Supplementary file for:

Prognostic utility of targeted circulating cell-free DNA versus formalin-fixed paraffin-embedded DNA mutation analysis for advanced lung cancer

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SUPPLEMENTARY METHODS

S1. Modified QIAamp Circulating Nucleic Acid protocol for purification circulating DNA from 2 mL plasma

*Noted with * marks modified steps.*

1. Pipet 200 µL QIAGEN Proteinase K into a 15 mL centrifuge tube (not provided).

2. Add 2 mL plasma to the tube.

3. Add 1.6 mL Buffer ACL. Close the cap and mix by pulse-vortexing for 30 s. Make sure that a visible vortex forms in the tube. To ensure efficient lysis, it is essential that the sample and Buffer ACL are mixed thoroughly to yield a homogeneous solution.
Note: Do not interrupt the procedure at this time. Proceed immediately to step 4 to start the lysis incubation.

*4. Incubate at 60 °C for 60 min.

5. Place the tube back on the lab bench and unscrew the cap.

6. Add 3.6 mL Buffer ACB to the lysate in the tube. Close the cap and mix thoroughly by pulse-vortexing for 15–30 s.

7. Incubate the lysate–Buffer ACB mixture in the tube for 5 min on ice.

8. Insert the QIAamp Mini column into the VacConnector on the QIAvac 24 Plus. Insert a 20 mL tube extender into the open QIAamp Mini column. Make sure that the tube extender is firmly inserted into the QIAamp Mini column in order to avoid leakage of sample.
Note: Keep the collection tube for the dry spin in step 13.

9. Carefully apply the lysate–Buffer ACB mixture from step 7 into the tube extender of the QIAamp Mini column. Switch on the vacuum pump (800 – 900 mbar). When all lysates have been drawn through the columns completely, switch off the vacuum pump and release the pressure to 0 mbar. Carefully remove and discard the tube extender.
Note: To avoid cross-contamination, be careful not to move the tube extenders over neighboring QIAamp Mini Columns.

10. Apply 600 µL Buffer ACW1 to the QIAamp Mini column. Leave the lid of the column open, and switch on the vacuum pump (800 – 900 mbar). After all of Buffer ACW1 has been drawn through the QIAamp Mini column, switch off the vacuum pump and release the pressure to 0 mbar.

*11. Apply 700 µL Buffer ACW2 to the QIAamp Mini column. Leave the lid of the column open, and switch on the vacuum pump (800 – 900 mbar). After all of Buffer ACW2 has been drawn through the QIAamp Mini column, switch off the vacuum pump and release the pressure to 0 mbar.

12. Apply 750 µL of ethanol (96–100%, not provided) to the QIAamp Mini column. Leave the lid of the column open, and switch on the vacuum pump (800 – 900 mbar). After all of ethanol has been drawn through the spin column, switch off the vacuum pump and release the pressure to 0 mbar.

*13. Close the lid of the QIAamp Mini column. Remove it from the vacuum manifold, and discard the VacConnector. Place the QIAamp Mini column in a clean 2 mL collection tube, and centrifuge at full speed (13,000 x g) for 3 min.

14. Place the QIAamp Mini Column into a new 2 mL collection tube. Open the lid, and incubate the assembly at 56 °C for 10 min to dry the membrane completely.

*15. Place the QIAamp Mini column in a clean 1.5 mL elution tube (provided) and discard the 2 mL collection tube from step 14. Carefully apply 60 µL of warm Buffer AVE to the center of the QIAamp Mini membrane. Close the lid and incubate at room temperature for 3 min.

*16. Centrifuge in a microcentrifuge at full speed (13,000 x g) for 1 min to elute the nucleic acids. Repeat the elution.

S2. Selected amplicons

A) Selected genes, exons, and amplicon coordinates

Gene	Exon	Coordinates (GRCh37)
AKT1	**3**	chr14:105246530-105246577
ALK	**22**	chr2:29445211-29445277
	23	chr2:29443607-29443659
BRAF	**11**	chr7:140481384-140481425
	15	chr7:140453119-140453164
EGFR	**18**	chr7:55241663-55241733
	19	chr7:55242459-55242519
	20	chr7:55248966-55249039
	20	chr7:55249058-55249131
	21	chr7:55259500-55259555
ERBB2	**19**	chr17:37880196-37880259
	20	chr17:37880986-37881034
KRAS	**2**	chr12:25398269-25398309
	3	chr12:25380240-25380280
MET	**2**	chr7:116339621-116339674
	14	chr7:116411852-116412047
	14	chr7:116411982-116412047
NRAS	**2**	chr1:115258730-115258791
	3	chr1:115256508-115256558

PIK3CA	**9**	chr3:178936070-178936114
	20	chr3:178952049-178952087

B) Primer sequences

Illumina® overhanging adapters added to the 5' end:

forward overhang: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-[locus-specific primer];

reverse overhang: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-[locus-specific primer].

Primer name	**Sequence 5'-3' (with adapter sequence)**	**Product size (bp)**	**Product size + adapter (bp)**	**+ index (bp)**
AKT1ex3F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCGGGTAGAGTGTGCGTGG	86	153	222
AKT1ex3R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTTGAGGAGGAAGTAGCGTG			
ALKex22F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACCCTCCCCTTCTCTGCC	106	173	242
ALKex22R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTGTCTCTCTGTGGCTTTACC			
ALKex23F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTTCGCTGCATTGGGGTGAG	95	162	231
ALKex23R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTCTCTCGGAGGAAGGACTTGA			
BRAFex11F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTGGGCAGATTACAGTGGGACA	88	155	224
BRAFex11R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCACATTACATACTTACCATGCCAC			
BRAFex15F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATTTCTTCATGAAGACCTCACAGTA	92	159	228
BRAFex15R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGACTGTTCAAACTGATGGGACC			
EGFRex18F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGCTCCCAACCAAGCTCTCT	111	178	247
EGFRex18R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGTGCCAGGGACCTTACCTT			
EGFRex19F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTGAGAAAGTTAAAATTCCCGTCGC	105	172	241

EGFRex19R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCCCACACAGCAAAGCAGAA			
EGFRex20mut1F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATGCGAAGCCACACTGAC	110	177	246
EGFRex20mut1R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGTGGAGGTGAGGCAGAT			
EGFRex20mut2F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATCTGCCTCACCTCCACC	111	178	247
EGFRex20mut2R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTTGAGCAGGTACTGGGAG			
EGFRex21F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAACACCGCAGCATGTCAAGA	99	166	235
EGFRex21R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCACCTCCTTACTTTGCCTCCTTC			
ERBB2ex19F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTCCCTGATGGGGAGAATGTGA	104	171	240
ERBB2ex19R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGTGGAGGGGCTTACGTCT			
ERBB2ex20F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTACCCTTGTCCCCAGGAAGCAT	91	158	227
ERBB2ex20R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCACCGTGGATGTCAGGCAG			
KRASex2F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGGCCTGCTGAAAATGACTGAA	87	154	223
KRASex2R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGAATTAGCTGTATCGTCAAGGCA			
KRASex3F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTTGGATATTCTCGACACAGCA	83	150	219
KRASex3R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAAAGAAAGCCCTCCCCAGTC			
METex2F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCAGTCGGAGGTTCACTGCAT	94	161	230
METex2R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAAAGGACTTTGGCTCCCAGG			
METex14F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTTGTAAGTGCCCGAAGTGT	113	180	249
METex14R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGTATTTCTCAGAACAATAAACTGAAAT			
METex14delF	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCACTGGGTCAAAGTCTCCTG	105	172	241
METex14delR	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGTATTTCTCAGAACAATAAACTGAAAT			
NRASex2F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACTGGTTTCCAACAGGTTCTTG	106	173	242

NRASex2R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGGATTAGCTGGATTGTCAGTG			
NRASex3F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGTGGTTATAGATGGTGAAACCTG	97	164	233
NRASex3R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCCTGTCCTCATGTATTGGTCT			
PIK3CAex9F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCAGCTCAAAGCAATTTCTACACGA	94	161	230
PIK3CAex9R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCCATTTTAGCACTTACCTGTGACT			
PIK3CAex20F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGCCTTAGATAAAACTGAGCAAGAGG	85	152	221
PIK3CAex20R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTTTTGTTGTCCAGCCACCAT			

C) Primer multiplex groups

Group 1	AKT1ex3	BRAFex15	EGFRex21	ERBB2ex19	ERBB2ex20	METex14	NRASex2
Group 2	BRAFex11	EGFRex18	EGFRex19	EGFRex20mut2	KRASex2	KRASex3	METex2
Group 3	ALKex22	ALKex23	EGFRex20mut1	METex14del	NRASex3	PIK3CAex9	PIK3CAex20

S3. Amplicon PCR protocol

HOT FIREPol® DNA polymerase activation 95°C for 12 minutes

34 cycles of:

- 95°C for 30 seconds
- 58°C for 30 seconds
- 72°C for 30 seconds

72°C for 5 minutes

Storage, hold at 4°C

S4. Modified Illumina's 16S Metagenomic Sequencing Library Preparation Index PCR protocol

*Noted with * marks modified steps.*

* 98°C for 10 minutes

8 cycles of:

- 95°C for 30 seconds
- 55°C for 30 seconds
- 72°C for 30 seconds
- 72°C for 5 minutes

Storage, hold at 4°C

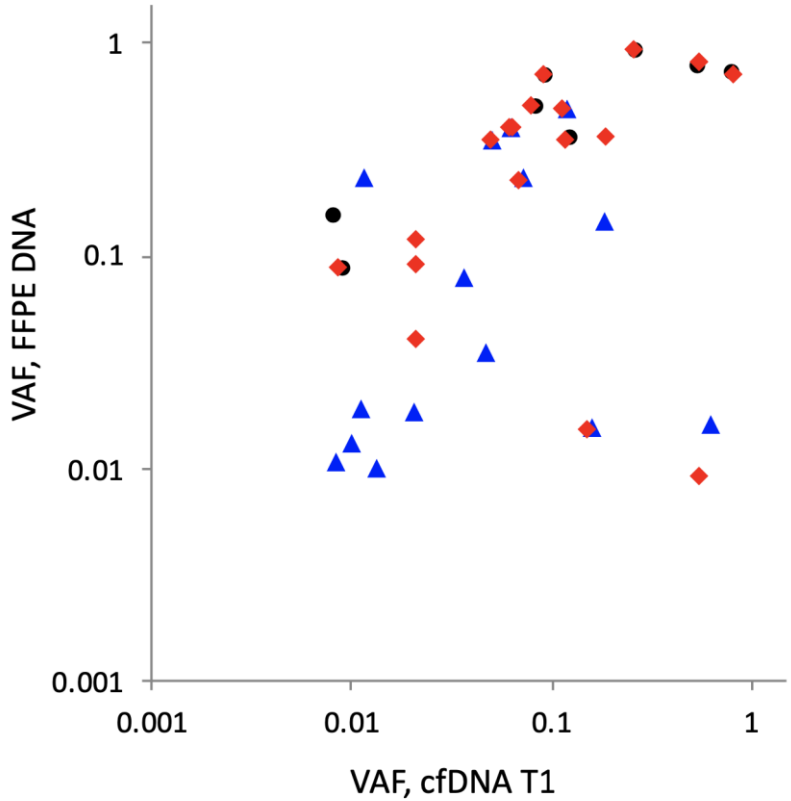
SUPPLEMENTARY RESULTS

FIGURES

F1. Concordance of mutation spectra between matched plasma and tumor (FFPE) samples (*n* = 75)

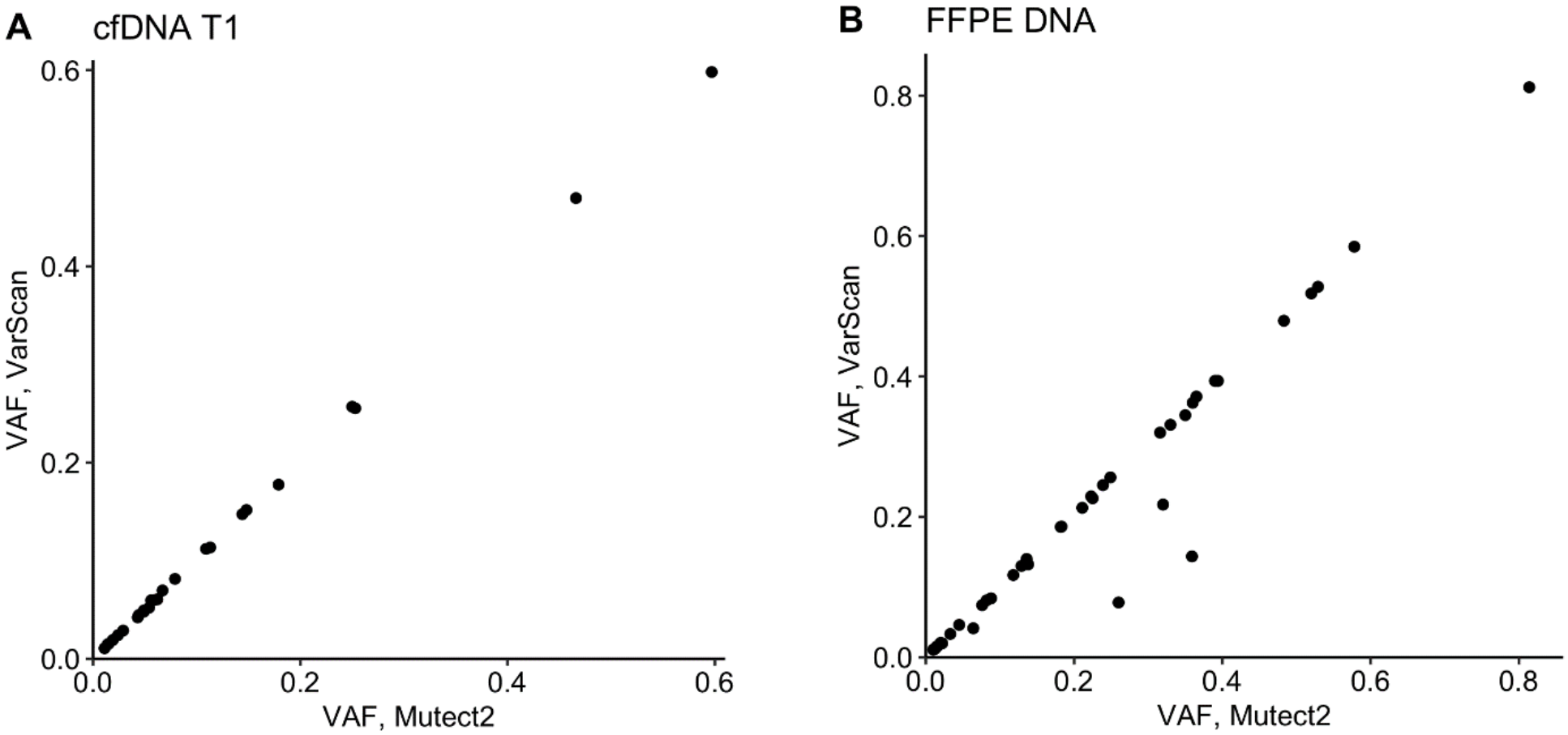
VAF estimates for the mutations detected in both cfDNA T1 and FFPE DNA are shown. Pearson's correlation coefficient between cfDNA T1 and FFPE DNA VAF estimates by calling algorithms were 0.48, 0.10, and 0.64 for Mutect2, VarScan, and AIH, respectively.

Red, Mutect2; blue, VarScan; black, AIH.

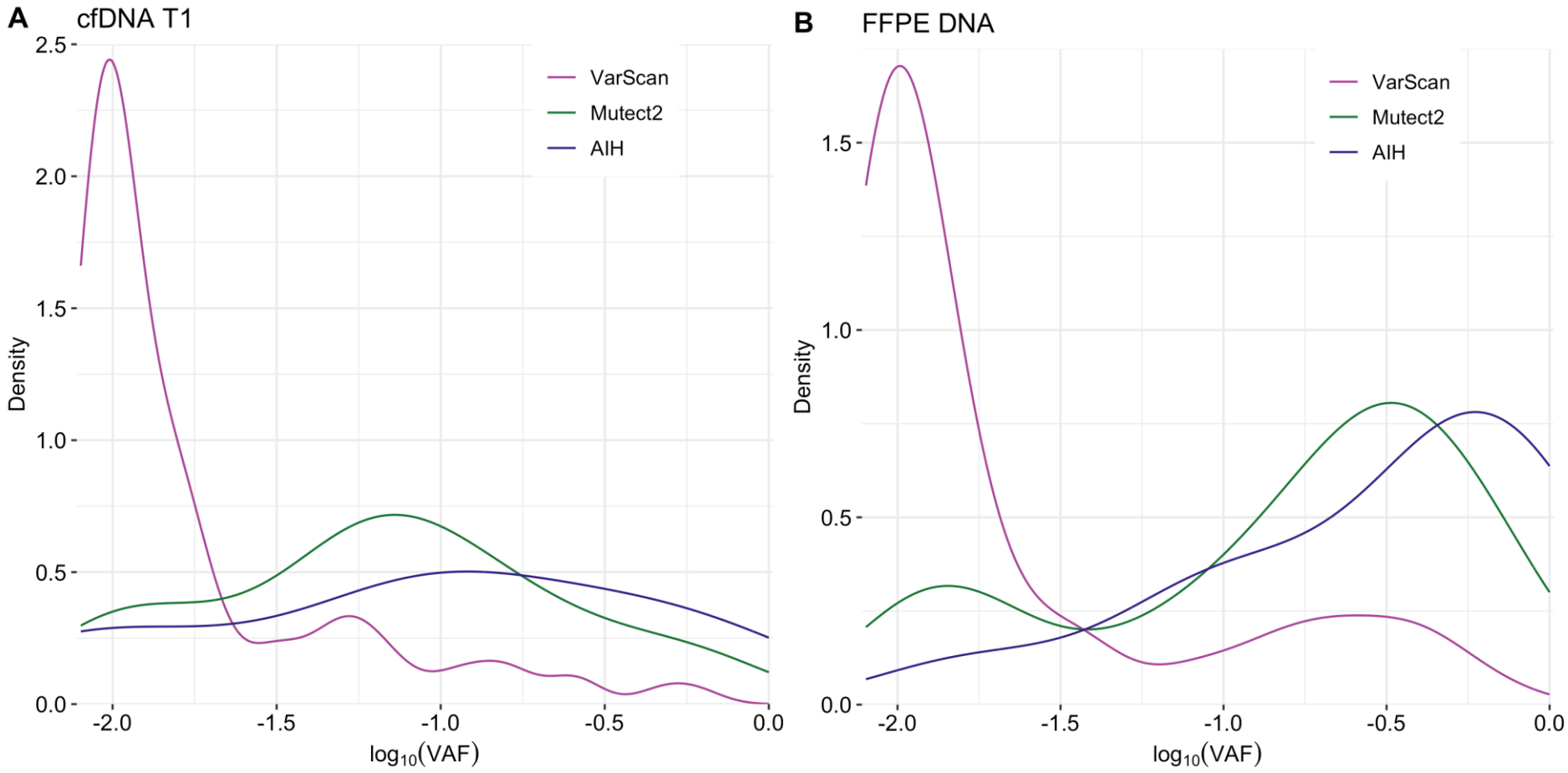


F2. Concordance of mutation spectra between calling algorithms

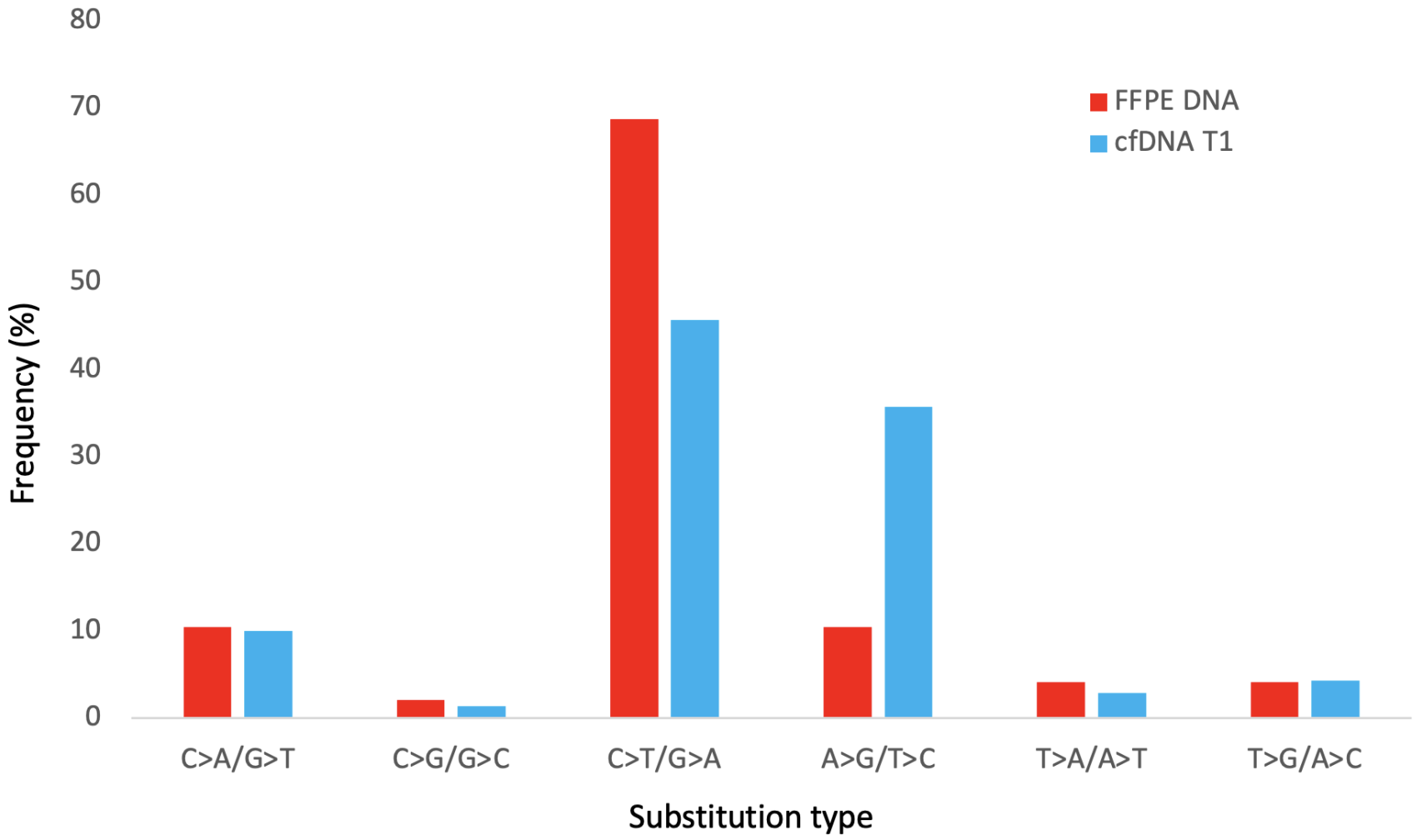
VAF estimates detected with both Mutect2 and VarScan calling algorithms in A) cfDNA T1 (26 mutations) and B) FFPE DNA (41 mutations). Correlations between Mutect2 and VarScan VAF estimates by DNA materials were 1 ($P < 0.001$) and 0.97 ($P < 0.001$) from cfDNA T1 and FFPE DNA, respectively.



F3. Variant allele frequency distributions called with Mutect2, VarScan and Amplicon Indel Hunter from cfDNA T1 and FFPE DNA



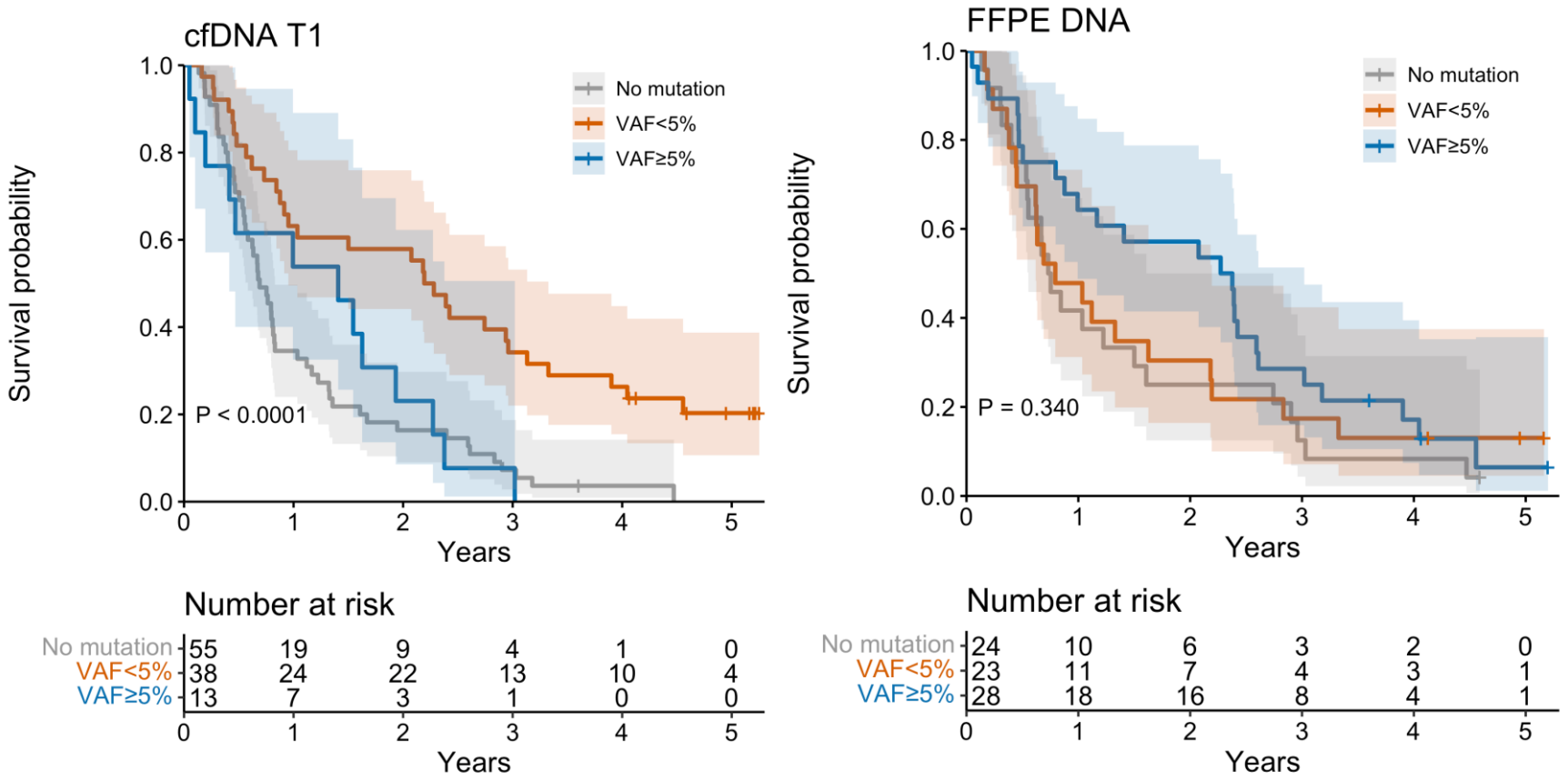
F4. The frequency of different types of substitutions observed in FFPE DNA and cfDNA T1 samples (*n* = 75)



F5. Kaplan-Meier estimate of overall survival of subjects stratified by VAF groups according to detected mutations in eight genes in cfDNA T1 and FFPE DNA

VAF groups: no mutation vs. VAF < 5% vs. VAF ≥ 5%

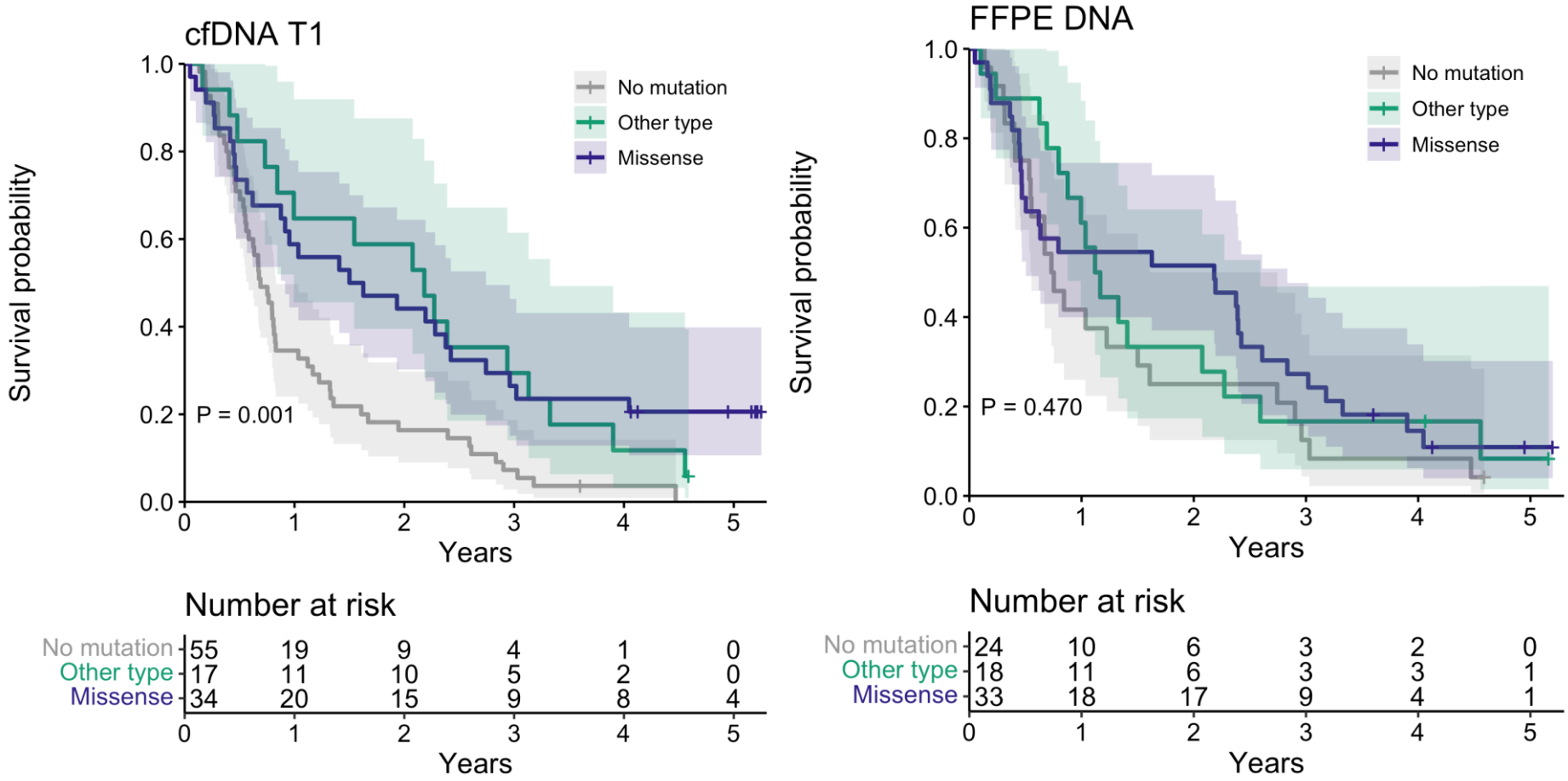
Mutations in genes: *ALK*, *AKT1*, *BRAF*, *EGFR*, *ERBB2*, *MET*, *NRAS*, and *PIK3CA*



F6. Kaplan-Meier estimate of overall survival of subjects stratified by mutation types according to detected mutations in eight genes in cfDNA T1 and FFPE DNA

Mutation types: no mutation vs. missense mutation vs. other type

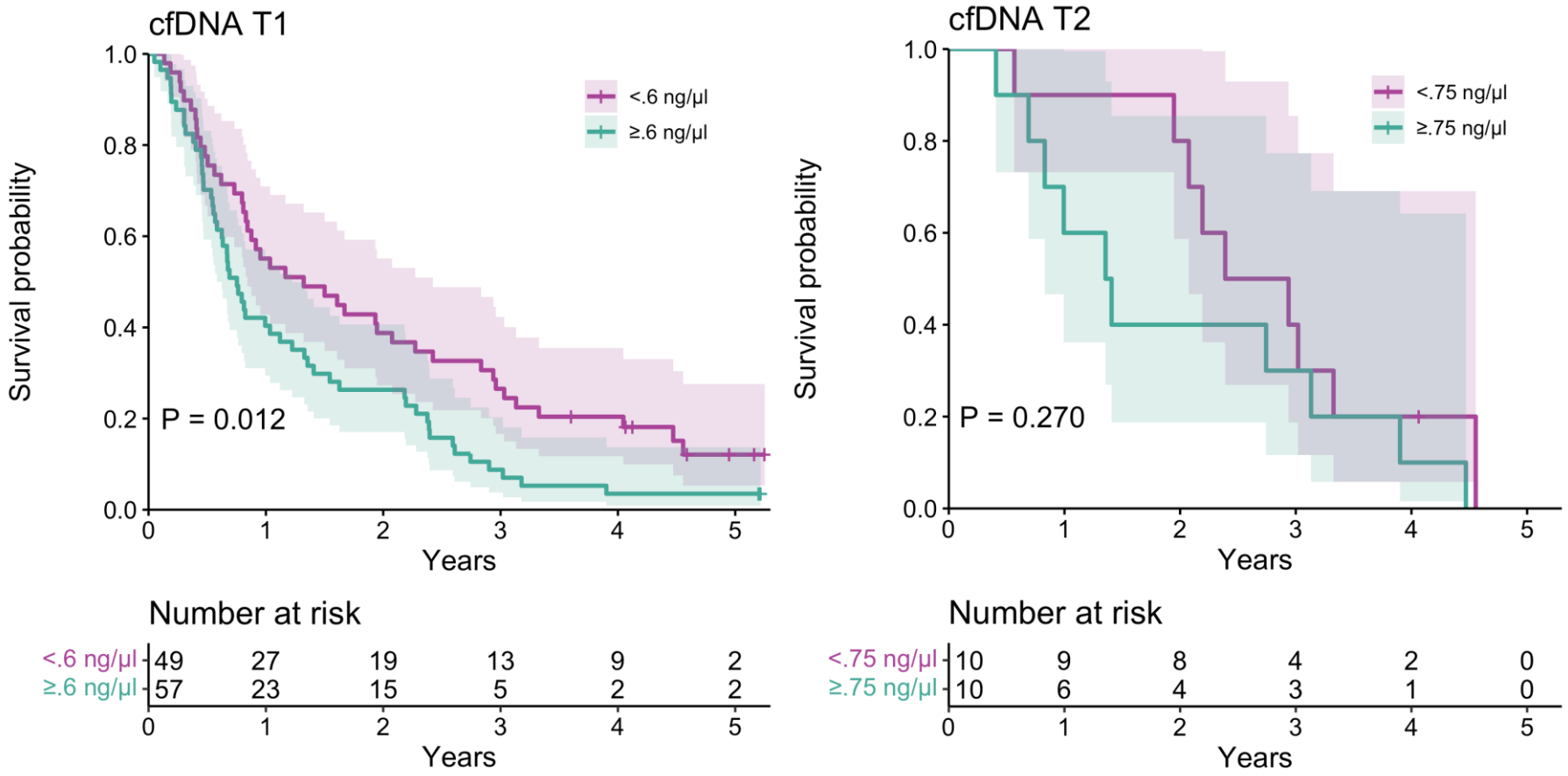
Mutations in genes: *ALK*, *AKT1*, *BRAF*, *EGFR*, *ERBB2*, *MET*, *NRAS*, and *PIK3CA*



F7. Kaplan-Meier estimate of overall survival according to cfDNA concentration measured at time points T1 and T2

cfDNA T1: < 0.60 ng/µL vs. ≥ 0.60 ng/µL

cfDNA T2: < 0.75 ng/µL vs. ≥ 0.75 ng/µL



TABLES

T1. Overview of called mutations

	cfDNA T1	cfDNA T2	FFPE DNA
Number of patients	106	22	75
Number of mutations			
Mutect2	36	4	51
VarScan	102	8	148
AIH	12	1	11
Mutect2 + VarScan + AIH	111	10	157
Number of overlapping mutations			
Mutect2 + VarScan	27	2	42
Mutect2 + AIH	12	1	11
VarScan + AIH	3	0	3
Mutect2 + VarScan + AIH	3	0	3
Number of mutations by VAF			
VAF < 5%	89	6	117
VAF > 5%	22	4	40

Number of mutations by type			
Intron	2	0	2
Stop gained	2	0	4
Inframe indel	13	1	12
Synonymous	24	1	49
Missense	70	8	90

T2. Detected unique mutations over the three different sample materials

., mutation not detected in that sample type or no dbSNP Reference SNP number available

Chr	Pos (GRCh37)	Ref	Alt	Mutated samples	Mutated cfDNA samples	Mutated FFPE samples	Gene	ID	Codon change	AA change	Mutation type	Previously described
1	115256515	C	T	2	.	2	*NRAS*	.	Gcc/Acc	p.A66T	missense variant	Known
1	115256529	T	C	1	.	1	*NRAS*	rs11554290	cAa/cGa	p.Q61R	missense variant	Known
1	115258731	G	A	1	.	1	*NRAS*	.	agC/agT	p.S17S	synonymous variant	Novel
1	115258738	C	T	1	.	1	*NRAS*	.	gGg/gAg	p.G15E	missense variant	Known
1	115258739	C	T	1	.	1	*NRAS*	.	Ggg/Agg	p.G15R	missense variant	Novel
1	115258744	C	T	1	.	1	*NRAS*	rs121434596	gGt/gAt	p.G13D	missense variant	Known
1	115258748	C	A	1	1	.	*NRAS*	rs121913250	Ggt/Tgt	p.G12C	missense variant	Known
1	115258754	C	T	1	1	.	*NRAS*	.	Gga/Aga	p.G10R	missense variant	Known
1	115258757	C	T	1	.	1	*NRAS*	.	Gtt/Att	p.V9I	missense variant	Known
2	29443608	G	T	1	.	1	*ALK*	.	gaC/gaA	p.D1203E	missense variant	Novel
2	29443617	C	T	1	1	.	*ALK*	rs56247462	gcG/gcA	p.A1200A	synonymous variant	Known
2	29443629	C	T	1	.	1	*ALK*	rs1418386125	ctG/ctA	p.L1196L	synonymous variant	Known
2	29443631	G	A	1	.	1	*ALK*	.	Ctg/Ttg	p.L1196L	synonymous variant	Novel
2	29443632	C	T	1	.	1	*ALK*	.	ctG/ctA	p.L1195L	synonymous variant	Novel
2	29443642	C	T	1	.	1	*ALK*	rs113994089	cGg/cAg	p.R1192Q	missense variant	Known
2	29443643	G	A	2	.	2	*ALK*	rs534852056	Cgg/Tgg	p.R1192W	missense variant	Known
2	29443655	G	A	1	.	1	*ALK*	.	Caa/Taa	p.Q1188*	stop gained	Known
2	29445211	T	C	1	1	.	*ALK*	.	Agc/Ggc	p.S1172G	missense variant	Known
2	29445218	C	T	1	.	1	*ALK*	.	ctG/ctA	p.L1169L	synonymous variant	Novel
2	29445221	G	A	1	1	.	*ALK*	.	gcC/gcT	p.A1168A	synonymous variant	Novel

2	29445242	T	C	1	.	1	*ALK*	.	gaA/gaG	p.E1161E	synonymous variant	Novel
2	29445244	C	T	1	.	1	*ALK*	rs145194836	Gaa/Aaa	p.E1161K	missense variant	Known
2	29445245	G	A	1	1	.	*ALK*	rs888578421	gaC/gaT	p.D1160D	synonymous variant	Known
2	29445246	T	C	1	1	.	*ALK*	.	gAc/gGc	p.D1160G	missense variant	Novel
2	29445248	C	T	1	1	.	*ALK*	.	caG/caA	p.Q1159Q	synonymous variant	Novel
2	29445259	A	G	1	.	1	*ALK*	.	Tgc/Cgc	p.C1156R	missense variant	Novel
2	29445267	G	A	3	1	2	*ALK*	.	cCt/cTt	p.P1153L	missense variant	Novel
2	29445268	G	A	4	2	2	*ALK*	.	Cct/Tct	p.P1153S	missense variant	Novel
2	29445269	C	T	8	6	2	*ALK*	.	ctG/ctA	p.L1152L	synonymous variant	Novel
2	29445270	A	G	10	9	1	*ALK*	.	cTg/cCg	p.L1152P	missense variant	Novel
2	29445272	C	T	5	3	2	*ALK*	rs145061595	acG/acA	p.T1151T	synonymous variant	Known
2	29445273	G	A	8	4	4	*ALK*	rs113994091	aCg/aTg	p.T1151M	missense variant	Known
2	29445274	T	C	12	12	.	*ALK*	.	Acg/Gcg	p.T1151A	missense variant	Novel
3	178936082	G	A	2	.	2	*PIK3CA*	rs121913273	Gaa/Aaa	p.E542K	missense variant	Known
3	178936091	G	A	1	1	.	*PIK3CA*	rs104886003	Gag/Aag	p.E545K	missense variant	Known
3	178936091	G	C	1	.	1	*PIK3CA*	rs104886003	Gag/Cag	p.E545Q	missense variant	Known
3	178936094	C	A	3	2	1	*PIK3CA*	rs121913286	Cag/Aag	p.Q546K	missense variant	Known
3	178952068	A	G	1	1	.	*PIK3CA*	.	aaA/aaG	p.K1041K	synonymous variant	Novel
3	178952085	A	G	1	1	.	*PIK3CA*	rs121913279	cAt/cGt	p.H1047R	missense variant	Known
3	178952085	A	T	1	.	1	*PIK3CA*	rs121913279	cAt/cTt	p.H1047L	missense variant	Known
7	55241663	T	C	3	2	1	*EGFR*	.	tTg/tCg	p.L704S	missense variant	Novel
7	55241664	G	A	1	.	1	*EGFR*	.	ttG/ttA	p.L704L	synonymous variant	Known
7	55241666	G	T	1	1	.	*EGFR*	.	aGg/aTg	p.R705M	missense variant	Novel

7	55241675	A	G	1	.	1	*EGFR*	.	aAg/aGg	p.K708R	missense variant	Known
7	55241676	G	A	1	.	1	*EGFR*	.	aaG/aaA	p.K708K	synonymous variant	Novel
7	55241683	G	A	1	.	1	*EGFR*	.	Gaa/Aaa	p.E711K	missense variant	Known
7	55241685	A	G	2	1	1	*EGFR*	.	gaA/gaG	p.E711E	synonymous variant	Known
7	55241695	A	G	1	1	.	*EGFR*	.	Atc/Gtc	p.I715V	missense variant	Novel
7	55241703	G	A	1	.	1	*EGFR*	.	gtG/gtA	p.V717V	synonymous variant	Novel
7	55241706	G	T	1	.	1	*EGFR*	rs397517088	ctG/ctT	p.L718L	synonymous variant	Known
7	55241707	G	A	2	1	1	*EGFR*	rs28929495	Ggc/Agc	p.G719S	missense variant	Known
7	55241713	G	A	1	.	1	*EGFR*	.	Ggt/Agt	p.G721S	missense variant	Known
7	55241715	T	C	1	.	1	*EGFR*	.	ggT/ggC	p.G721G	synonymous variant	Novel
7	55241717	C	T	1	1	.	*EGFR*	rs762494280	gCg/gTg	p.A722V	missense variant	Known
7	55241718	G	A	1	.	1	*EGFR*	rs367694667	gcG/gcA	p.A722A	synonymous variant	Known
7	55241719	T	C	1	1	.	*EGFR*	.	Ttc/Ctc	p.F723L	missense variant	Known
7	55241727	G	A	4	.	4	*EGFR*	rs55959834	acG/acA	p.T725T	synonymous variant	Known
7	55242464	AGGAATTAAGAGAAGC	A	17	9	8	*EGFR*	.	aaGGAATTAAGAGAAGCa/aaa	p.KELREA745-750K	inframe indel	Known
7	55242464 and 55242478	AGGAATTAAGAGAAGC and G	A and C	3	2	1	*EGFR*	. and rs121913229	aaGGAATTAAGAGAAGCa/aaa and Gca/Cca	p.KELREA745-750K and p.A750P	inframe indel	Known
7	55242464 and 55242478 and 55242480	AGGAATTAAGAGAAGC and G and A	A and C and C	1	.	1	*EGFR*	. and rs121913229 and .	aaGGAATTAAGAGAAGCa/aaa and Gca/Cca and gcA/gcC	p.KELREA745-750K and p.A750P and p.A750A	inframe indel	Novel
7	55242464 and 55242495	AGGAATTAAGAGAAGC and C	A and T	1	1	.	*EGFR*	.	aaGGAATTAAGAGAAGCa/aaa and gcC/gcT	p.KELREA745-750K and p.A755A	inframe indel	Novel
7	55242488	C	T	2	.	2	*EGFR*	rs559717059	cCg/cTg	p.P753L	missense variant	Known
7	55242489	G	A	1	.	1	*EGFR*	rs764064214	ccG/ccA	p.P753P	synonymous variant	Known
7	55242510	C	T	1	1	.	*EGFR*	rs397517103	ctC/ctT	p.L760L	synonymous variant	Known

7	55242511	G	A	2	.	2	*EGFR*	rs121913418	Gat/Aat	p.D761N	missense variant	Known
7	55248982	C	T	1	1	.	*EGFR*	rs766533982	.	.	intron variant	Known
7	55248986	G	A	1	.	1	*EGFR*	.	Gaa/Aaa	p.E762K	missense variant	Known
7	55248991	C	T	1	.	1	*EGFR*	.	gcC/gcT	p.A763A	synonymous variant	Known
7	55248994	C	T	1	1	.	*EGFR*	rs755011268	taC/taT	p.Y764Y	synonymous variant	Known
7	55248995	G	A	1	.	1	*EGFR*	rs374873413	Gtg/Atg	p.V765M	missense variant	Known
7	55248997	G	A	1	.	1	*EGFR*	.	gtG/gtA	p.V765V	synonymous variant	Novel
7	55248998	A	ATGGCCAGCG	2	1	1	*EGFR*	rs727504263	atg/aTGGCCAGCGtg	p.M766MASV	inframe indel	Known
7	55249003	C	T	1	1	.	*EGFR*	.	gcC/gcT	p.A767A	synonymous variant	Known
7	55249006	C	T	2	.	2	*EGFR*	rs778199483	agC/agT	p.S768S	synonymous variant	Known
7	55249021	C	T	1	1	.	*EGFR*	rs768468531	caC/caT	p.H773H	synonymous variant	Known
7	55249032	T	C	1	.	1	*EGFR*	.	cTg/cCg	p.L777P	missense variant	Novel
7	55249036	G	A	1	1	.	*EGFR*	rs397517118	ctG/ctA	p.L778L	synonymous variant	Known
7	55249038	G	A	1	.	1	*EGFR*	.	gGc/gAc	p.G779D	missense variant	Known
7	55249039	C	T	1	.	1	*EGFR*	rs1487193140	ggC/ggT	p.G779G	synonymous variant	Known
7	55249058	G	A	1	1	.	*EGFR*	rs762672864	Gtg/Atg	p.V786M	missense variant	Known
7	55249063	G	A	5	.	5	*EGFR*	rs1050171	caG/caA	p.Q787Q	synonymous variant	Known
7	55249071	C	T	1	1	.	*EGFR*	rs121434569	aCg/aTg	p.T790M	missense variant	Known
7	55249072	G	A	3	.	3	*EGFR*	rs376452156	acG/acA	p.T790T	synonymous variant	Known
7	55249078	C	T	1	1	.	*EGFR*	.	ctC/ctT	p.L792L	synonymous variant	Known
7	55249087	C	T	1	.	1	*EGFR*	rs375332959	ttC/ttT	p.F795F	synonymous variant	Known
7	55249088	G	A	1	.	1	*EGFR*	rs754426793	Ggc/Agc	p.G796S	missense variant	Known
7	55249099	G	A	1	.	1	*EGFR*	.	ctG/ctA	p.L799L	synonymous variant	Novel
7	55249100	G	A	1	1	.	*EGFR*	.	Gac/Aac	p.D800N	missense variant	Known

7	55249109	C	T	1	.	1	*EGFR*	rs1215679186	Cgg/Tgg	p.R803W	missense variant	Known
7	55249110	G	A	2	.	2	*EGFR*	rs1263602634	cGg/cAg	p.R803Q	missense variant	Known
7	55249123	C	T	1	.	1	*EGFR*	.	gaC/gaT	p.D807D	synonymous variant	Novel
7	55259501	C	T	1	.	1	*EGFR*	.	atC/atT	p.I853I	synonymous variant	Known
7	55259515	TG	GT	1	.	1	*EGFR*	rs1057519848	cTG/cGT	p.L858R	missense variant	Known
7	55259515	T	G	7	2	5	*EGFR*	rs121434568	cTg/cGg	p.L858R	missense variant	Known
7	55259522	A	G	1	1	.	*EGFR*	.	aaA/aaG	p.K860K	synonymous variant	Known
7	55259523	C	T	1	.	1	*EGFR*	.	Ctg/Ttg	p.L861L	synonymous variant	Known
7	55259524	T	A	1	.	1	*EGFR*	rs121913444	cTg/cAg	p.L861Q	missense variant	Known
7	55259530	G	A	1	.	1	*EGFR*	rs909797662	gGt/gAt	p.G863D	missense variant	Known
7	55259534	G	A	1	.	1	*EGFR*	rs397517131	gcG/gcA	p.A864A	synonymous variant	Known
7	116339626	C	T	1	.	1	*MET*	.	tCc/tTc	p.S163F	missense variant	Novel
7	116339672	C	T	1	.	1	*MET*	rs35775721	agC/agT	p.S178S	synonymous variant	Known
7	116412011	A	G	1	1	.	*MET*	.	gAa/gGa	p.E999G	missense variant	Novel
7	116412022	T	C	2	1	1	*MET*	.	Tac/Cac	p.Y1003H	missense variant	Novel
7	116412025	C	T	1	.	1	*MET*	rs766865557	Cga/Tga	p.R1004*	stop gained	Known
7	116412026	G	A	1	.	1	*MET*	.	cGa/cAa	p.R1004Q	missense variant	Known
7	116412043	G	C	1	.	1	*MET*	.	Gat/Cat	p.D1010H	missense variant	Known
7	140453128	G	A	4	2	2	*BRAF*	rs104886015	Cga/Tga	p.R603*	stop gained	Known
7	140453136	A	T	3	1	2	*BRAF*	rs113488022	gTg/gAg	p.V600E	missense variant	Known
7	140453145	A	T	2	1	1	*BRAF*	rs121913366	cTa/cAa	p.L597Q	missense variant	Known
7	140453157	C	T	1	.	1	*BRAF*	.	gGt/gAt	p.G593D	missense variant	Known
7	140481400	T	C	1	.	1	*BRAF*	.	Aca/Gca	p.T470A	missense variant	Known
12	25380251	G	A	1	.	1	*KRAS*	.	gaC/gaT	p.D69D	synonymous variant	Known

12	25380275	T	A	2	1	1	*KRAS*	rs17851045	caA/caT	p.Q61H	missense variant	Known
12	25380275	T	G	2	1	1	*KRAS*	rs17851045	caA/caC	p.Q61H	missense variant	Known
12	25380275	T	C	1	.	1	*KRAS*	.	caA/caG	p.Q61Q	synonymous variant	Known
12	25398279	C	T	2	2	.	*KRAS*	rs104894365	Gta/Ata	p.V14I	missense variant	Known
12	25398280	G	A	1	1	.	*KRAS*	rs397517040	ggC/ggT	p.G13G	synonymous variant	Known
12	25398281	C	T	1	1	.	*KRAS*	rs112445441	gGc/gAc	p.G13D	missense variant	Known
12	25398284	C	A	5	2	3	*KRAS*	rs121913529	gGt/gTt	p.G12V	missense variant	Known
12	25398284	C	T	5	2	3	*KRAS*	rs121913529	gGt/gAt	p.G12D	missense variant	Known
12	25398284	C	G	3	2	1	*KRAS*	rs121913529	gGt/gCt	p.G12A	missense variant	Known
12	25398285	C	A	18	10	8	*KRAS*	rs121913530	Ggt/Tgt	p.G12C	missense variant	Known
12	25398287	G	A	1	.	1	*KRAS*	.	gCt/gTt	p.A11V	missense variant	Known
12	25398295	TACC	T	2	1	1	*KRAS*	.	gtGGTa/gta	p.VV7-8V	inframe indel	Novel
12	25398299	A	G	1	.	1	*KRAS*	.	gTg/gCg	p.V7A	missense variant	Known
14	105246532	C	T	3	3	.	*AKT1*	.	cGg/cAg	p.R23Q	missense variant	Known
14	105246533	G	A	3	.	3	*AKT1*	.	Cgg/Tgg	p.R23W	missense variant	Possible known artefact
14	105246541	T	C	1	.	1	*AKT1*	.	aAg/aGg	p.K20R	missense variant	Novel
14	105246543	G	A	2	.	2	*AKT1*	.	atC/atT	p.I19I	synonymous variant	Possible known artefact
14	105246546	G	A	1	.	1	*AKT1*	rs1317567840	taC/taT	p.Y18Y	synonymous variant	Known
14	105246550	T	C	1	.	1	*AKT1*	.	gAg/gGg	p.E17G	missense variant	Known
14	105246561	C	T	2	.	2	*AKT1*	rs770565457	.	.	intron variant	Known
14	105246562	G	A	1	1	.	*AKT1*	rs776342530	.	.	intron variant	Known
17	37880218	G	A	1	.	1	*ERBB2*	.	gtG/gtA	p.V754V	synonymous variant	Novel
17	37880224	G	A	1	1	.	*ERBB2*	.	agG/agA	p.R756R	synonymous variant	Known

17	37880227	A	G	1	1	.	*ERBB2*	.	gaA/gaG	p.E757E	synonymous variant	Novel
17	37880229	A	G	1	1	.	*ERBB2*	.	aAc/aGc	p.N758S	missense variant	Novel
17	37880238	C	T	1	.	1	*ERBB2*	.	cCc/cTc	p.P761L	missense variant	Known
17	37880247	A	G	1	1	.	*ERBB2*	.	aAc/aGc	p.N764S	missense variant	Novel
17	37880987	C	T	1	.	1	*ERBB2*	rs748318804	taC/taT	p.Y772Y	synonymous variant	Known
17	37880988	G	A	2	.	2	*ERBB2*	rs772054394	Gtg/Atg	p.V773M	missense variant	Known
17	37881003	G	A	1	.	1	*ERBB2*	.	Ggc/Agc	p.G778S	missense variant	Known
17	37881019	C	A	1	.	1	*ERBB2*	.	tCc/tAc	p.S783Y	missense variant	Novel

T3. Number of times somatic mutation was called according to the sample type

-, no individuals with mutations

	0	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***13***
cfDNA T1	43	33	20	6	2	1	-	1	-	-	-	-
cfDNA T2	15	4	3	-	-	-	-	-	-	-	-	-
FFPE DNA	15	28	13	6	5	2	1	-	1	2	1	1

T4. Detected *EGFR* gene mutations

Neg, no EGFR mutation detected; x, no FFPE material available.

Patient	Diagnostic labs	Next generation sequencing	
		cfDNA T1	FFPE DNA
kA02	ex19del	ex19del	ex19del
kA10	p.L858R	neg	x
kA16	neg	neg	L861Q
kA19	p.L858R	neg	p.L858R
kA20	ex19del	ex19del	x
kA24	p.L858R	neg	p.L858R
kA26	ex19del	ex19del	x
kA27	neg	neg	ex19del
kA28	ex19del	ex19del	ex19del
kA33	ex19del	ex19del and p.T790M	ex19del
kA47	p.L858R	p.L858R	p.L858R
kA48	ex19del	ex19del	ex19del
kA54	neg	neg	ex19del
kA59	ex19del	ex19del	ex19del
kA62	ex19del	ex19del	x
kA63	ex19del	ex19del	ex19del
kA67	ex19del	ex19del	ex19del
kA74	neg	neg	p.L858R

kA78	p.L858R	neg	p.L858R
kA82	ex19del	ex19del	ex19del
kA84	ex20ins	ex20ins	ex20ins
kA92	p.G719S	p.G719S	p.G719S
kB03	neg	p.L858R	p.L858R

T5. Mutations found in patients' cfDNA who had cancer progression

Two patients had no mutations in their T1 or T2 cfDNA sample, and are therefore absent from the table.

AA, amino acid; T1, time point at baseline; T2, time point at progression; VAF, variant allele frequency; OS, overall survival; ., no mutation found at that time point

Sample ID	AA change	Gene Name	T1 VAF %	T2 VAF %	OS
kA05	p.A1168A	*ALK*	0,99%	.	1215
	p.H773H	*EGFR*	1,54%	.	
	p.Y764Y	*EGFR*	0,98%	.	
kA10	p.D1160D	*ALK*	1,04%	.	1144
kA20	p.KELREA745-750K	*EGFR*	4,90%	.	1073
	p.E545K	*PIK3CA*	.	0,83%	
kA25	p.L1152P	*ALK*	1,03%	.	1002
kA27	intron	*AKT1*	0,97%	.	1664
	p.L1152L	*ALK*	0,95%	.	
kA28	p.D1160G	*ALK*	0,91%	.	514
	p.R603*	*BRAF*	0,84%	.	
	p.KELREA745-750K and p.A750P	*EGFR*	54,00%	.	
kA38	p.G12C	*KRAS*	1,55%	6,22%	495
kA40	p.G12C	*KRAS*	6,20%	.	1633
kA47	p.L1152L	*ALK*	.	1,53%	1103
	p.Q1159Q	*ALK*	0,84%	.	
	p.F723L	*EGFR*	0,87%	.	
	p.I715V	*EGFR*	1,04%	.	

	p.L858R	*EGFR*	5,97%	.	
	p.N764S	*ERBB2*	.	0,82%	
kA49	p.L704S	*EGFR*	0,97%	.	801
	p.E757E	*ERBB2*	0,87%	.	
kA51	p.T1151A	*ALK*	.	0,84%	149
	p.T1151T	*ALK*	1,41%	.	
	p.G12C	*KRAS*	11,35%	12,74%	
kA59	p.KELREA745-750K	*EGFR*	0,81%	.	873
kA63	p.KELREA745-750K	*EGFR*	12,20%	53,80%	171
	p.Q546K	*PIK3CA*	6,97%	45,10%	
kA67	intron	*EGFR*	0,83%	.	758
	p.KELREA745-750K	*EGFR*	1,00%	.	
kA75	p.L1152P	*ALK*	1,09%	.	1485
	p.S1172G	*ALK*	0,90%	.	
	p.E711E	*EGFR*	3,49%	.	
	p.L792L	*EGFR*	0,80%	.	
	p.R705M	*EGFR*	0,92%	.	
	p.V14I	*KRAS*	0,88%	.	
	p.E999G	*MET*	1,45%	.	
kA76	p.R23Q	*AKT1*	.	0,98%	252
kA82	p.T1151A	*ALK*	.	1,01%	363
	p.KELREA745-750K	*EGFR*	79,40%	.	

kA84	p.M766MASV	*EGFR*	0,90%	.	1425
kB01	p.V14I	*KRAS*	0,87%	.	195
kB07	p.T1151A	*ALK*	1,42%	.	207
	p.T1151T	*ALK*	1,52%	.	