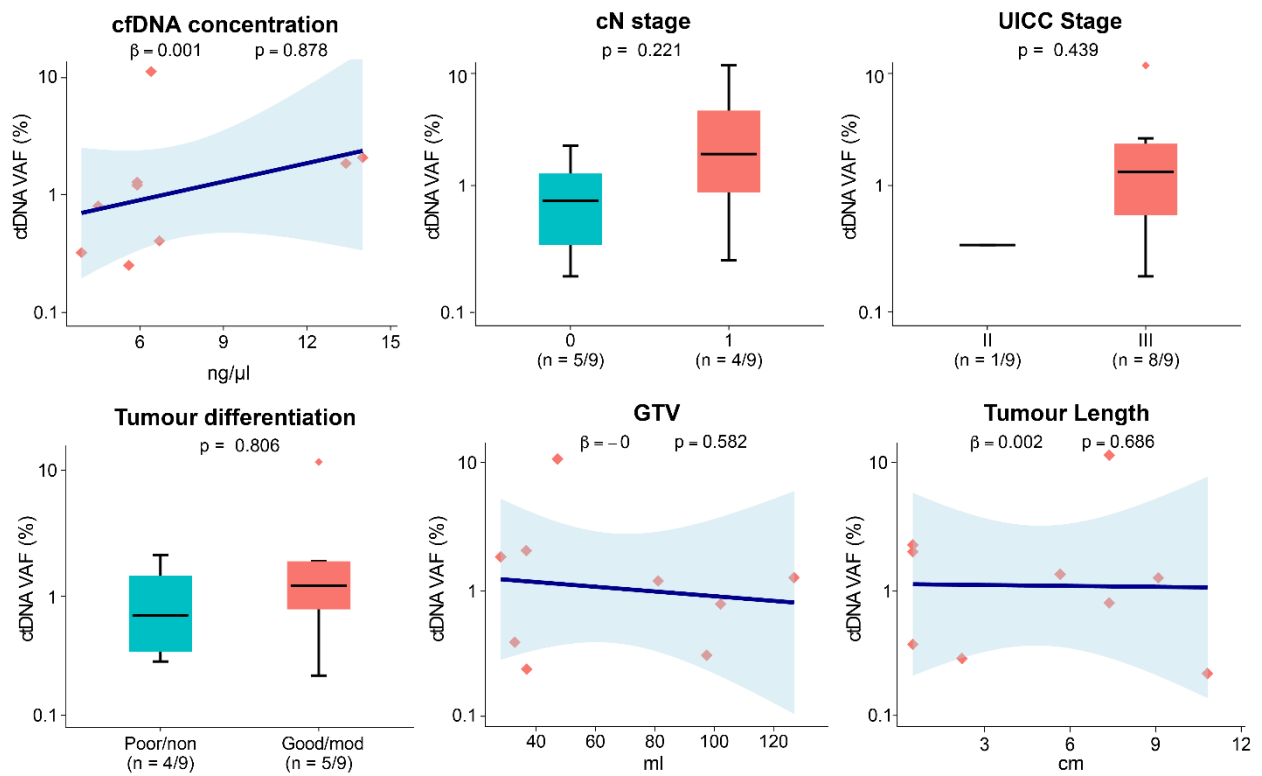


# Detection of circulating tumour DNA after neoadjuvant chemoradiotherapy in patients with locally advanced oesophageal cancer

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## Supplementary Figure S1

### Supplementary Tables S1–S2



**Figure S1.** Correlation of baseline tumour characteristics with variant allele frequency (VAF) of nine pre-treatment ctDNA-positive patients. Patients for whom data were missing were excluded from the analysis. cN, clinical nodal; UICC, Union for International Cancer Control; GTV, gross tumour volume.

**Table S1.** Input information of the analysed tissue and plasma samples.

Study ID	Tissue samples for NGS				cfDNA samples for NGS-UMI			cfDNA samples for dPCR		
	t = 0 tumour biopsies		Resection specimens		t = 0	t = 1	t = 2	t = 0	t = 1	t = 2
	Tumour area (%)	DNA concentration (ng/μl)	Tumour area (%)	DNA concentration (ng/μl)	DNA input (ng)	DNA input (ng)	DNA input (ng)	DNA input (ng)	DNA input (ng)	DNA input (ng)
1	60%	1.57	NTR	–	20.0	21.0	21.0	25.7	39.1	38.7
2	30%	1.70	70%	1.85	20.0	15.0	10.0	–	–	–
3	90%	1.06	NTR	–	14.0	20.0	21.0	1.1	5.5	6.9
4	80%	4.16	60%	1.03	21.5	20.0	14.0	–	–	–
5	50%	3.98	NR	–	3.8	15.0	3.8	15.7	18.9	29.5
6	50%	1.70	70%	13.50	3.8	10.2	20.0	–	–	–
7	40%	1.40	40–50%	4.10	4.0	5.1	–	9.6	12.5	–
8	60%	7.10	70–80%	15.30	4.0	18.2	5.0	8.4	24.8	28.3
9	40%	1.80	NR	–	4.0	–	–	–	–	–
10	70%	10.80	70%	15.80	4.0	31.8	–	9.3	17.6	–
11	60%	5.50	80%	15.50	4.0	5.9	–	–	–	–
12	70%	16.10	NR	–	4.0	4.5	N/A	19.0	29.8	26.4
13	80%	9.31	90%	17.60	–	–	–	–	–	–
14	80%	3.90	NR	–	–	–	–	–	–	–
15	80%	6.92	NR	–	4.0	–	–	–	–	–
16	60%	12.10	NR	–	–	–	–	–	–	–
17	50%	4.02	70%	0.96	20.0	20.0	–	–	–	–
18	60%	7.31	NTR	–	–	–	–	14.8	5.9	4.8
19	N/A	2.79	NTR	–	15.3	23.0	21.0	–	–	–
20	N/A	N/A	80%	5.34	N/A	21.0	–	–	–	–
21	60%	20.70	NTR	–	–	–	–	–	–	–
22	70%	5.04	80%	6.91	–	–	–	–	–	–
23	70%	9.65	NTR	–	–	–	–	–	–	–
24	50%	0.76	70%	0.48	20.7	20.0	20.0	0.0	3.5	21.3
25	70%	2.89	95%	10.10	20.8	20.0	21.0	19.1	20.2	2.9
26	70%	1.15	60%	1.42	–	–	–	–	–	–
27	70%	0.91	60%	13.10	15.4	24.0	–	–	–	–
28	80%	4.05	60%	2.30	4.0	3.3	1.0	9.3	13.0	14.9
29	70%	12.30	60%	1.75	3.9	4.9	–	6.6	13.3	–
30	80%	5.90	NR	–	3.9	–	–	20.9	–	–
31	70%	7.10	NR	–	3.9	130.2	N/A	13.3	21.7	N/A

NGS, next-generation sequencing; cfDNA, circulating cell-free DNA; NGS-UMI, next-generation sequencing with unique molecular identifiers; dPCR, digital polymerase chain reaction; NTR, no tumour residue in resection specimen; NR, no resection performed; ND, no data; N/A, not available.

**Table S2.** Mutations identified in baseline ( $t = 0$ ) tissue that were potentially detectable in circulating cell-free DNA (cfDNA) with corresponding findings in cfDNA at the baseline ( $t = 0$ ) and 6 weeks ( $t = 1$ ) and 12 weeks ( $t = 2$ ) after neoadjuvant chemoradiotherapy. Findings shown in bold were considered to be ctDNA-positive.

ID	$t = 0$ tissue				cfDNA analysis	$t = 0$ cfDNA						$t = 1$ cfDNA						$t = 2$ cfDNA					
	NGS					NGS-UMI			dPCR			NGS-UMI			dPCR			NGS-UMI			dPCR		
	Gene	CDS mutation	AA mutation	VAF (%)		Mol cov	Mut mol	VAF (%)	WT cop	Mut cop	VAF (%)	Mol cov	Mut mol	VAF (%)	WT cop	Mut cop	VAF (%)	Mol cov	Mut mol	VAF (%)	WT cop	Mut cop	VAF (%)
1	TP53	c.524G>A	p.R175H	69.2%	O/P	2968	1	0.03%	5558	0	0.00%	5091	2	0.04%	13980	0	0.00%	4433	0	0.00%	11922	0	0.00%
2	TP53	c.527G>T	p.C176F	45.1%	O	1248	0	0.00%	–	–	–	2637	0	0.00%	–	–	–	1391	0	0.00%	–	–	–
3	TP53	c.742C>T	p.R248W	41.9%	O/P	1377	0	0.00%	1168	0	0.00%	2598	0	0.00%	3606	0	0.00%	N/A	N/A	N/A	4340	0	0.00%
4	TP53	c.709_733dup	p.G245Dfs*3	48.2%	O	1563	0	0.00%	–	–	–	3457	0	0.00%	–	–	–	2532	0	0.00%	–	–	–
5	TP53	c.455C>T	p.P152L	64.1%	O/P	1536	1	0.07%	2733	0	0.00%	2259	0	0.00%	0	0	0.00%	3362	2	0.06%	0	0	0.00%
6	TP53	c.659A>G	p.Y220C	68.9%	O	888	0	0.00%	–	–	–	1513	0	0.00%	–	–	–	1802	0	0.00%	–	–	–
7	AKT1	c.49G>A	p.E17K	55.3%	O	1162	0	0.00%	–	–	–	791	0	0.00%	–	–	–	–	–	–	–	–	–
7	TP53	c.524G>A	p.R175H	50.5%	O/P	1314	0	0.00%	2654	0	0.00%	950	0	0.00%	1075	0	0.00%	–	–	–	–	–	–
8	TP53	c.818G>A	p.R273H	54.3%	O/P	1094	1	0.09%	1282	4	0.30%	1886	0	0.00%	4068	0	0.00%	568	0	0.00%	416	0	0.00%
9	TP53	c.637C>T	p.R213*	51.3%	O	696	0	0.00%	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
10	TP53	c.817C>T	p.R273C	27.0%	O/P	707	2	0.28%	2117	0	0.00%	569	0	0.00%	87	0	0.00%	–	–	–	–	–	–
11	TP53	c.764_766del	p.I255del	58.1%	O	<b>1214</b>	<b>3</b>	<b>0.25%</b>	–	–	–	<b>1574</b>	<b>6</b>	<b>0.38%</b>	–	–	–	–	–	–	–	–	–
12	TP53	c.586C>T	p.R196*	44.2%	O/P	1032	2	0.19%	<b>5113</b>	<b>16</b>	<b>0.32%</b>	834	0	0.00%	<b>7235</b>	<b>20</b>	<b>0.28%</b>	<b>795</b>	<b>43</b>	<b>5.41%</b>	<b>5135</b>	<b>300</b>	<b>5.51%</b>
15	APC	c.4391_4394del	p.E1464Vfs*8	68.3%	O	<b>1728</b>	<b>22</b>	<b>1.27%</b>	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
17	TP53	c.413C>T	p.A138V	60.2%	O	2663	0	0.00%	–	–	–	3261	0	0.00%	–	–	–	–	–	–	–	–	–
18	TP53	c.916C>T	p.R306*	11.2%	P	–	–	–	5438	0	0.00%	–	–	–	3800	4	0.10%	–	–	–	4550	0	0.00%
19	KRAS	c.182A>T	p.Q61L	23.7%	O	1658	0	0.00%	–	–	–	2313	0	0.00%	–	–	–	1832	0	0.00%	–	–	–
20	TP53	c.578A>C	p.H193P	44.0%	O	1080	0	0.00%	–	–	–	2012	0	0.00%	–	–	–	–	–	–	–	–	–
24	TP53	c.818G>A	p.R273H	41.4%	O/P	<b>1710</b>	<b>7</b>	<b>0.40%</b>	N/A	N/A	N/A	2550	0	0.00%	1417	2	0.14%	2855	0	0.00%	762	0	0.00%
25	TP53	c.524G>A	p.R175H	79.1%	O/P	<b>2016</b>	<b>24</b>	<b>1.20%</b>	<b>5273</b>	<b>27</b>	<b>0.51%</b>	3579	0	0.00%	9733	0	0.00%	3442	0	0.00%	2296	0	0.00%
27	TP53	c.772G>A	p.E258K	48.0%	O	3259	0	0.00%	–	–	–	3760	0	0.00%	–	–	–	–	–	–	–	–	–
28	TP53	c.524G>A	p.R175H	89.8%	O/P	<b>1435</b>	<b>141</b>	<b>9.83%</b>	<b>2133</b>	<b>272</b>	<b>11.32%</b>	722	0	0.00%	801	0	0.00%	673	0	0.00%	2425	0	0.00%
29	TP53	c.524G>A	p.R175H	61.6%	O/P	<b>1149</b>	<b>4</b>	<b>0.35%</b>	<b>1260</b>	<b>10</b>	<b>0.79%</b>	828	0	0.00%	3758	0	0.00%	–	–	–	–	–	–
30	TP53	c.733G>A	p.G245S	57.5%	O/P	<b>4466</b>	<b>70</b>	<b>1.57%</b>	<b>5758</b>	<b>122</b>	<b>2.07%</b>	–	–	–	–	–	–	–	–	–	–	–	–
31	TP53	c.818G>A	p.R273H	65.3%	O/P	<b>2764</b>	<b>47</b>	<b>1.70%</b>	<b>2397</b>	<b>45</b>	<b>1.85%</b>	<b>5408</b>	<b>327</b>	<b>6.05%</b>	<b>6199</b>	<b>386</b>	<b>5.86%</b>	–	–	–	–	–	–
31	ERBB2	c.2524G>A	p.V842I	36.2%	O	<b>3730</b>	<b>21</b>	<b>0.56%</b>	–	–	–	<b>7153</b>	<b>181</b>	<b>2.53%</b>	–	–	–	–	–	–	–	–	–
31	KRAS	c.34G>T	p.G12C	19.9%	O/P	3457	0	0.00%	4446	0	0.00%	12010	0	0.00%	18301	0	0.00%	–	–	–	–	–	–
31	KRAS	c.35G>T	p.G12V	15.9%	O/P	<b>5212</b>	<b>95</b>	<b>2.75%</b>	<b>5673</b>	<b>96</b>	<b>1.66%</b>	<b>8393</b>	<b>1258</b>	<b>14.99%</b>	<b>19384</b>	<b>2246</b>	<b>10.38%</b>	–	–	–	–	–	–

NGS, next-generation sequencing; NGS-UMI, next-generation sequencing with unique molecular identifiers; dPCR, digital polymerase chain reaction; Resec spec, resection specimen; CDS, coding DNA sequence; AA, amino acid; VAF, variant allele frequency; Mol cov, molecular coverage; Mut mol, mutant molecules; WT cop, wild-type copies; Mut cop, mutant copies; O, OncoPrint NGS; P, digital PCR; N/A, not available.