

Figure S1 Linear regression of bTMB and used plasma volume in mL (A) and input DNA in ng (B). bTMB, blood tumor mutational burden.

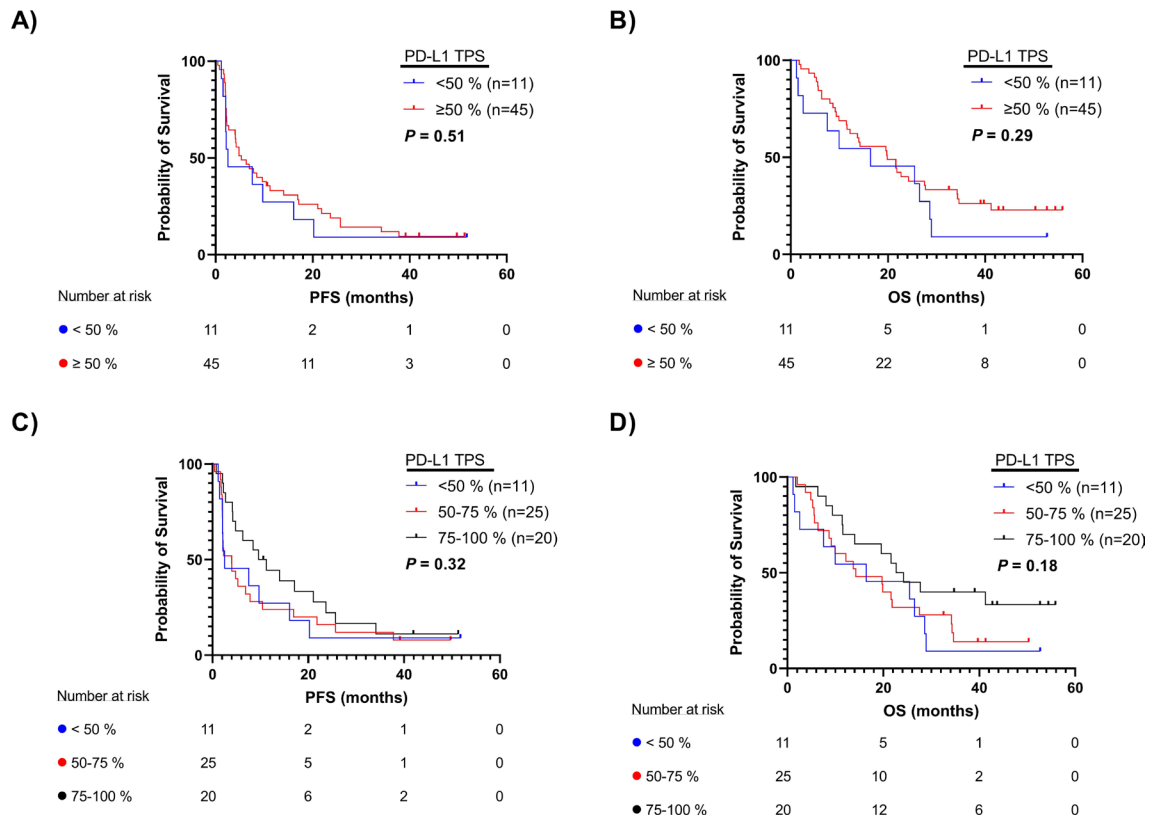


Figure S2 Kaplan-Meier survival analysis according to PD-L1 TPS. (A) and (B) Patients stratified based on a threshold of 50% PD-L1 TPS. (C) and (D) Patients stratified based on either below 50%, 50–75% and 75–100% PD-L1 TPS. OS, overall survival; PD-L1, programmed cell death ligand 1; PFS, progression-free survival; TPS, tumor proportion score.

Table S1 Univariate and multivariate Cox regression analysis

Variable	Univariate		Multivariate	
	P value	HR (95% CI)	P value	HR (95% CI)
PFS				
Age [cont.]	0.03	1.04 (1.01–1.08)	0.01	1.07 (1.02–1.13)
Histology [Sq. cell]	0.07	1.92 (0.89–3.78)		
Sex [F]	0.71	0.90 (0.51–1.58)		
Smoking [Active]	0.95	0.98 (0.52–1.76)		
PD-L1 TPS [>50%]	0.41	0.75 (0.40–1.55)		
Treatment line [2. line]	0.21	1.53 (0.76–2.89)		
PS				
[1]	0.16	1.61 (0.85–3.28)		
[2]	0.37	1.57 (0.54–4.07)		
Co-morbidity [cont.]	0.30	1.11 (0.90–1.32)		
TP53 [mut.]	0.39	0.78 (0.45–1.38)		
KRAS [mut.]	0.37	0.77 (0.43–1.36)		
Baseline bTMB [High]	0.02	0.49 (0.28–0.87)	0.005	0.31 (0.13–0.68)
Baseline aMM [High]	0.36	1.30 (0.74–2.28)		
ctDNA dynamics [Clearing]	0.006	0.35 (0.16–0.72)	0.007	0.25 (0.09–0.67)
aMM dynamics [Increase]	<0.001	7.17 (3.08–16.58)	0.001	4.60 (1.83–11.63)
OS				
Age [cont.]	0.31	1.02 (0.98–1.06)		
Histology [Sq. cell]	0.16	1.71 (0.77–3.45)		
Sex [F]	0.32	0.74 (0.41–1.34)		
Smoking [Active]	0.95	0.98 (0.49–1.84)		
PD-L1 TPS [>50%]	0.23	0.66 (0.34–1.37)		
Treatment line [2. Line]	0.09	1.78 (0.88–3.38)		
PS				
[1]	0.43	1.32 (0.68–2.76)		
[2]	0.49	0.64 (0.14–2.06)		
Co-morbidity score [cont.]	0.44	1.08 (0.88–1.29)		
TP53 [mut.]	0.65	1.15 (0.63–2.10)		
KRAS [mut.]	0.53	0.82 (0.43–1.50)		
Baseline bTMB [High]	0.04	0.52 (0.27–0.95)	0.05	0.48 (0.23–0.97)
Baseline aMM [High]	0.25	1.42 (0.78–2.59)		
ctDNA dynamics [Clearing]	0.05	0.48 (0.22–0.98)		
aMM dynamics [Increase]	0.003	3.05 (1.41–6.24)	0.002	3.45 (1.55–7.40)

Variable tested is shown in brackets. Independent variables with P values below 0.05 was included in the multivariate Cox regression analysis. aMM, average number of mutant molecules per mL of plasma; CI, confidence interval; cont., continuous variable; ctDNA, circulating tumor DNA; F, female; HR, hazard ratio; mut., mutated; PD-L1, programmed cell death ligand 1; PFS, progression-free survival; PS; performance status; sq. cell; squamous cell carcinoma; TPS, tumor proportion score.

Table S3 Best overall response for the low and high bTMB groups

Best overall response	Low bTMB (n = 31)	High bTMB (n = 25)	P value
Objective response, n (%)	7 (22.58)	15 (60.00)	
Complete response	0 (0)	0 (0)	
Partial response	7 (22.58)	15 (60.00)	
No objective response, n (%)	21 (67.74)	10 (40.00)	
Stable disease	7 (22.58)	6 (24.00)	
Progressive disease	14 (45.16)	4 (16.00)	
Unknown, n (%) [†]	3 (9.68)	0 (0)	0.01

[†], patients with no available radiologic assessments of response, who were excluded from comparison. Association between bTMB and response was calculated using Fisher's exact test. bTMB, blood tumor mutational burden.

Table S5 Genetic alterations in the ctDNA after 3-6 weeks of treatment (T_x)

Pt	Gene	Type	Coding change	Amino acid change	AF	Variant depth	Unique depth	MM	COSMIC ID
2	<i>EGFR</i>	SNV	c.391G>A	p.Gly131Arg	0.22%	11	4901	15.6	COSM5575629
	<i>GPR139</i>	SNV	c.436C>T	p.Arg146Trp	0.14%	10	7086	9.83	COSM5641101
	<i>TP53</i>	SNV	c.613T>G	p.Tyr205Asp	0.14%	7	4920	9.91	COSM1564188
3	<i>CDH9</i>	SNV	c.2116C>A	p.Leu706Met	2.84%	126	4433	241	COSM1200510
	<i>KPRP</i>	SNV	c.296C>A	p.Ala99Glu	4.05%	214	5289	343	COSM6121185
	<i>KRAS</i>	SNV	c.35G>A	p.Gly12Asp	10.31%	542	5256	875	COSM1135366
4	<i>KIT</i>	SNV	c.1657T>A	p.Tyr553Asn	15.89%	675	4247	13400	COSM133763
6									
7	<i>BRAF</i>	SNV	c.1415A>G	p.Tyr472Cys	0.13%	4	3083	3.15	COSM1133046
	<i>FBXL7</i>	SNV	c.411C>G	p.Cys137Trp	0.14%	3	2146	3.39	N/A
	<i>KRAS</i>	SNV	c.182A>T	p.Gln61Leu	0.16%	5	3150	3.85	COSM1140131
8	<i>CSMD3</i>	SNV	c.4463C>T	p.Ser1488Phe	0.25%	13	5114	10.1	COSM4892642
	<i>TP53</i>	SNV	c.892G>T	p.Glu298 [†]	0.45%	21	4629	18.1	COSM10710
9	<i>CDH8</i>	SNV	c.1178C>T	p.Ser393Leu	0.26%	12	4627	19.1	COSM1302087
	<i>KRAS</i>	SNV	c.35G>A	p.Gly12Asp	1.70%	99	5829	125	COSM1135366
	<i>KRAS</i>	SNV	c.57G>C	p.Leu19Phe	0.10%	6	6057	7.29	COSM12703
	<i>NFE2L2</i>	SNV	c.72G>C	p.Trp24Cys	6.75%	252	3733	496	COSM132852
	<i>NPAP1</i>	SNV	c.2033C>A	p.Thr678Asn	1.01%	59	5827	74.5	COSM3732695
	<i>PIK3CA</i>	SNV	c.1624G>A	p.Glu542Lys	1.41%	107	7611	103	COSM125369
	<i>PIK3CA</i>	SNV	c.1633G>A	p.Glu545Lys	1.23%	94	7646	90.4	COSM125370
	<i>TP53</i>	SNV	c.517G>A	p.Val173Met	17.31%	607	3506	1270	COSM11084
10	<i>SLC39A12</i>	SNV	c.1357G>T	p.Gly453 [†]	0.16%	8	5073	3.47	N/A
11	<i>EYS</i>	SNV	c.171C>A	p.Cys57 [†]	0.68%	45	6586	38.8	COSM3782087
	<i>FBXL7</i>	SNV	c.496G>A	p.Val166Met	0.39%	19	4847	22.3	COSM5424885
	<i>GJA8</i>	SNV	c.769G>C	p.Val257Leu	0.15%	10	6711	8.47	N/A
	<i>TP53</i>	SNV	c.406C>T	p.Gln136 [†]	0.51%	30	5889	29	COSM11166
12	<i>TP53</i>	SNV	c.644G>T	p.Ser215Ile	0.63%	26	4145	24.4	COSM11450
15	<i>KRAS</i>	SNV	c.34G>T	p.Gly12Cys	0.10%	4	3835	2.89	COSM1140136
16									
19	<i>KRAS</i>	SNV	c.35G>T	p.Gly12Val	0.13%	6	4484	5.21	COSM1140133
	<i>P2RY10</i>	SNV	c.904C>A	p.Pro302Thr	0.22%	13	5824	8.69	N/A
	<i>TP53</i>	SNV	c.469G>T	p.Val157Phe	0.12%	5	4205	4.63	COSM10670
20									
21	<i>EGFR</i>	SNV	c.1124C>T	p.Ala375Val	0.22%	14	6237	8.23	N/A
22									
23	<i>LPPR4</i>	SNV	c.1132C>T	p.Arg378 [†]	0.36%	20	5525	78.2	N/A
	<i>NRAS</i>	SNV	c.35G>A	p.Gly12Asp	0.11%	7	6109	24.7	COSM564
	<i>USP29</i>	SNV	c.788G>A	p.Ser263Asn	0.27%	19	6934	59.2	N/A
24	<i>PHACTR1</i>	SNV	c.760C>G	p.Pro254Ala	0.19%	8	4204	17	COSM5061407
25	<i>TP53</i>	SNV	c.559+1G>T	‡	0.32%	5	1564	3.72	COSM1649387
26	<i>ERBB2</i>	INDEL	c.2313_2324dup	p.Ala775_	3.70%	36	1918	98.4	N/A
	<i>TP53</i>	SNV	c.814G>A	Gly776insTyrValMetAla p.Val272Met	0.76%	18	2353	20.3	COSM10891
27									
28	<i>MET</i>	SNV	c.2770G>A	p.Glu924Lys	0.11%	8	7332	5.11	COSM5576816
29									
30	<i>GRM5</i>	SNV	c.2131C>A	p.Leu711Ile	4.63%	173	3733	100	COSM5788131
	<i>KRAS</i>	SNV	c.34G>T	p.Gly12Cys	4.18%	153	3658	90.7	COSM1140136
	<i>TP53</i>	SNV	c.404G>T	p.Cys135Phe	4.12%	113	2746	89.2	COSM10647
31	<i>KRAS</i>	SNV	c.35G>A	p.Gly12Asp	0.21%	14	6822	6.49	COSM1135366
33	<i>KRAS</i>	SNV	c.35G>A	p.Gly12Asp	5.76%	451	7827	893	COSM1135366
	<i>LPPR4</i>	SNV	c.1205G>T	p.Arg402Leu	1.14%	71	6251	176	COSM6702659
34									
35									
36									
37	<i>FAM71B</i>	SNV	c.995G>C	p.Gly332Ala	0.20%	8	4060	6.34	COSM737062
38									
39	<i>BRINP3</i>	SNV	c.2156G>A	p.Arg719His	0.24%	8	3286	8.55	COSM4026218
	<i>KEAP1</i>	SNV	c.700C>T	p.Arg234Trp	7.78%	194	2494	273	COSM4855177
40	<i>BRAF</i>	SNV	c.1405G>T	p.Gly469 [†]	0.39%	32	8281	20.9	N/A
	<i>BRAF</i>	SNV	c.1406G>C	p.Gly469Ala	0.41%	33	8118	22	COSM460
	<i>TP53</i>	SNV	c.475G>C	p.Ala159Pro	0.24%	14	5856	12.9	COSM1649380
	<i>ZIC4</i>	SNV	c.425G>T	p.Ser142Ile	0.11%	7	6287	6.02	COSM4742816
41	<i>KPRP</i>	SNV	c.935G>A	p.Arg312His	0.73%	35	4824	32.5	COSM1212763
	<i>KRAS</i>	SNV	c.34G>T	p.Gly12Cys	2.70%	43	1590	125	COSM1140136
	<i>TP53</i>	SNV	c.473G>T	p.Arg158Leu	1.97%	94	4762	93.5	COSM10714
42									
43	<i>USP29</i>	SNV	c.217C>T	p.Arg73Cys	0.32%	23	7132	150	N/A
44									
45									
46	<i>TP53</i>	SNV	c.151G>T	p.Glu51 [†]	1.76%	80	4539	243	COSM1564161
48	<i>KRAS</i>	SNV	c.183A>T	p.Gln61His	0.93%	43	4638	406	COSM1146992
49	<i>KEAP1</i>	SNV	c.821A>G	p.His274Arg	4.67%	70	1499	792	COSM5816784

[†], premature stop codon. [‡], splice site mutation, underscore = patients with T_x sample taken after 1 cycle of treatment. AF, allele frequency, COSMIC, catalogue of somatic mutations in cancer; ctDNA, circulating tumor DNA; MM, number of mutant molecules per mL of plasma; Pt, patient.

Table S6 Best overall response for patients stratified based on increase in ctDNA

Best overall response	Increase (n=12)	No increase (n=29)	P value
Objective response, n (%)	1 (8.33)	18 (62.07)	
Complete response	0 (0)	0 (0)	
Partial response	1 (8.33)	18 (62.07)	
No objective response, n (%)	9 (75.00)	11 (37.93)	
Stable disease	2 (16.67)	8 (27.59)	
Progressive disease	7 (58.33)	3 (10.34)	
Unknown, n (%) [†]	2 (16.67)	0 (0)	0.008

[†], patients with no available radiologic assessments of response, who were excluded from comparison. Association between ctDNA increase and response was calculated using Fisher's exact test. ctDNA, circulating tumor DNA.