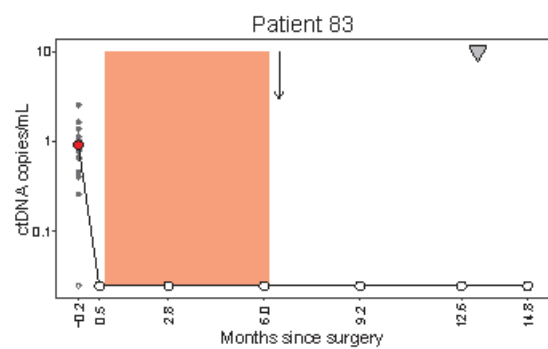
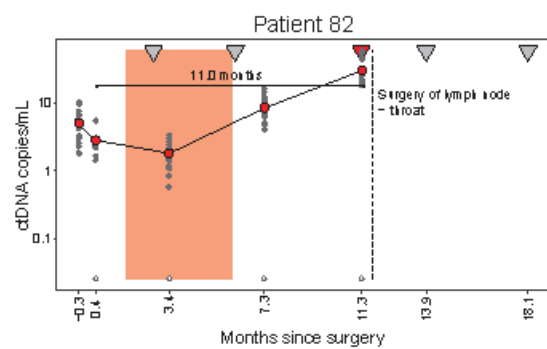
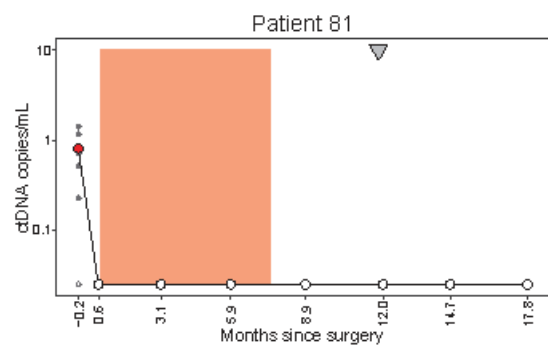
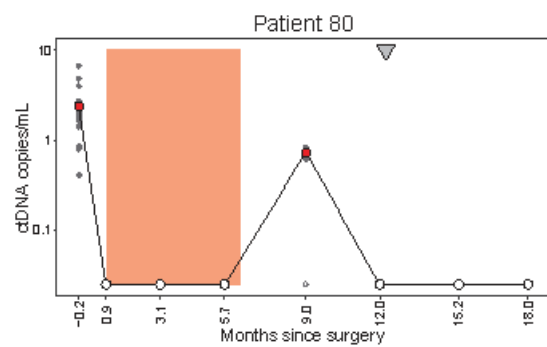
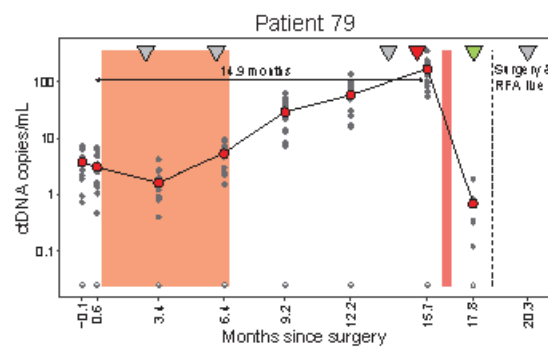
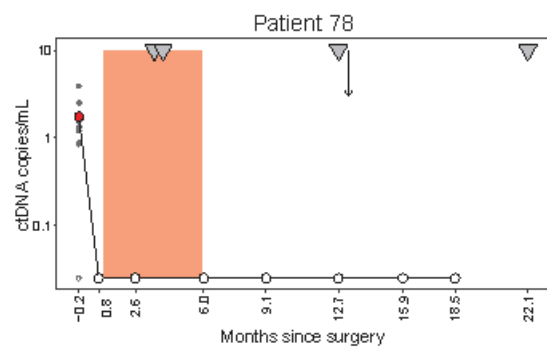
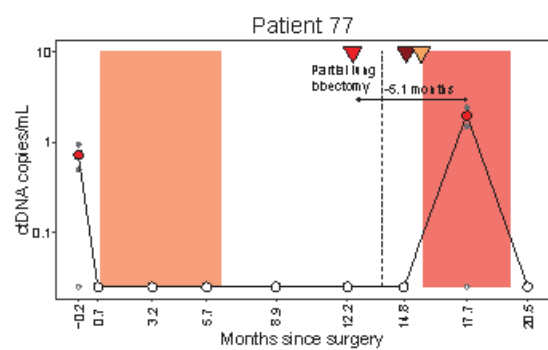
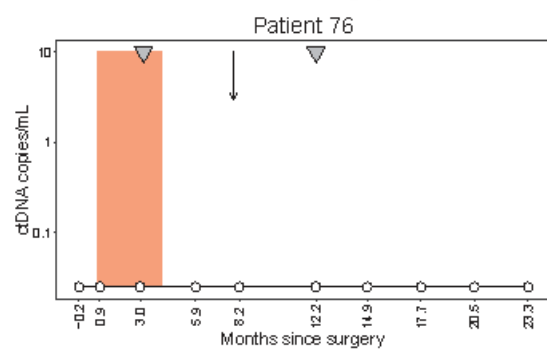
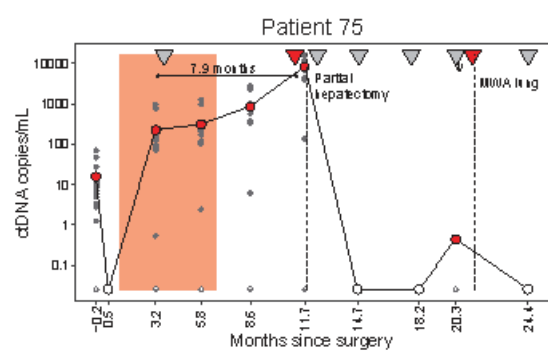
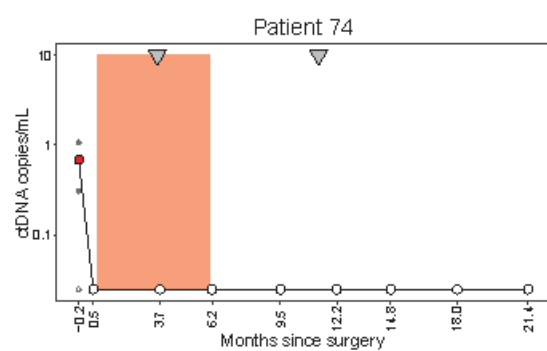


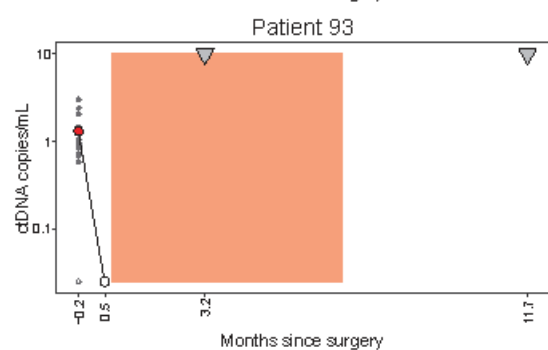
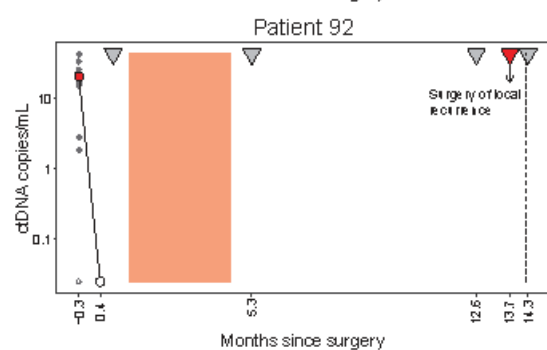
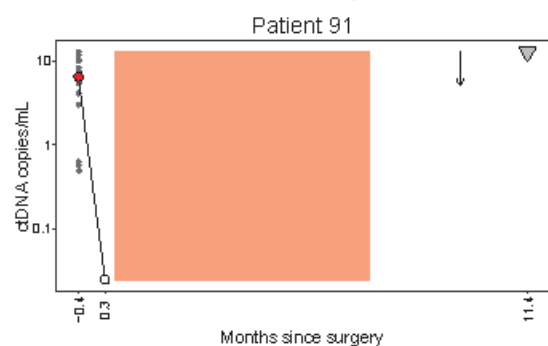
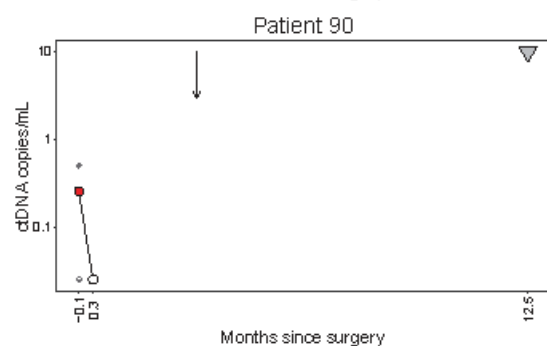
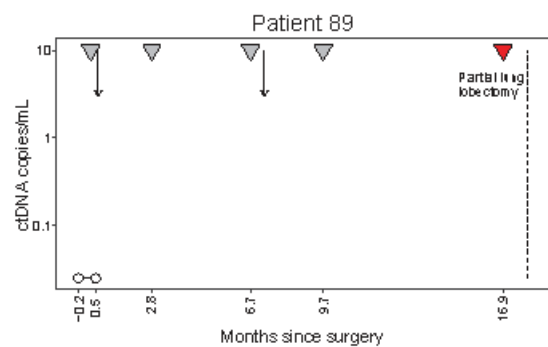
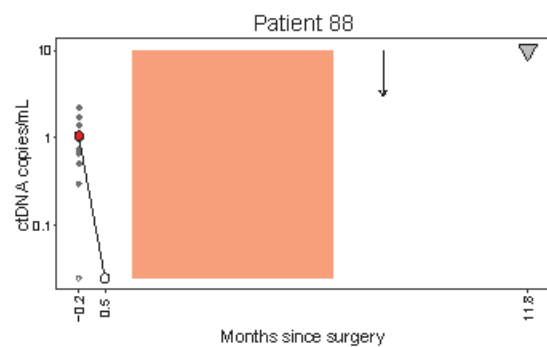
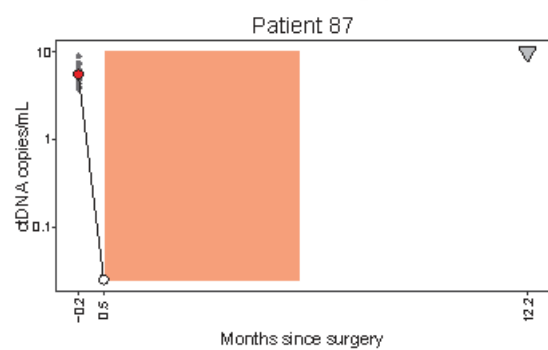
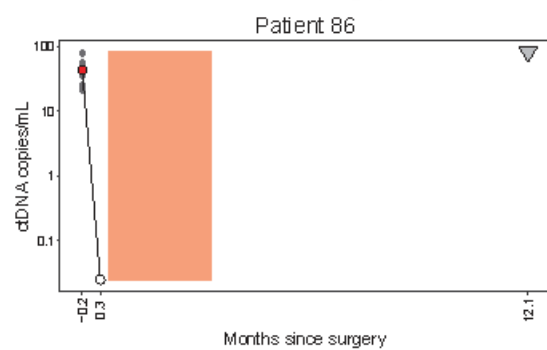
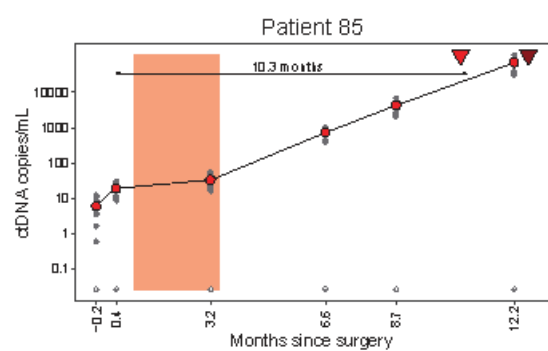
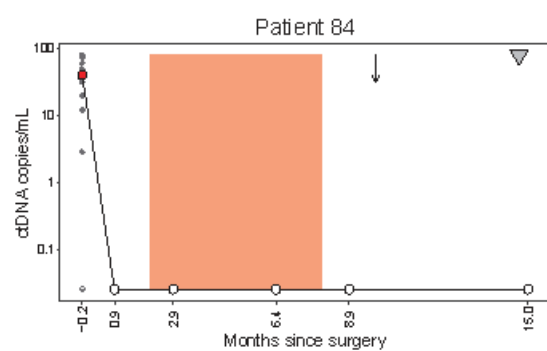


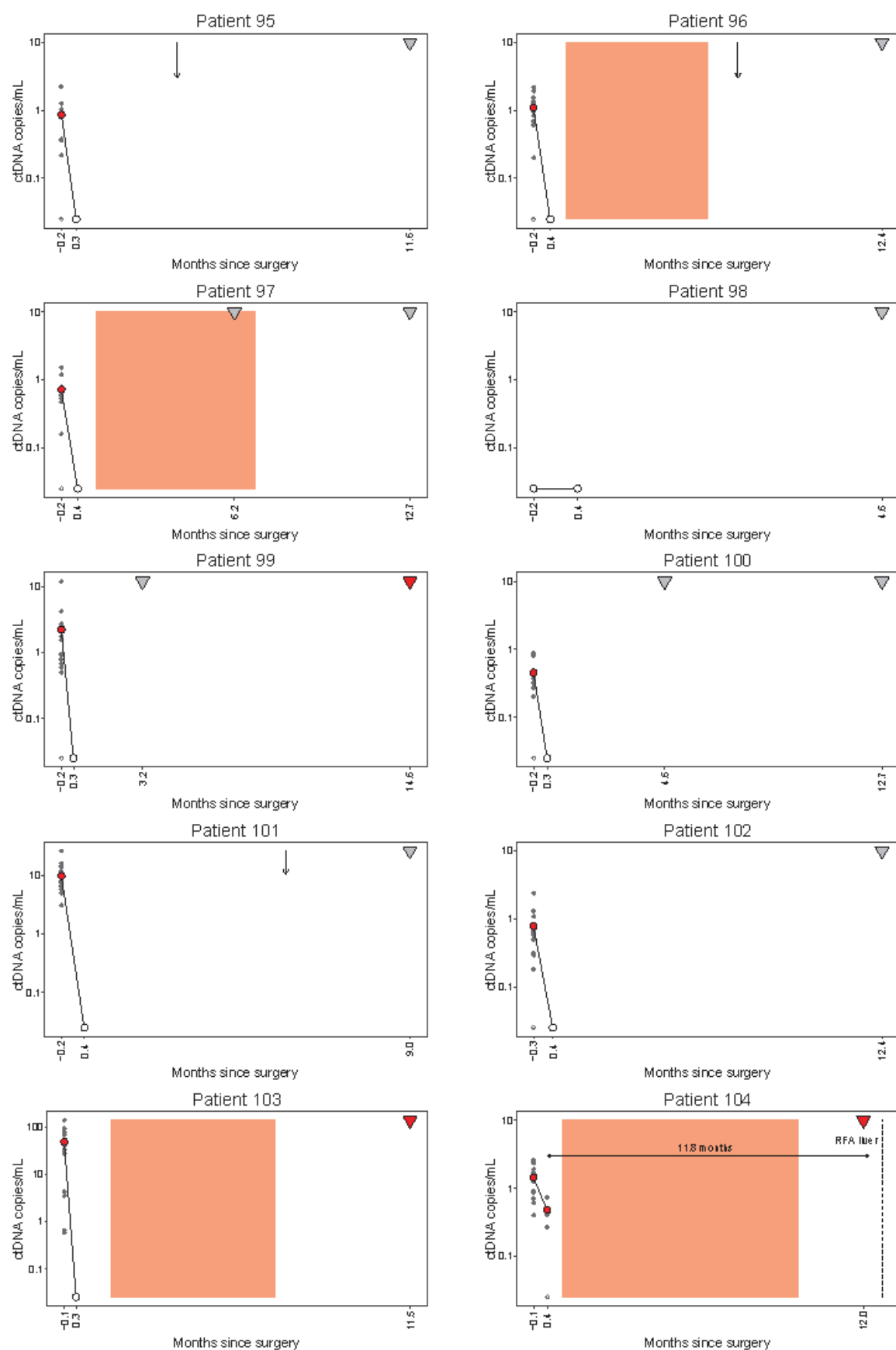


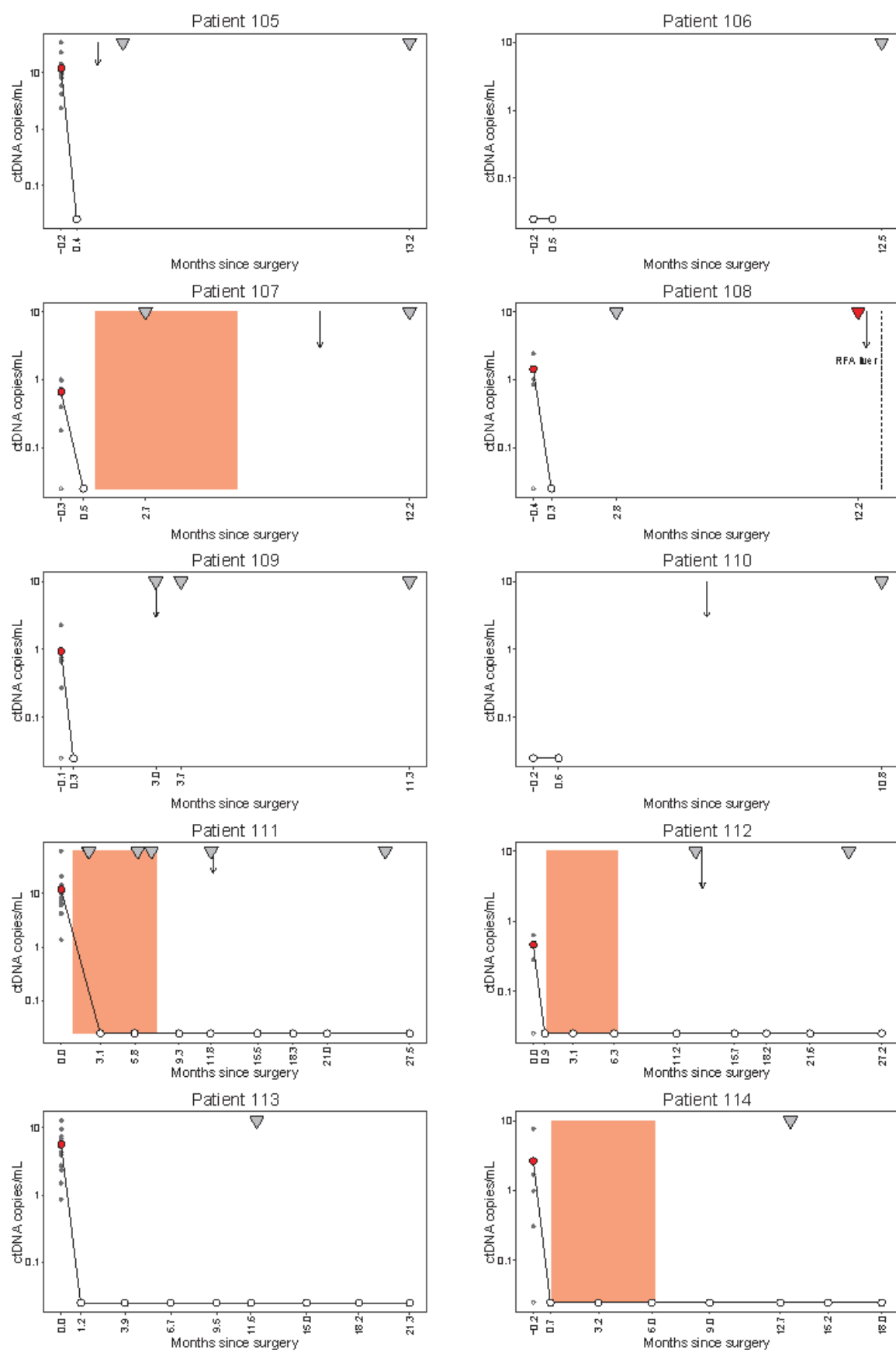


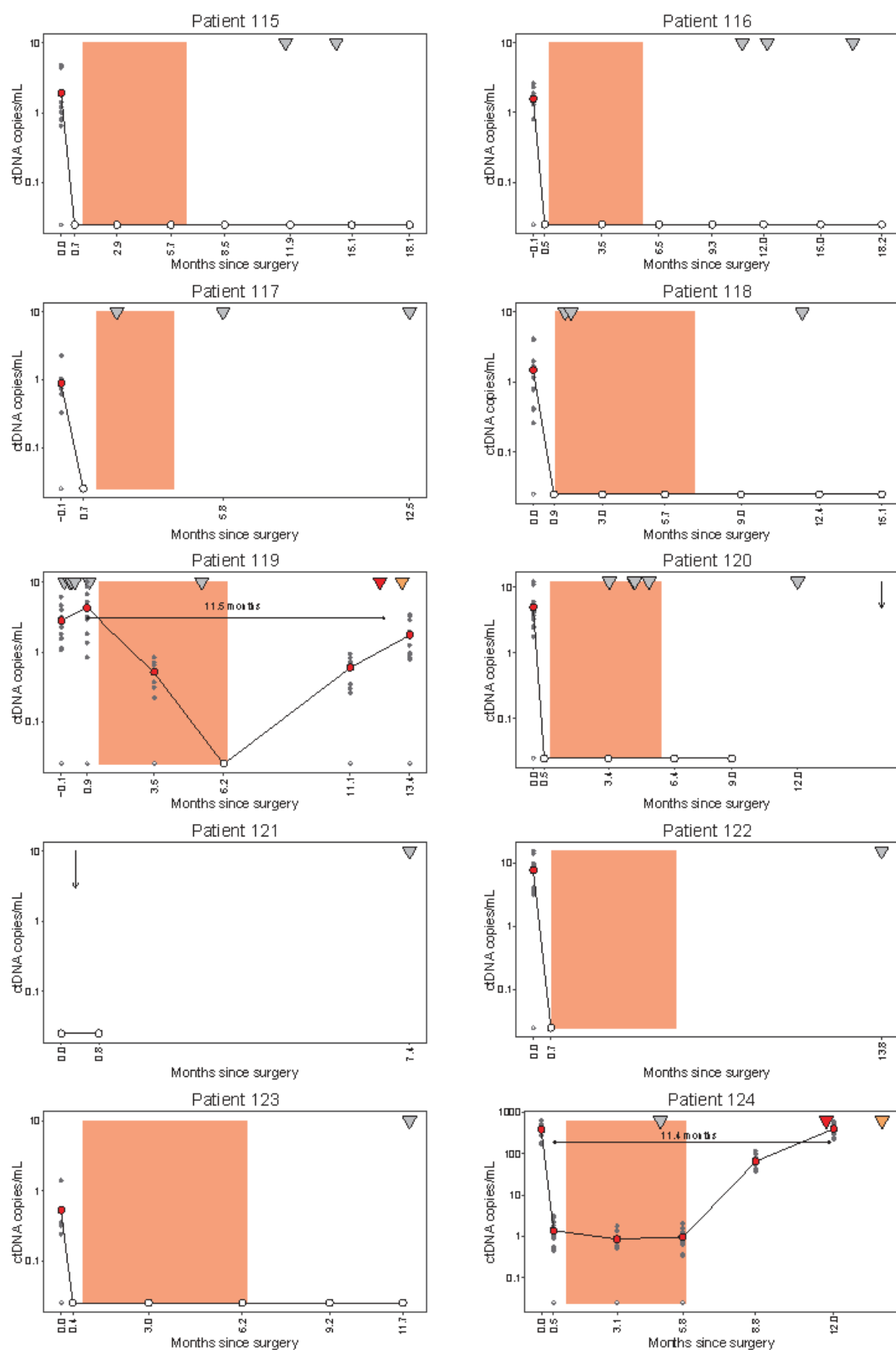


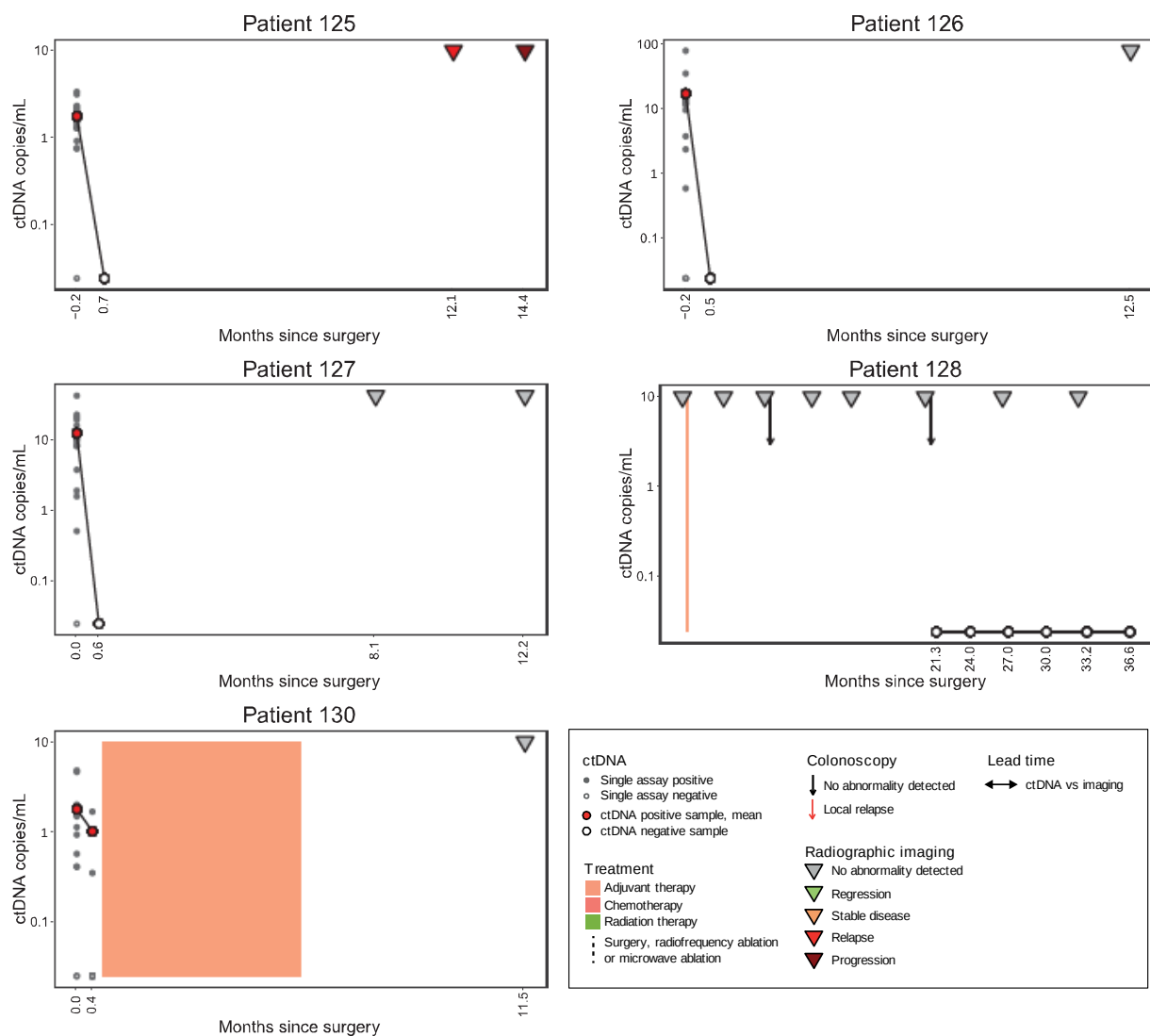




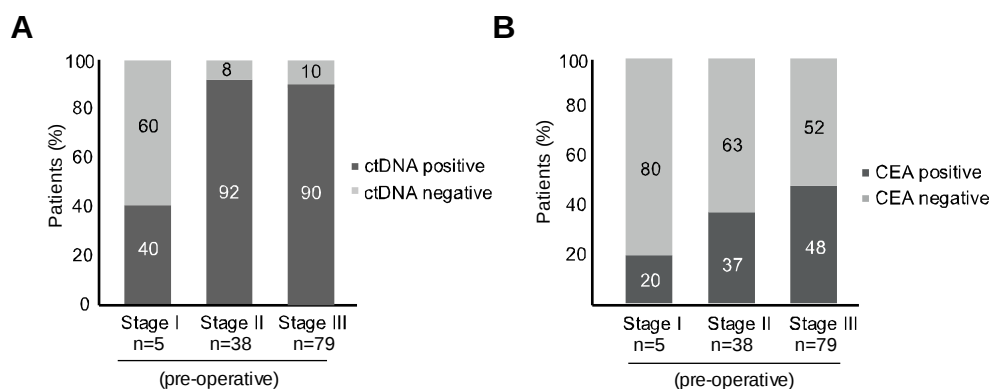




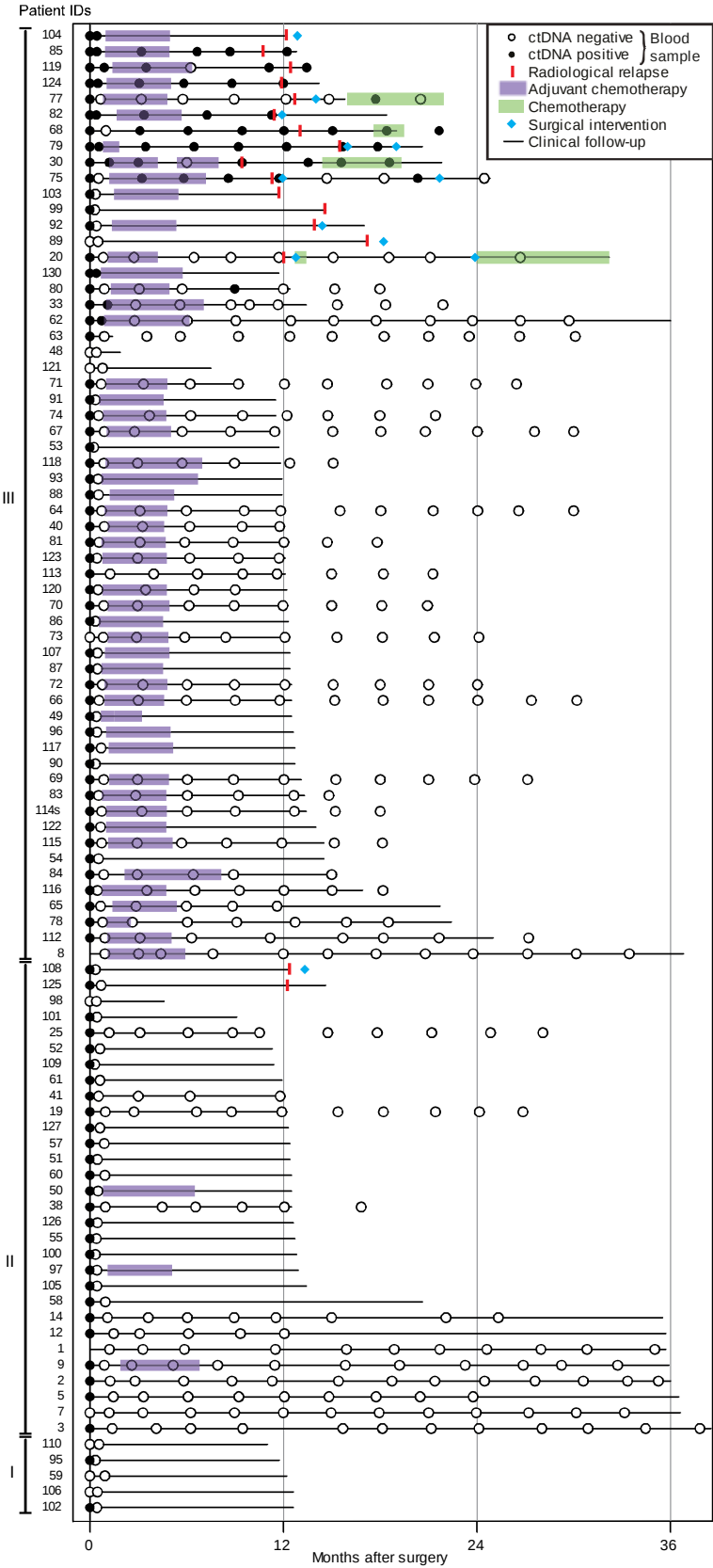




eFigure 4. Pre-operative Detection of ctDNA and CEA in 122 Stage I-III CRC Patients.

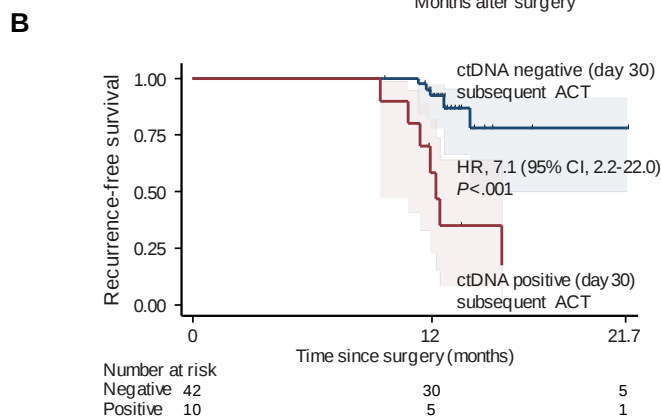
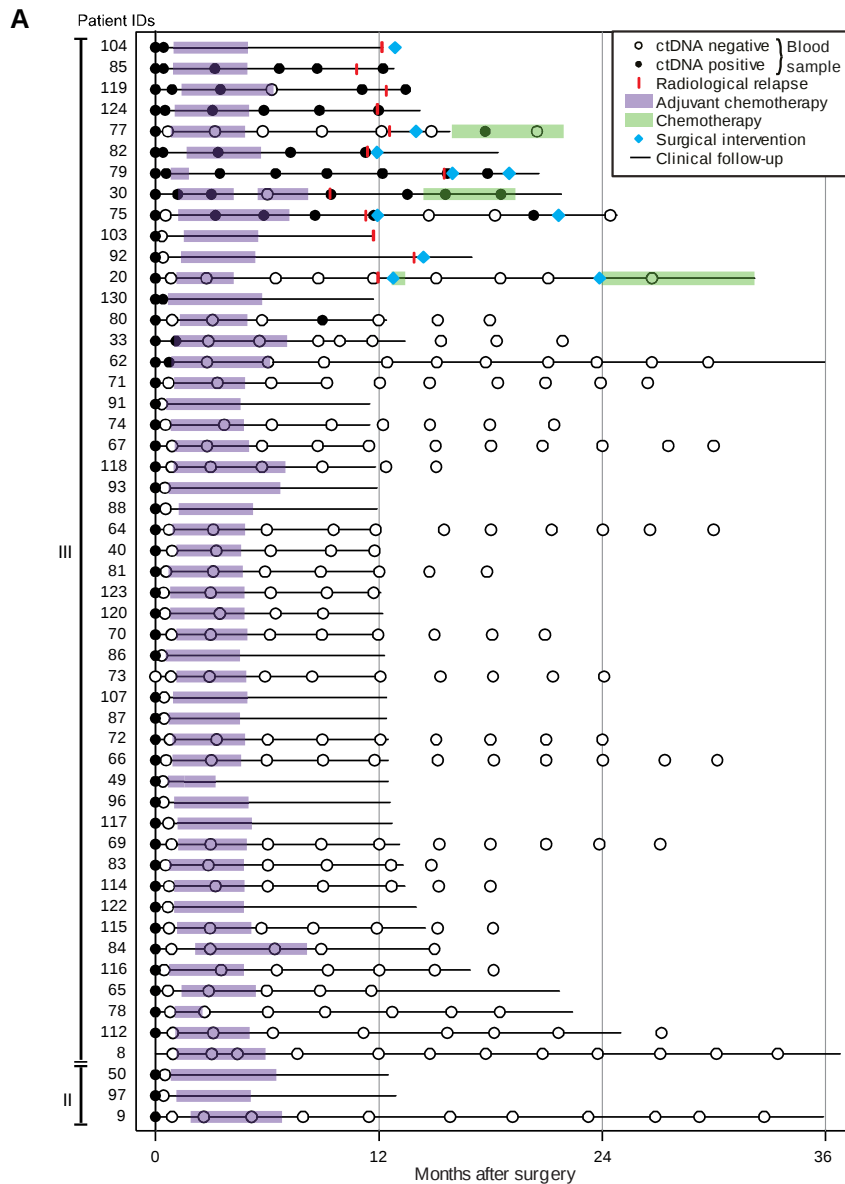


eFigure 5. ctDNA Profiling Results From the 94 Patients Included in the Day 30 ctDNA Analysis. Patients are ordered by recurrence status and disease stage.



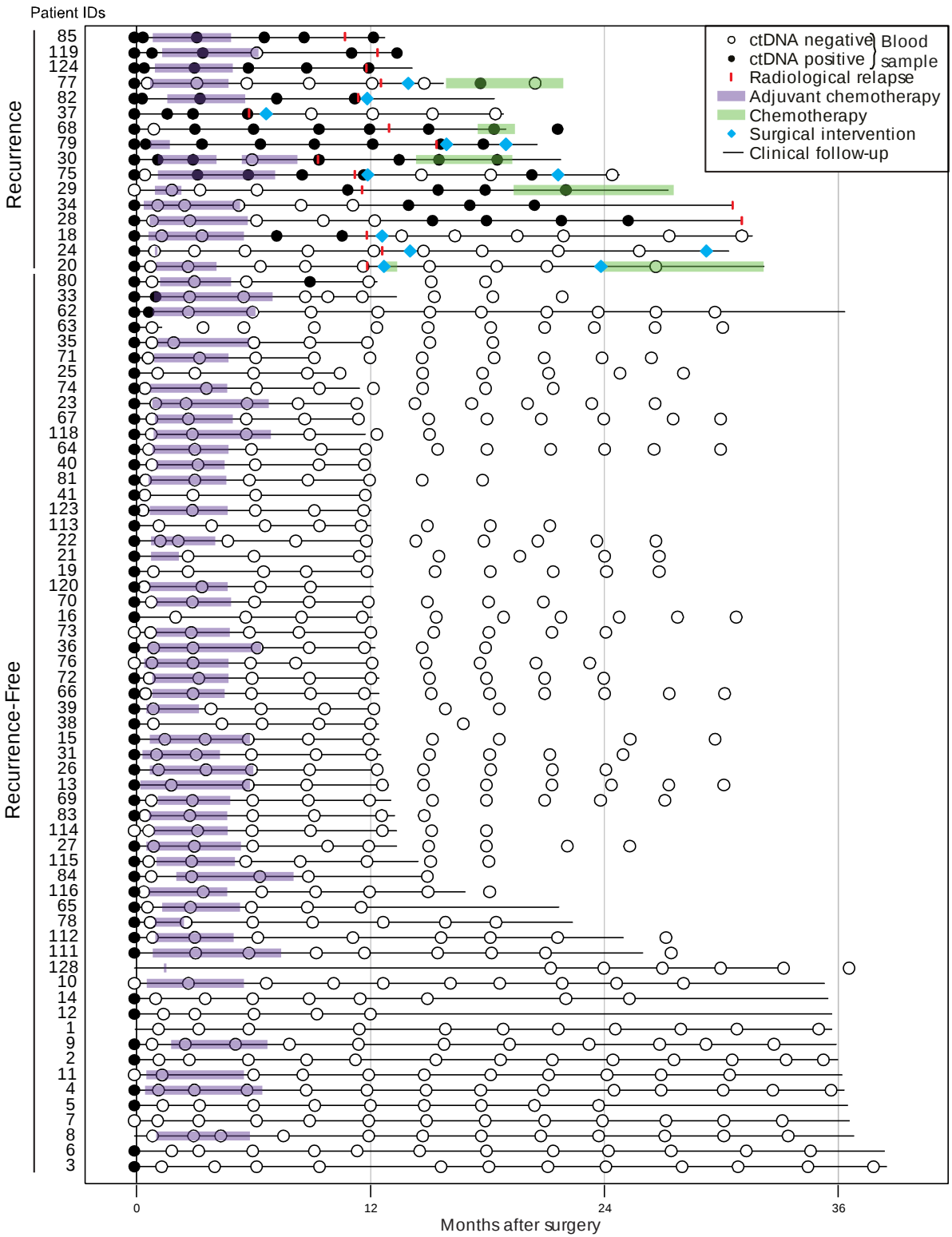
eFigure 6. ctDNA Profiling Results of the ACT Treated Fraction of Patients (n=52) Included in the Day 30 ctDNA Analysis.

A) Schematic overview of the ctDNA results. Patients are ordered by recurrence status and disease stage. B) Kaplan-Meier estimates (CI=95%) of recurrence free survival the 52 ACT treated patients, stratified by post-operative day 30 ctDNA status. The Kaplan Meier plot were halted when the proportion of patients in follow-up was under 10%.

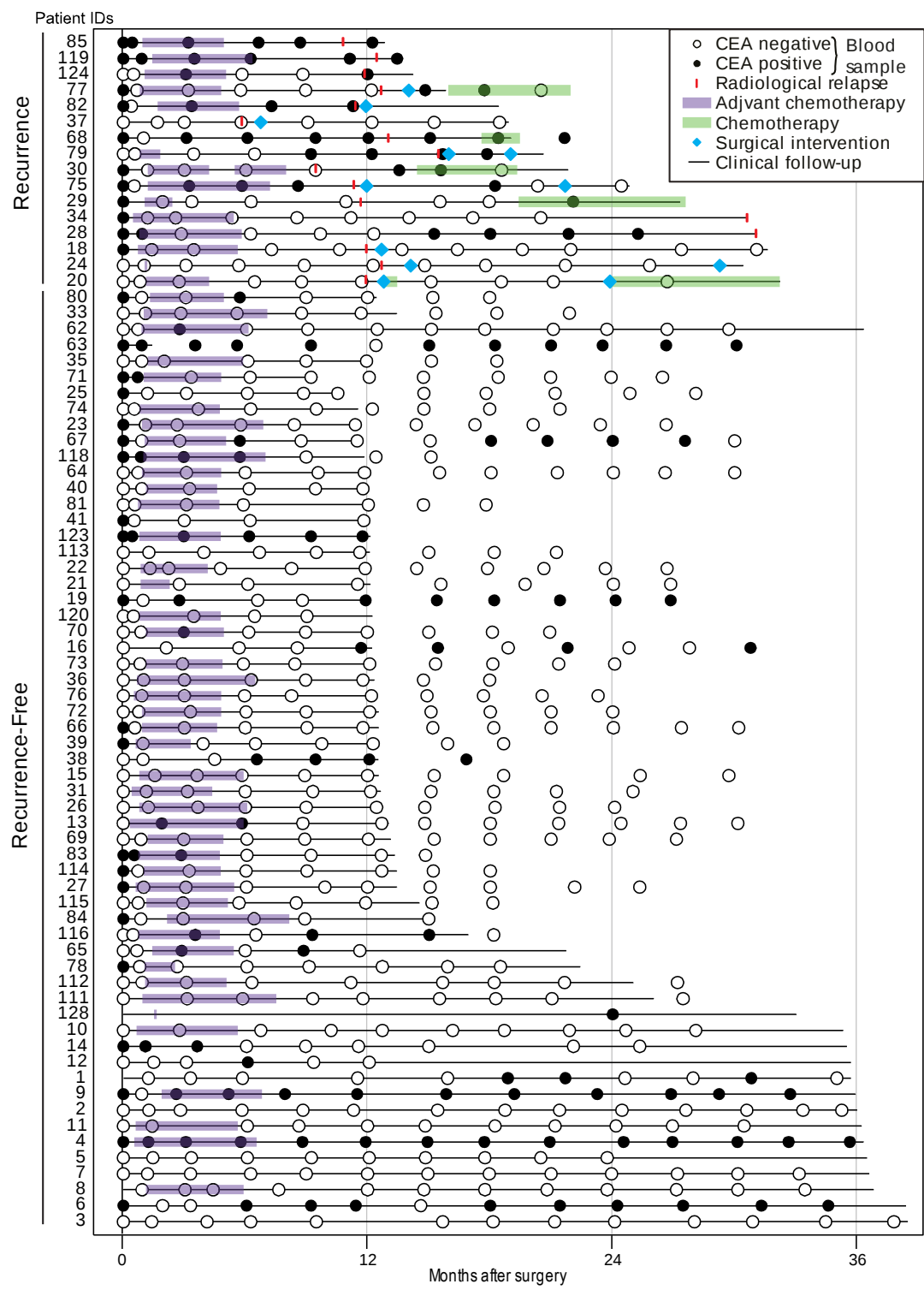


eFigure 7. ctDNA Profiling Results From the 75 Patients Included in the Longitudinal Post-Definitive-Treatment ctDNA Analysis.

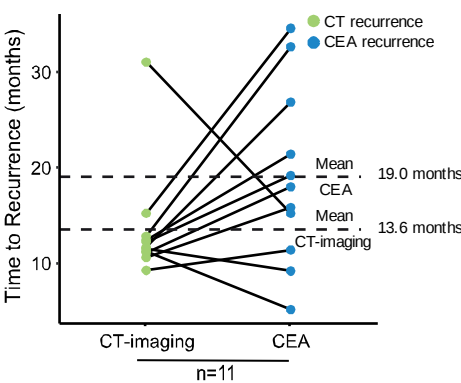
Patients are ordered by recurrence status. Patients were considered positive if one or more plasma samples post-definitive-treatment was ctDNA-positive. Patient 80, which had a transient ctDNA-positive call at month 9 is likely false positive. From eFigure 3 it can be seen that only 2/16 mutations were called positive at month 9. All other post-operative samples from patient 80 were negative. In all other ctDNA-positive patients positivity persisted in the longitudinal samples and ctDNA status only changed to negative in case of clinical intervention.



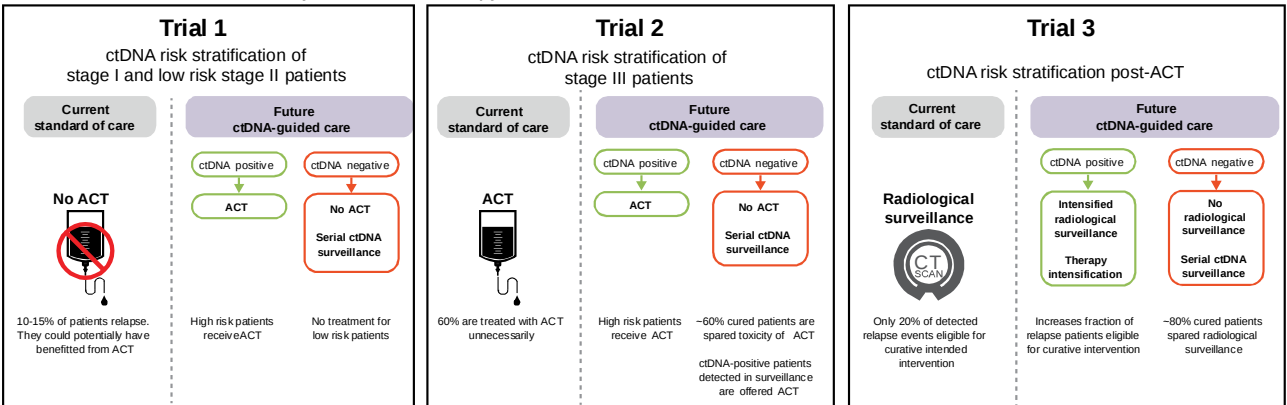
eFigure 8. CEA Profiling Results From the 75 Patients Included in the Post-Definitive Treatment ctDNA Surveillance Analysis. Patients are ordered by recurrence status. Patients were considered positive if one or more surveillance samples were CEA positive.



eFigure 9. Comparison of Time to Recurrence by CEA and Standard-of-Care CT Imaging. The average time from surgery to relapse detection was 19 months for CEA and 13.6 months for CT imaging



eFigure 10. Clinical Trial Proposals for Investigating the Clinical Benefit of ctDNA-Guided Post-Operative Management of CRC Patients. In the present study, we show that detection of circulating tumor DNA post-operatively, defines a patient subgroup with residual disease and very high risk of clinical recurrence, while ctDNA negative patients have low risk of recurrence. Based on these findings we suggest three clinical trials, aimed at investigating the clinical benefit of using ctDNA to guide the post-operative management. Trial 1: here we suggest investigating the effect of offering adjuvant chemotherapy to post-operative ctDNA-positive stage I and low risk stage II patients, who currently would not receive adjuvant chemotherapy. Trial 2: here we suggest investigating the effect of withholding adjuvant chemotherapy from ctDNA negative stage III patients, and hence likely cured patients, with the aim to spare them from unnecessary toxicity. Instead, the patients may be monitored by ctDNA based surveillance. Trial 3: here we suggest investigating the effect of differentiating the intensity of follow-up based on ctDNA-risk-stratification after adjuvant chemotherapy.



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