Supplementary figure legends

Outcomes with and without RAS mutation as estimated from Cox regression, keeping the following factors constant; high differentiation, no perforation of the peritoneum, dMMR, no neural or vascular invasion, stage II, no BRAF mutation in serum combined with pMMR in tissue, and BRAF wild type in serum.

Figure S1: OS with/without RAS in tissue, based on Cox regression.

Black line: Three-year OS without a mutation in tissue: 85%, 95%CI=78-92%.

Red line: Three-year OS with a mutation in tissue: 83%, 95%CI=76-91%.

Figure S2: DFS with/without RAS in tissue, based on Cox regression.

Black line: Three-year DFS without a mutation in tissue: 79%, 95%CI=71-88%.

Red line: Three-year DFS with a mutation in tissue: 75%, 95%CI=67-85%.

Figure S3: OS with/without RAS in serum, based on Cox regression.

Black line: Three-year OS without a mutation in serum: 86%, 95%CI=80-92%.

Red line: Three-year OS with a mutation in serum: 70%, 95%CI=57-87%.

Figure S4: DFS with/without RAS in serum, based on Cox regression.

Black line: Three-year DFS without a mutation in serum: 79%, 95%CI=73-87%.

Red line: Three-year DFS with a mutation in serum: 61%, 95%CI=46-80%.

Supplementary table legends

Table S1: The three rounds of mutation testing.

Table S2: BioRad PrimePCR assays.

Validation data including MIQE context sequences can be found on www.bio-rad.com.

Table S3: Frequencies of the specific mutations in the cohort.

Table S4: Mutational load divided into quartiles and distribution in disease stages.

Supplementary tables

Table S1:

1st round			2nd round			3rd round		
KRAS	**Exon 2**	G12D	**KRAS**	**Exon 2**	G12A	**KRAS**	**Exon 3**	Q61H A>C
						KRAS	**Exon 3**	Q61H A>T
KRAS	**Exon 2**	G12V	**KRAS**	**Exon 2**	G12C	**KRAS**	**Exon 3**	Q61R
						KRAS	**Exon 3**	Q61L
KRAS	**Exon 2**	G13D	**KRAS**	**Exon 2**	G12S			
						KRAS	**Exon 2**	G12R
BRAF		V600E	**KRAS**	**Exon 4**	A146T	**KRAS**	**Exon 2**	G13C
			KRAS	**Exon 4**	A146V	**KRAS**	**Exon 4**	K117N A>C
			KRAS	**Exon 4**	A146P	**KRAS**	**Exon 4**	K117N A>T
						NRAS	**Exon 2**	G12D
						NRAS	**Exon 2**	G12C
						NRAS	**Exon 2**	G12V
						NRAS	**Exon 2**	G13D
						NRAS	**Exon 2**	G13R
						NRAS	**Exon 3**	Q61K
						NRAS	**Exon 3**	Q61R
						NRAS	**Exon 3**	Q61H
						NRAS	**Exon 3**	Q61L

Table S2:

Gene	Assay number	Assay length	CDS mutation	AA mutation
KRAS	dHsaCP2500586	57	c.35G>C	p.G12A
KRAS	dHsaCP2500584	57	c.34G>T	p.G12C
KRAS	dHsaCP2500596	57	c.35G>A	p.G12D
KRAS	dHsaCP2500590	57	c.34G>C	p.G12R
KRAS	dHsaCP2500588	57	c.34G>A	p.G12S
KRAS	dHsaCP2500592	57	c.35G>T	p.G12V
KRAS	dHsaCP2500594	57	c.37G>T	p.G13C
KRAS	dHsaCP2500598	57	c.38G>A	p.G13D
KRAS	dHsaCP2000133	61	c.183A>C	p.Q61H
KRAS	dHsaCP2000131	61	c.183A>T	p.Q61H
KRAS	dHsaCP2000101	61	c.182A>T	p.Q61L

KRAS	dHsaCP2000135	61	c.182A>G	p.Q61R
KRAS	custom made		c.351A>T	p.K117N/T
KRAS	custom made		c.351A>C	p.K117N/C
KRAS	dHsaCP2000079	80	c.436G>A	p.A146T
KRAS	custom made	80	c.436G>C	p.A146P
KRAS	custom made	80	c.436A>T	p.A146V
BRAF	dHsaCP2000027	91	c.1799T>A	p.V600E
NRAS	dHsaCP2500530	70	c.34G>T	p.G12C
NRAS	dHsaCP2000095	70	c.35G>A	p.G12D
NRAS	dHsaCP2500528	70	c.35G>T	p.G12V
NRAS	dHsaCP2500526	70	c.38G>A	p.G13D
NRAS	dHsaCP2500534	70	c.37G>C	p.G13R
NRAS	dHsaCP2000065	65	c.183A>T	p.Q61H
NRAS	dHsaCP2000067	65	c.181C>A	p.Q61K
NRAS	dHsaCP2000069	65	c.182A>T	p.Q61L
NRAS	dHsaCP2000071	65	c.182A>G	p.Q61R

Table S3:

Mutation	Quantity	Frequency (%)
KRAS G12D	37	12,59
KRAS G12V	26	8,84
KRAS G13D	22	7,48
BRAF V600E	76	25,85
KRAS G12C	8	2,72
KRAS G12S	4	1,36

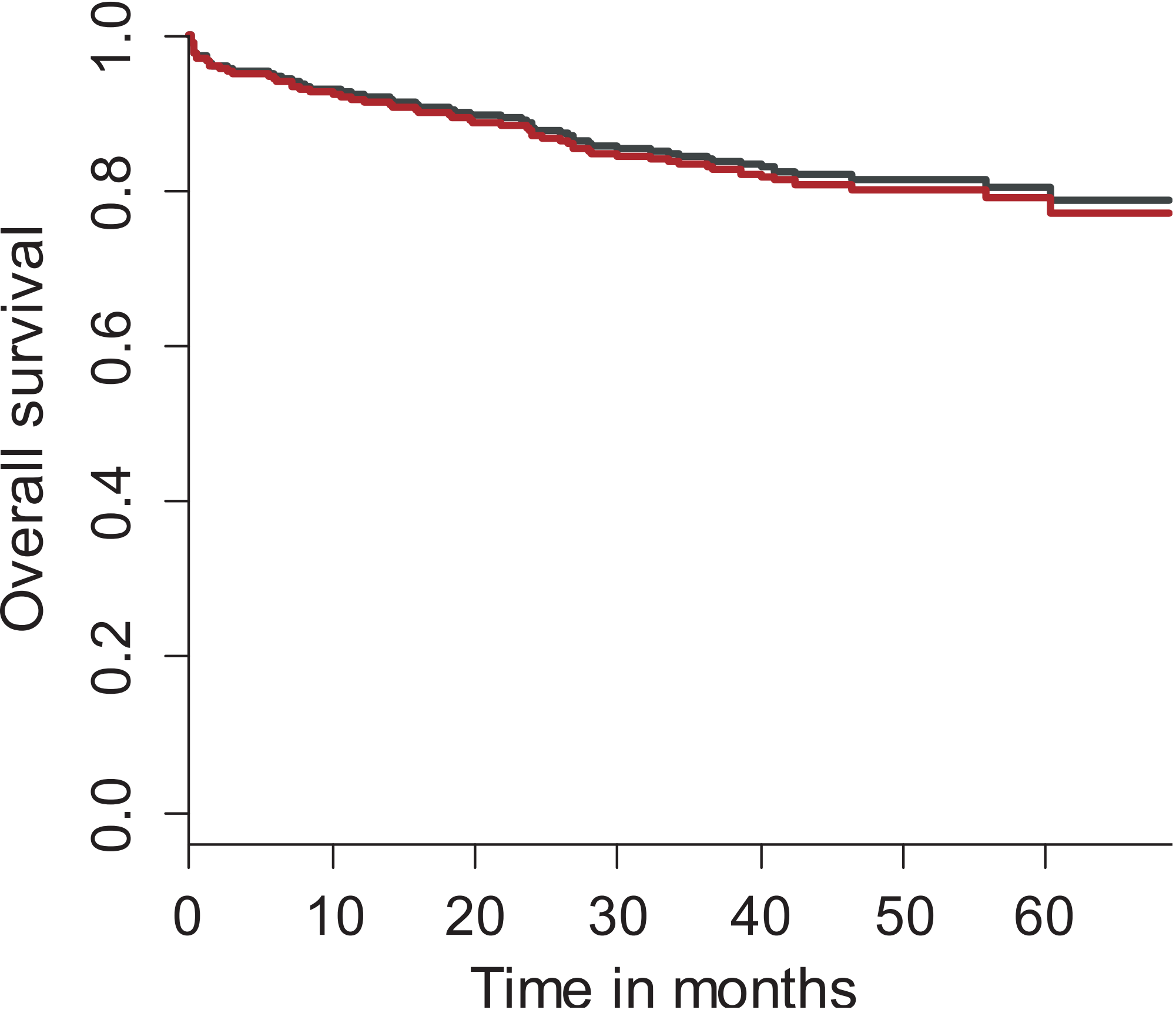
KRAS A146T	4	1,36
KRAS A146V	1	0,34
KRAS A146P	1	0,34
KRAS Q61R	2	0,68
KRAS Q61L	1	0,34
KRAS G12R	1	0,34
NRAS G12D	2	0,68
NRAS Q61K	1	0,34
NRAS Q61R	2	0,68
NRAS Q61L	1	0,34

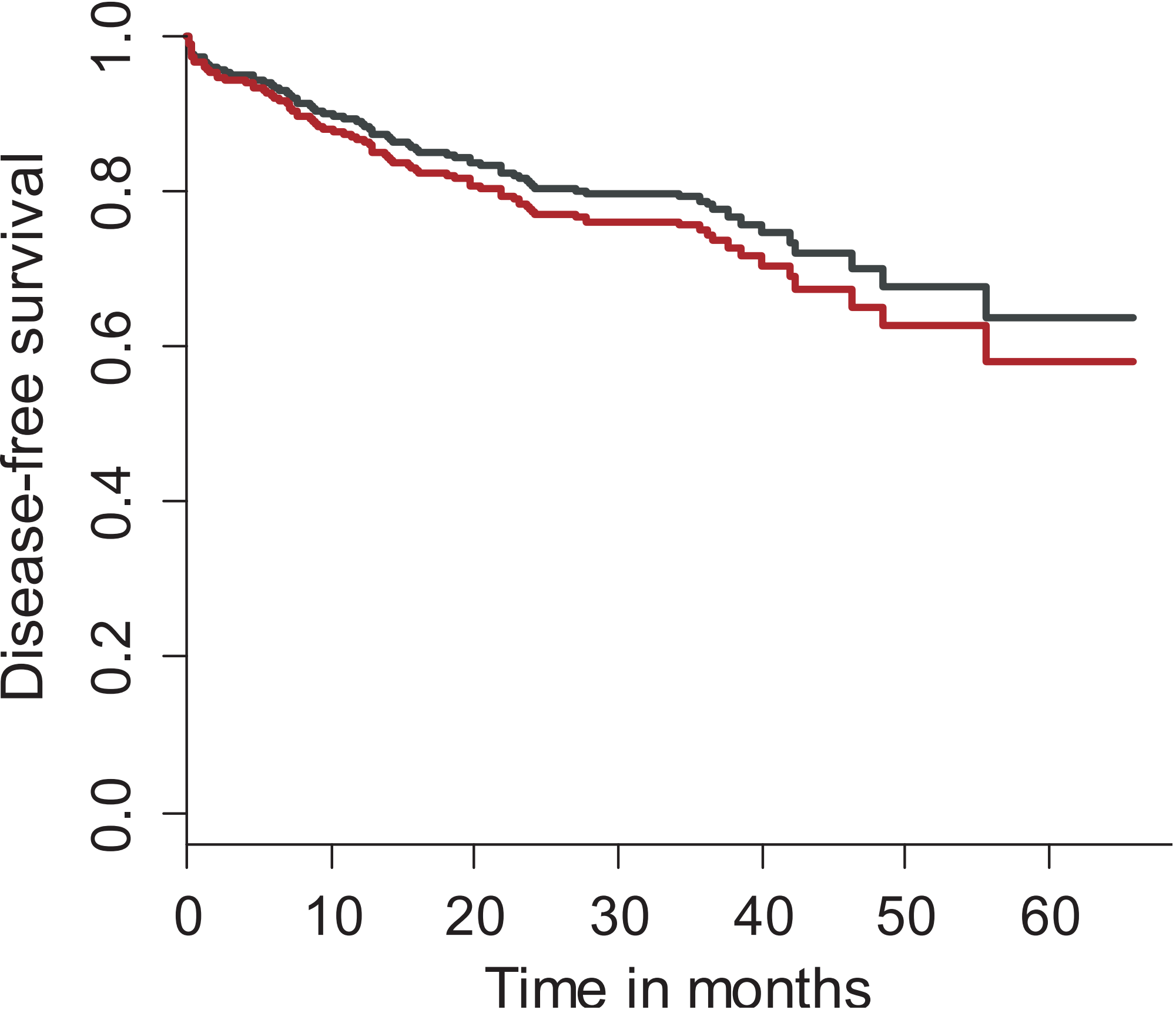
NOTE: Calculations based on the total number of patients (n=294).

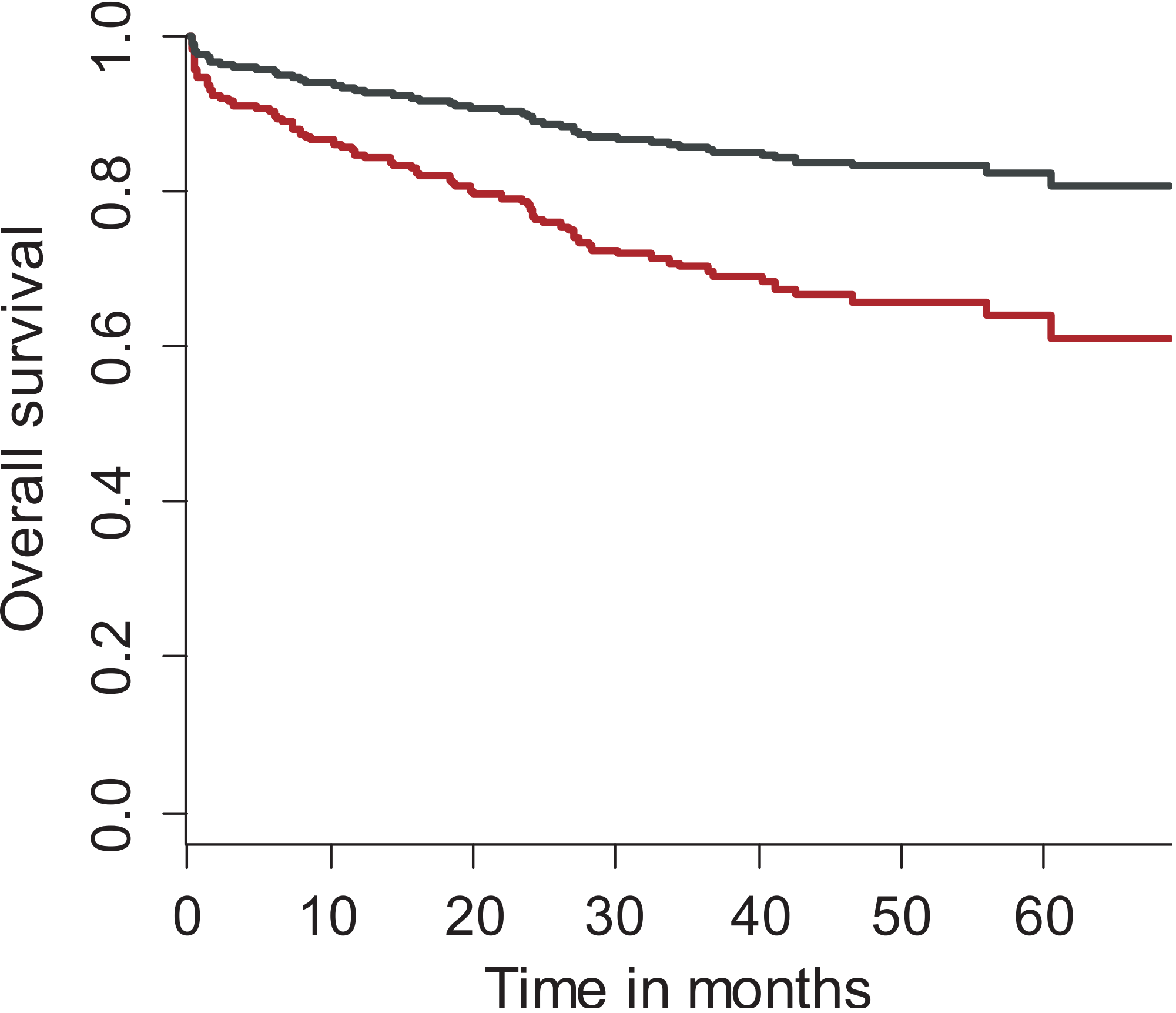
Table S4:

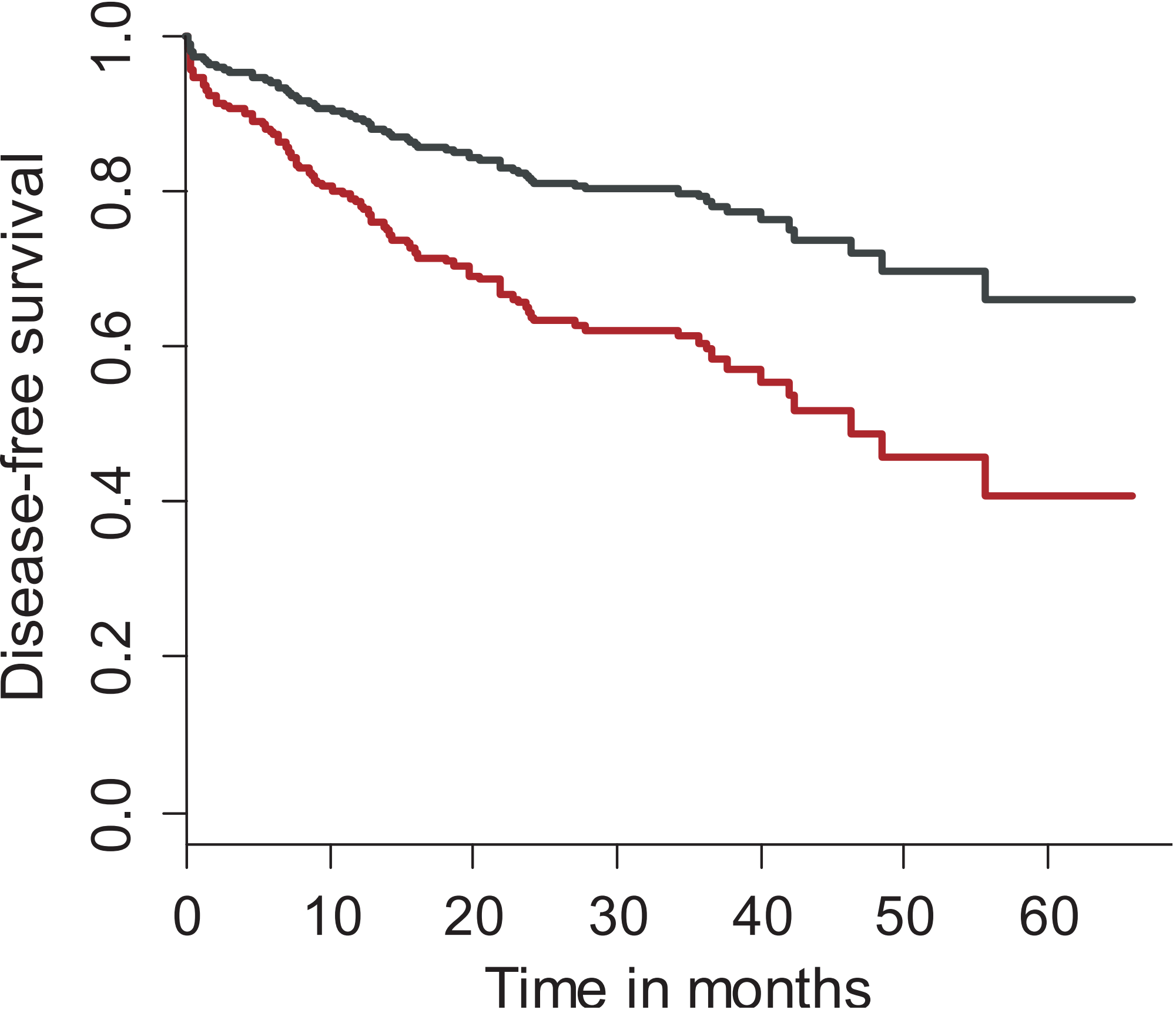
Quartile	Mutated DNA (%)	Stage I (n)	Stage II (n)	Stage III (n)
<25%	<0.035	2	11	6
25-50%	0.035-0.098	1	12	8
50-75%	0.098-0.35	1	11	8
>75%	>0.35	0	11	9

Percentage of mutated DNA ranged from 0.005-11.23%.


Overall survival
0.0
0.2
0.4
0.6
0.8
1.0
0
10
20
30
40
50
60
Time in months


Disease-free survival
0.0
0.2
0.4
0.6
0.8
1.0
0
10
20
30
40
50
60
Time in months


Overall survival
0.0
0.2
0.4
0.6
0.8
1.0
0
10
20
30
40
50
60
Time in months


Disease-free survival
0.0
0.2
0.4
0.6
0.8
1.0
0
10
20
30
40
50
60
Time in months