

Figure S1. Relation between LDH levels (U/l) and baseline plasma cfDNA levels (ng/ $\mu$ l) evaluated using a linear regression model (n=40). cfDNA, cell-free DNA; LDH, lactate dehydrogenase.

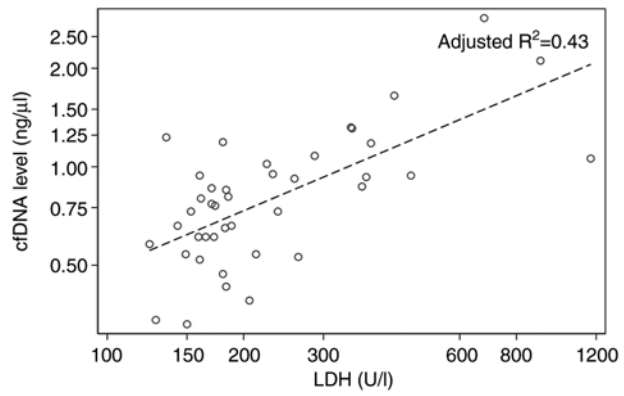


Table S1. Cox proportional hazards regression for PFS and OS.

Variable	Univariate			Multivariate with cfDNA as a continuous variable			
	PFS		OS	PFS		OS	
	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
Baseline cfDNA level, $\geq 75$ th vs. $< 75$ th percentile	3.03 (1.46-6.29)	0.003	3.48 (1.44-8.44)	6.67 (2.22-3.74)	0.006	7.39 (1.61-33.73)	0.01
Age, above vs. below median	0.69 (0.35-1.33)	0.27	0.80 (0.34-1.89)		0.61		
Sex, female vs. male	1.10 (0.57-2.10)	0.78	1.35 (0.57-3.20)		0.49		
WHO performance status, $\geq 2$ vs. $< 2$	3.76 (0.84-16.91)	0.08	11.43 (2.05-63.64)		0.005	5.77 (0.75-44.37)	0.09
Localisation primary tumor, rectum vs. colon	0.98 (0.48-1.99)	0.95	0.72 (0.26-1.98)		0.53		
Sideness primary tumor, left vs. right	0.83 (0.43-1.60)	0.57	0.92 (0.39-2.17)		0.84		
Resection status primary tumor, unresected vs. resected	1.27 (0.64-2.55)	0.50	0.73 (0.28-1.89)		0.52		
Number of lines of previous anticancer therapies, $\geq 1$ vs. 0	8.14 (1.67-39.60)	0.009	7.00 (1.44-33.73)	4.69 (0.94-23.37)	0.016	3.49 (0.62-19.55)	0.16
Time of metastases, metachronous vs. synchronous	0.68 (0.33-1.38)	0.28	1.43 (0.60-3.38)		0.42		
Number of metastatic sites, $\geq 1$ vs. 1	0.94 (0.48-1.87)	0.87	1.32 (0.52-3.30)		0.56		
Liver involvement, yes vs. no	2.26 (1.11-4.60)	0.025	1.44 (0.60-3.46)	1.33 (0.60-2.97)	0.41		
Lung involvement, yes vs. no	0.72 (0.37-1.39)	0.33	1.30 (0.55-3.06)		0.55		
Tissue mutation status, RAS/BRAF mutation vs. wild-type	1.26 (0.55-2.90)	0.58	1.66 (0.49-5.63)		0.42		
LDH, $>$ vs. $<$ ULN	1.81 (0.92-3.58)	0.09	2.48 (1.04-5.92)		0.04	1.19 (0.42-3.37)	0.75

OS, overall survival; PFS, progression-free survival; cfDNA, cell-free DNA; WHO, World Health Organization; LDH, lactate dehydrogenase; ULN, upper limit of normal ( $> 205$  U/l).

Table SII. Best treatment response according to RECIST v. 1.1 and association with cfDNA levels at baseline.

Response	cfDNA, ng/ $\mu$ l [median (95% CI)]
PR	0.73 (0.52-0.97)
SD	0.70 (0.57-0.82)
PD <sup>a</sup>	1.05 (0.50-1.96)
DC <sup>a</sup>	0.73 (0.61-0.84)

<sup>a</sup>Compared by Mann-Whitney U-test, P=0.04. None of the patients achieved complete response on study treatment. PR, partial response; SD, stable disease; PD, progressive disease; DC, disease control (PR+SD); cfDNA, cell-free DNA.