Supplementary Table 1 Clinical characteristics of rectal cancer patients

		ctDNA analysis (n = 9)	%	**Not included (n=21)**	%	**Total (n = 30)**	%
Age Median (min - max)		65.8 (44.0 – 81.0)		67.0 (39.0 – 81.0)			
Gender	Male	3	33.3%	14	66.6%	17	56.7
	Female	6	67.7%	7	32.4%	13	43.3
Tumor size, sm (mean +/- SD)		4.0 +/ 1.47		3.8 +/0.93			
Distance from anal verge, sm (mean ± SD)		6.43 +/- 1.09		7.14 +/- 0.58			
cT	2	0	0.0%	2	9.5%	2	6.7%
	3	7	77.8%	19	90.5%	26	86.6%
	4	2	22.2%	0	0.0%	2	6.7%
cN	0	0	0.0%	2	9.5%	2	6.7%
	1	6	66.7%	16	76.2%	22	73.3%
	2	3	33.3%	3	14.3%	6	20.0%
Clinical stage							

	IIA	0	0.0%	1	4.8%	1	3.3%
	IIIA	0	0.0%	1	4.8%	1	3.3%
	IIIB	9	100.0%	18	85.7%	27	90.0%
	IV	0	0.0%	1	4.8%	1	3.3%
ypT							
	0	0	0.0%	3	14.3%	3	20.0%
	2	0	0.0%	5	23.8%	5	33.3%
	3	4	44.4%	2	9.5%	6	40.0%
	4	1	11.1%	0	0.0%	1	6.7%
	NA	4	44.4%	11	52.4%	15	50.0%
Grade							
	1	3	33.3%	7	33.3%	10	33.3%
	2	3	33.3%	6	28.6%	9	30.0%
	3	1	11.1%	2	9.5%	3	10.0%
	NA	2	22.2%	6	28.6%	8	26.7%
CRM+							
	Yes	4	44.4%	4	23.5%	8	29.6%
	No	5	55.6%	13	76.5%	19	70.4%
EMV+							
	Yes	3	33.3%	5	29.4%	8	29.6%
	No	6	66.7%	12	70.6%	19	70.4%

NA – not assessed, CRM - circumferential resection margin, EMV – extramural venous invasion

ypT – post-neoadjuvant T

Supplementary Table 2 Treatment results summary

		ctDNA analysis (n = 9)	%	Not included (n=21)	%	Total (n = 30)	%	*P*-value[1]
Response evaluated								
	Yes	9	100	12	61.9%	21	70.0%	
Clinical response								
	CR	0	0.0%	1	8.3%	1	4.8%	Fisher exact test P
	PR	3	33.3%	10	83.3%	13	61.9%	= 0.019
	SD	4	44.5%	1	8.3%	5	23.8%	
	PD	2	22.2%	0	0.0%	2	9.5%	
	NA	0		9		9		
Surgery performed								
	Yes	5	55.5%	11	52.4%	16	53.3%	
TRG (MRI)								
	TRG 1	0	0.0%	1	9.1%	1	5.0%	Fisher exact test P
	TRG 2	1	11.1%	4	26.4%	5	25.0%	= 0.364
	TRG 3	5	55.6%	5	45.5%	10	50.0%	
	TRG 4	3	33.3%	1	9.1%	4	20.0%	
	NA	0		10		10		

PFS, months (95%CI)	Not achieved	12.7 (9.8 – 15.5)	12.7 (10.3 – 15.1)	Log-rank P = 0.409

[1]Comparison of patients included in the ctDNA monitoring vs. patients excluded from the analysis.

PFS – recurrence free survival; TRG - tumor regression grade; RECIST abbreviations: CR - complete response, PR - partial response, SD - stable disease, PD - progressive disease; NA - not evaluated.

Supplementary Table 3 ddPCR assays for mutation detection, the primers and probe design for ddPCR

Mutation	Target-gene probe (wt)	Target-gene probe (mut)	Forward primer	Reverse primer
BRAF	[FAM]TCTAGCTACAGTGAAAT	[HEX]TCTAGCTACAGAGAAAT	GAAGACCTCACAGTA	ATAGCCTCAATTCT
V600E	CTCGATGG[BHQ]	CTCGATGG[BHQ1]	AAAATAG	TACCATCC
KRAS	[FAM]CCTACGCCACCAGCTC[B	[R6G]CCTACGCAACCAGCTC[B	AAATGACTGAATATA	ATTAGCTGTATCGT
G13C	HQ1]	HQ1]	AACTTGT	CAAGG
NRAS	[R6G]ACACCACCTGCTCCAACC	[R6G]ACACCATCTGCTCCAACC	CTTGCTGGTGTGAAAT	ATTGTCAGTGCGCT
G12D	AC[BHQ1]	AC[BHQ1]	GAC	TTTCC
NRAS	[FAM]ACAGCTGGACAAGAAG	[JOE]ACAGCTGGACTAGAAGA	ACCTGTTTGTTGGACA	ATTGGTCTCTCATG
Q61L	AGTACAGT[BHQ1]	GTACAGT[BHQ1]	TACT	GCACT

The ddPCR Supermix for Probes (no UTP) kit (Bio-Rad Laboratories) was utilized in all reactions. The primers and probes were at concentration of 100 µM. ddPCR reactions were performed according to Oskina et al., 2017 (doi:10.1007/s40291-017-0281-0)

Supplementary Table 4 Bio-Rad ddPCR assays

Mutation	**Kit**
KRAS G13D	PrimePCR™ ddPCR™ Mutation Detection Assay Kit: KRAS WT for p.G13D, and KRAS p.G13D, Bio-Rad Laboratories, Inc.
KRAS Q61R	ddPCR™ KRAS Q61 Screening Kit, Bio-Rad Laboratories, Inc.
KRAS G12A	PrimePCR™ ddPCR™ Mutation Detection Assay Kit: KRAS WT for p.G12A, and KRAS p.G12A, Bio-Rad Laboratories, Inc.
KRAS G12D	PrimePCR™ ddPCR™ Mutation Detection Assay Kit: KRAS WT for p.G12D, and KRAS p.G12D, Bio-Rad Laboratories, Inc.
KRAS G12S	PrimePCR™ ddPCR™ Mutation Detection Assay Kit: KRAS WT for p.G12S, and KRAS p.G12S, Bio-Rad Laboratories, Inc.