

Circulating tumor DNA detection in lung cancer patients before and after surgery

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Supplementary Table S1. Mutations and mutation frequencies identified in tDNA and plasma ctDNA before and after surgery.

Patient no.	Gene	Mutation type	AA mutation	% mutation in tDNA (reads)	% mutation in pre-op ctDNA (reads)	% mutation in post-op ctDNA (reads)	% mutation in WBC (reads)	Detected in tDNA/ctDNA
1	EGFR	SNP	p.L858R	4.00 (23606)	2.33 (17676)	0.41 (15955)	0.00 (19588)	Y/Y
2	EGFR	DEL	p.K745_E749del	11.17 (20207)	0.00 (27639)	0.00 (22599)	0.00 (13271)	Y/N
3	TP53	SNP	p.E204*	34.84 (11212)	0.10 (37966)	0.10 (12839)	0.00 (20164)	Y/Y
	TP53	SNP	p.V203V	35.04 (11255)	0.15 (37975)	0.06 (12840)	0.00 (20138)	Y/Y
4	EGFR	SNP	p.L858R	19.54 (9970)	1.33 (21980)	0.02 (4975)	0.00 (7505)	Y/Y
6	TP53	DEL	p.S303fs*42	17.47 (3681)	6.80 (9809)	0.00 (4987)	0.00 (2047)	Y/Y
9	EGFR	DEL	p.E746_A750delELREA	13.46 (9685)	4.06 (9997)	0.00 (10000)	0.00 (9799)	Y/Y
10	EGFR	SNP	p.L858R	4.44 (17785)	0.94 (28900)	0.50 (12618)	0.00 (9527)	Y/Y
	EGFR	SNP	p.G810D	4.32 (14110)	0.16 (65062)	0.07 (37789)	0.00 (8381)	Y/Y
11	KRAS	SNP	p.G13C	19.64 (560)	0.01 (15793)	0.01 (20036)	0.00 (851)	Y/N
	BRAF	SNP	p.S605G	0.00 (189)	0.99 (15824)	0.32 (13517)	0.00 (219)	N/Y
12	EGFR	DEL	p.E746_A750delELREA	6.63 (7149)	0.00 (22096)	0.00 (18587)	0.00 (2460)	Y/N
	EGFR	SNP	p.L858R	0.06 (16602)	26.44 (16791)	0.02 (22712)	0.00 (3463)	N/Y
14	BRAF	SNP	p.V600E	1.38 (799)	0.01 (7174)	0.01 (11450)	0.00 (619)	Y/N
	EGFR	SNP	p.L858R	6.34 (6909)	81.06 (4689)	0.33 (23855)	0.00 (2548)	Y/Y
15	EGFR	SNP	p.S768I	7.82 (307)	0.01 (15966)	0.00 (2236)	0.00 (351)	Y/N
16	EGFR	DEL	p.L747_P753>S	15.48 (1169)	0.00 (51613)	0.00 (21521)	0.00 (1180)	Y/N
18	PIK3CA	SNP	p.K111R	9.16 (764)	0.46 (7671)	0.36 (7291)	0.00 (280)	Y/Y
19	EGFR	DEL	p.E746_A750delELREA	26.02 (2563)	0.00 (26959)	0.00 (11927)	0.00 (6964)	Y/N
21	KRAS	SNP	p.G12D	0.16 (1256)	2.26 (19251)	0.15 (15248)	0.00 (1569)	N/Y
	EGFR	SNP	p.L858R	10.26 (10608)	0.16 (18354)	0.05 (14744)	0.00 (5944)	Y/Y
22	EGFR	SNP	p.L858R	0.10 (4919)	1.31 (15007)	0.08 (18930)	0.00 (5398)	N/Y
24	EGFR	DEL	p.L747_P753>S	17.87 (414)	0.00 (21464)	0.00 (22992)	0.00 (11647)	Y/N
27	EGFR	SNP	p.L858R	30.06 (6663)	0.19 (14512)	0.02 (11352)	0.00 (6387)	Y/Y
28	EGFR	SNP	p.L858R	29.67 (6930)	0.29 (14815)	0.01 (14138)	0.00 (10015)	Y/Y
30	EGFR	SNP	p.L858R	83.71 (23376)	75.45 (81301)	0.13 (11140)	0.00 (6041)	Y/Y
33	EGFR	SNP	p.L858R	2.44 (4018)	0.035 (22648)	0.036 (18954)	0.00 (9395)	Y/N
34	EGFR	DEL	p.L747_P753>S	73.26 (25063)	0.04 (28813)	0.04 (21551)	0.00 (3241)	Y/N
	TP53	SNP	p.C238F	34.95 (8931)	0.00 (57159)	0.01 (59452)	0.00 (7188)	Y/N
35	ERBB2	INS	p.A775_G776insYVMA	54.7 (8931)	0.00 (57159)	0.00 (59452)	0.00 (5756)	Y/N
36	EGFR	SNP	p.L858R	16.92 (4220)	0.78 (17892)	0.14 (13248)	0.00 (11387)	Y/Y
	EGFR	DEL	p.E746_A750delELREA	0.00 (3164)	3.53 (25172)	0.06 (24795)	0.00 (8245)	N/Y
37	EGFR	SNP	p.L858R	25.39 (2225)	1.36 (15867)	0.24 (15465)	0.00 (2847)	Y/Y
	EGFR	SNP	p.L861Q	0.00 (2242)	1.34 (15918)	0.19 (15554)	0.00 (2825)	N/Y
38	EGFR	SNP	p.L858R	2.60 (5452)	1.46 (16551)	3.01 (11418)	0.00 (5505)	Y/Y
44	KRAS	SNP	p.G13D	20.71 (1811)	0.26 (25839)	0.02 (13372)	0.00 (1735)	Y/Y

SNP: single nucleotide polymorphism; DEL: deletion; INS: insert; WBC: white blood cells

Supplementary Table S2. Concordance, specificity, sensitivity, and positive predictive value calculations for all samples in the study.

	Plasma mutation status		
	Positive	Negative	Total
Tumor mutation status			
Positive	18	8	26
Negative	1	14	15
Total	19	22	41

	n	Rate (%)	95% Confidence interval (%)	
Concordance	41	78.05		
Sensitivity	26	69.23	48.10	84.91
Specificity	15	93.33	66.03	99.65
Positive-predictive value	19	94.74	71.89	99.72

Supplementary Table S3. Concordance, specificity, sensitivity, and positive predictive value calculations for early stage (I-II) samples in the study.

	Plasma mutation status		
	Positive	Negative	Total
Tumor mutation status			
Positive	15	5	20
Negative	1	9	10
Total	16	14	30

	n	Rate (%)	95% Confidence interval (%)	
Concordance	30	80.00		
Sensitivity	20	75.00	50.59	90.41
Specificity	10	90.00	54.12	99.48
Positive-predictive value	16	93.75	67.71	99.67

Supplementary Table S4. Concordance, specificity, sensitivity, and positive predictive value calculations for late stage (III-IV) samples in the study.

	Plasma mutation status		
	Positive	Negative	Total
Tumor mutation status			
Positive	3	3	6
Negative	0	5	5
Total	3	8	11

	n	Rate (%)	95% Confidence interval (%)	
Concordance	11	72.73		
Sensitivity	6	50.00	13.95	86.05
Specificity	5	100.00	46.29	100.00
Positive-predictive value	3	100.00	40.00	100.00

Supplementary Table S5. Patient cfDNA concentrations before and after surgery (ng/μl).

Patient no.	Pre-op cfDNA (ng/μl)	Post-op cfDNA (ng/μl)	Change in cfDNA concentration (+/-)
1	0.964	1.07	+0.106
2	1.39	1.45	+0.06
3	0.848	1.36	+0.512
4	0.50	1.16	+0.66
5	0.464	0.738	+0.274
6	0.952	1.06	+0.108
7	1.04	0.994	-0.046
9	0.694	1.52	+0.826
10	0.968	1.06	+0.092
11	0.688	0.618	-0.07
12	0.664	0.602	-0.062
14	0.702	0.58	-0.122
15	0.636	0.796	+0.16
16	0.624	0.592	-0.032
17	0.428	0.654	+0.226
18	0.542	0.746	+0.204
19	0.478	1.02	+0.542
20	0.344	0.40	+0.056
21	0.516	0.853	+0.337
22	0.424	0.388	-0.036
23	0.444	0.91	+0.466
24	0.908	0.978	+0.07
25	0.79	1.25	+0.46
26	0.564	0.904	+0.34
27	0.442	0.618	+0.176
28	0.516	0.79	+0.274
29	0.34	1.85	+1.51
30	0.422	0.558	+0.136
31	0.492	0.686	+0.194
33	0.406	0.396	-0.01
34	0.36	0.38	+0.02
35	0.40	0.64	+0.24
36	0.334	0.41	+0.076
37	0.294	0.594	+0.30
38	0.478	0.386	-0.092
39	0.104	0.364	+0.26
40	0.49	1.57	+1.08
41	0.276	0.256	-0.02
42	0.284	0.176	-0.108
44	0.23	0.14	-0.09
45	0.14	0.246	+0.106

Supplementary Table S6. Tumor biomarker levels of patients. The positive cut-off values were defined as follows: CA125 >35 U/ml, CA19-9 >39 U/ml, CEA >4.7 ng/ml, CYFRA21-1 >3.3ng/ml, NSE >16.3 ng/ml, and SCC >1.5 ng/ml. (ND: not detected)

Patient no.	CEA (µg/L)	CA125 (U/mL)	CA19-9 (U/mL)	CYFRA21-1 (ng/ml)	NSE (ng/ml)	SCC (ng/ml)
1	16.67	26.69	65.79	4.40	11.04	0.70
2	ND	ND	ND	ND	ND	ND
3	2.58	ND	ND	3.16	12.34	0.70
4	10.59	11.46	7.39	2.15	9.35	0.90
5	16.57	18.33	8.02	2.47	12.5	0.60
6	95.98	11.93	13.36	19.33	26.0	10.60
7	3.76	4.77	6.39	4.54	10.45	0.60
9	4.39	25.84	9.8	2.08	13.03	1.00
10	28.68	10.1	11.05	4.34	11.56	1.10
11	6.3	14.46	7.32	1.02	13.78	1.10
12	2.62	29.61	16.9	0.94	11.19	1.10
14	3.35	19.51	1.00	1.65	13.62	0.80
15	3.91	12.44	18.52	1.80	9.81	0.90
16	ND	ND	ND	ND	ND	ND
17	3.45	9.51	8.07	1.62	6.75	0.70
18	3.77	24.09	9.92	11.91	26.77	1.10
19	3.95	12.23	4.61	2.31	12.44	0.80
20	1.78	3.17	7.80	2.63	12.78	0.90
21	2.49	7.96	7.26	2.09	8.93	0.60
22	0.82	15.34	18.87	2.62	12.27	0.60
23	2.11	10.87	14.22	1.39	14.78	0.60
24	40.7	164.9	29.61	6.33	11.44	0.90
25	2.46	9.94	11.83	1.96	15.96	0.70
26	8.51	5.08	5.05	2.11	16.00	0.40
27	1.78	20.41	8.71	1.77	9.06	0.40
28	ND	ND	ND	ND	ND	ND
29	ND	ND	ND	ND	ND	ND
30	154.9	26.94	16.13	11.55	16.49	0.60
31	ND	ND	ND	ND	ND	ND
33	7.04	19.33	8.41	118.7	26.11	4.50
34	1.28	5.26	10.2	3.75	13.71	0.60
35	18.56	12.8	11.07	10.25	14.79	1.20
36	ND	ND	ND	ND	ND	ND
37	4.35	8.14	4.32	2.03	10.45	0.40
38	1.29	6.00	7.19	1.23	9.13	0.60
39	2.02	11.23	16.18	3.07	7.65	3.50
40	3.51	26.66	42.91	2.38	16.42	0.90
41	4.55	174.5	24.4	25.79	19.81	1.90
42	0.76	13.16	5.34	2.13	11.84	1.1
44	2.00	4.53	7.52	3.32	8.19	1.4
45	3.49	20.29	10.04	4.62	10.89	3.5