

Supplementary Material

Tumor residue in patients with stage II–IVA nasopharyngeal carcinoma who received intensity-modulated radiation therapy: development and validation of a prediction nomogram integrating postradiotherapy plasma Epstein–Barr virus deoxyribonucleic acid, clinical stage, and radiotherapy dose

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sTable 1. Clinical characteristics of patients in the development and validation cohorts

Characteristics	Development cohort No. (%)	Validation cohort No. (%)	<i>P</i> ^e
Total	<i>n</i> =736 (70.1)	<i>n</i> =314 (29.9)	
Sex			0.713
Male	558 (75.8)	234 (74.5)	
Female	178 (24.2)	80 (25.5)	
Age, years			0.497
<45	420 (57.1)	187(59.6)	
≥45	316 (42.9)	127 (40.4)	
Histological type^a			1.000
I&II	8 (1.1)	4 (1.3)	
III	728 (98.9)	310 (98.7)	
T category^b			0.414
T2	183 (24.9)	89 (28.3)	
T3	411 (55.8)	172 (54.8)	
T4	142 (19.3)	53 (16.9)	
N category^b			0.782
N0	48 (6.5)	22 (7.0)	
N1	300 (40.8)	129 (41.1)	
N2	269 (36.5)	106 (33.8)	
N3	119 (16.2)	57 (18.2)	
AJCC TNM stage^b			0.875
II	107 (14.5)	49 (15.6)	
III	381 (51.8)	163 (51.9)	
IVA	248 (33.7)	102 (32.5)	
Pretreatment EBV DNA, copies/ml			0.585
0	195 (26.5)	80 (25.5)	
1-4999	273 (37.1)	127 (40.4)	
≥5000	268 (36.4)	107 (34.1)	
No of patients (%) with detectable pretreatment EBV DNA (>0), copies/ml	541 (73.5)	234 (74.5)	
Median (IQR)	4830 (1280-21150)	4135 (1098-18050)	0.569
Treatment modality			0.484
RT	66 (9.0)	25 (8.0)	
CCRT	320 (43.5)	149 (47.5)	
NACT+CCRT	350 (47.6)	140 (44.6)	
RT dose to nasopharynx ± metastatic cervical lymph node(s), Gy			0.916
68.00-69.96	352 (47.8)	152 (48.4)	
70.00-74.00	384 (52.2)	162 (51.6)	
Median (IQR)	70.00 (69.90-70.06)	70.00 (69.90-70.06)	0.810
Postradiotherapy EBV DNA, copies/ml			0.231
0	666 (90.5)	273 (86.9)	

1-499	31 (4.2)	18 (5.7)	
≥500	39 (5.3)	23 (7.3)	
No of patients (%) with detectable postradiotherapy EBV DNA (>0), copies/ml	70 (9.5)	41 (13.1)	
Median (IQR)	680 (113.8-3385)	950 (142.5-7710)	0.442
Residual tumor type^c			0.103
Local	30 (4.0)	20 (6.2)	
Regional	70 (9.2)	41 (12.7)	
Locoregional	22 (2.9)	7 (2.2)	

Abbreviations: T, tumor; N, lymph node(s); TNM, tumor-lymph node-metastasis; EBV, Epstein-Barr virus; DNA, deoxyribonucleic acid; IQR, interquartile range; RT, radiotherapy; CCRT, concurrent chemoradiotherapy; NACT, neoadjuvant chemotherapy.

^aAccording to the World Health Organization (WHO) histologic classification (2005)

^bAll patients' diseases were re-staged according to the 8th edition of the American Joint Committee on Cancer (AJCC)

^cThe definition of residual tumor types, including local residue: residual tumor in the nasopharynx, and(or) extension to the oropharynx, nasal cavity, parapharyngeal space, adjacent soft tissue, and/or infiltration of bony structures at the skull base, cervical vertebra, pterygoid structures, paranasal sinuses, intracranial extension; regional residue: residual tumor in retropharyngeal lymph nodes and/or cervical lymph nodes; locoregional residue: both local and regional residues

^ePearson's chi-squared test or Fisher's exact test for categorical variables and Mann–Whitney *U* test for non-normally distributed variables were used to analyse patient characteristics between the two groups

sTable 2. Distribution of pretreatment plasma EBV DNA by TNM stage (AJCC 8th edition)

No of patients (%)	All stage (II-IVA)	AJCC TNM stage II	AJCC TNM stage III	AJCC TNM stage IVA	<i>P</i>
Development cohort	736 (100.0)	107 (14.5)	381 (51.8)	248 (33.7)	<0.001 ^a
Undetectable pretreatment EBV DNA (0 copies/ml)	195 (26.5)	45 (42.1)	103 (27.0)	47 (18.9)	
Detectable pretreatment EBV DNA (>0 copies/ml)	541 (73.5)	62 (57.9)	278 (73.0)	201 (81.1)	
Median (copies/ml)	4830	2925	4450	6575	
Interquartile range	1280-21150	706.5-10185	1128-18125	1963-31300	
Validation cohort	314 (100.0)	49 (15.6)	163(51.9)	102 (32.5)	<0.001 ^a
Undetectable pretreatment EBV DNA (0 copies/ml)	80 (25.5)	22 (44.9)	44 (27.0)	14 (13.7)	
Detectable pretreatment EBV DNA (>0 copies/ml)	234 (74.5)	27 (55.1)	119 (63.0)	88 (86.3)	
Median (copies/ml)	4135	2680	3010	12900	
Interquartile range	1098-18050	498-15000	690-12200	2128-36850	

Abbreviations: EBV, Epstein-Barr virus; DNA, deoxyribonucleic acid; AJCC, American Joint Committee on Cancer (8th Edition); TNM, tumor-lymph node-metastasis

^aCorrelation of pretreatment EBV DNA (Undetectable vs. Detectable) and AJCC TNM Stage (II, III, IVA) was by Chi-squared test

sTable 3. Distribution of postradiotherapy plasma EBV DNA by TNM stage (AJCC 8th edition)

No of patients (%)	All stage (II-IVA)	AJCC TNM stage II	AJCC TNM stage III	AJCC TNM stage IVA	<i>P</i>
Development cohort	736 (100.0)	107 (14.5)	381 (51.8)	248 (33.7)	0.007 ^a
Undetectable postradiotherapy EBV DNA (0 copies/ml)	666 (90.5)	103 (96.3)	349 (91.6)	214 (86.3)	
Detectable postradiotherapy EBV DNA (>0 copies/ml)	70 (9.5)	4 (3.7)	32 (8.4)	34 (13.7)	
Median (copies/ml)	680	152.3	578.1	970	
Interquartile range	113.8-3385	37.6-912.8	92-3095	135.8-6028	
Validation cohort	314 (100.0)	49 (15.6)	163(51.9)	102 (32.5)	0.019 ^a
Undetectable postradiotherapy EBV DNA (0 copies/ml)	273 (86.9)	48 (98.0)	140 (85.9)	85 (83.3)	
Detectable postradiotherapy EBV DNA (>0 copies/ml)	41 (13.1)	1 (2.0)	23 (14.1)	17 (16.7)	
Median (copies/ml)	950	1750	488	1030	
Interquartile range	142.5-7710	-	136-10400	196-7585	

Abbreviations: EBV, Epstein-Barr virus; DNA, deoxyribonucleic acid; AJCC, American Joint Committee on Cancer (8th Edition); TNM, tumor-lymph node-metastasis

^aCorrelation of postradiotherapy EBV DNA (Undetectable vs. Detectable) and AJCC TNM Stage (II, III, IVA) was by Chi-squared test.

sTable 4. Comparison of AUC and OR of residue at different level of pretreatment EBV DNA in the development cohort (n=736)

Level of pretreatment EBV DNA cut-off (copies/ml)	AUC (95% CI)	OR (95% CI)	<i>P</i> ^a
EBV DNA: 2 groups (Undetectable vs. Detectable)	0.575 (0.523-0.627)	2.52 (1.47-4.33)	0.001
EBV DNA: 2 groups (0-4999 vs. ≥ 5000)	0.562 (0.505-0.618)	1.67 (1.13-2.48)	0.010
EBV DNA: 2 groups (0-19999 vs. ≥ 20000)	0.561 (0.503-0.619)	2.01 (1.29-3.12)	0.002
EBV DNA: 3 groups (0 vs. 1-4999 vs. ≥ 5000)	0.593 (0.541-0.646)	1.00	-
		2.23 (1.24-4.02)	0.007
		2.83 (1.59-5.04)	<0.001
EBV DNA: 3 groups (0-4999 vs. 5000-19999 vs. ≥ 20000)	0.572 (0.515-0.630)	1.00	-
		1.24 (0.72-2.12)	0.432
		2.11 (1.33-3.34)	0.002
EBV DNA: 4 groups (0 vs. 1-4999 vs. 5000-19999 vs. ≥ 20000)	0.604 (0.550-0.657)	1.00	-
		2.23 (1.24-4.02)	0.007
		2.09 (1.06-4.15)	0.034
		3.56 (1.90-6.64)	<0.001

Abbreviations: EBV, Epstein-Barr virus; DNA, deoxyribonucleic acid; AUC, area under curve; OR, odds ratio; CI, confidence interval

^aLogistic regression model was used to calculate the odds ratio, corresponding 95% CI and *P* value

sTable 5. Comparison of AUC and OR of residue at different level of postradiotherapy EBV DNA in the development cohort (n=736)

Level of postradiotherapy EBV DNA cut-off (copies/ml)	AUC (95% CI)	OR (95% CI)	<i>P</i> ^a
EBV DNA: 2 groups (Undetectable vs. Detectable)	0.625 (0.564-0.685)	7.66 (4.55-12.91)	<0.001
EBV DNA: 2 groups (0-499 vs. ≥ 500)	0.596 (0.535-0.656)	12.52 (6.22-25.21)	<0.001
EBV DNA: 2 groups (0-1999 vs. ≥ 2000)	0.556 (0.496-0.615)	12.16 (4.84-30.52)	<0.001
EBV DNA: 3 groups (0 vs. 1-499 vs. ≥ 500)	0.627 (0.567-0.688)	1.00	-
		3.76 (1.74-8.12)	0.001
		13.67 (6.76-27.63)	<0.001
EBV DNA: 3 groups (0-499 vs. 500-1999 vs. ≥ 2000)	0.596 (0.536-0.656)	1.00	-
		11.48 (4.15-31.76)	<0.001
		13.42 (5.33-33.75)	<0.001
EBV DNA: 4 groups (0 vs. 1-499 vs. 500-1999 vs. ≥ 2000)	0.604 (0.550-0.657)	1.00	-
		3.76 (1.74-8.12)	0.001
		12.53 (4.52-34.77)	<0.001
		14.65 (5.80-36.96)	<0.001

Abbreviations: EBV, Epstein-Barr virus; DNA, deoxyribonucleic acid; AUC, area under curve; OR, odds ratio; CI, confidence interval

^aLogistic regression model was used to calculate the odds ratio, corresponding 95% CI and *P* value

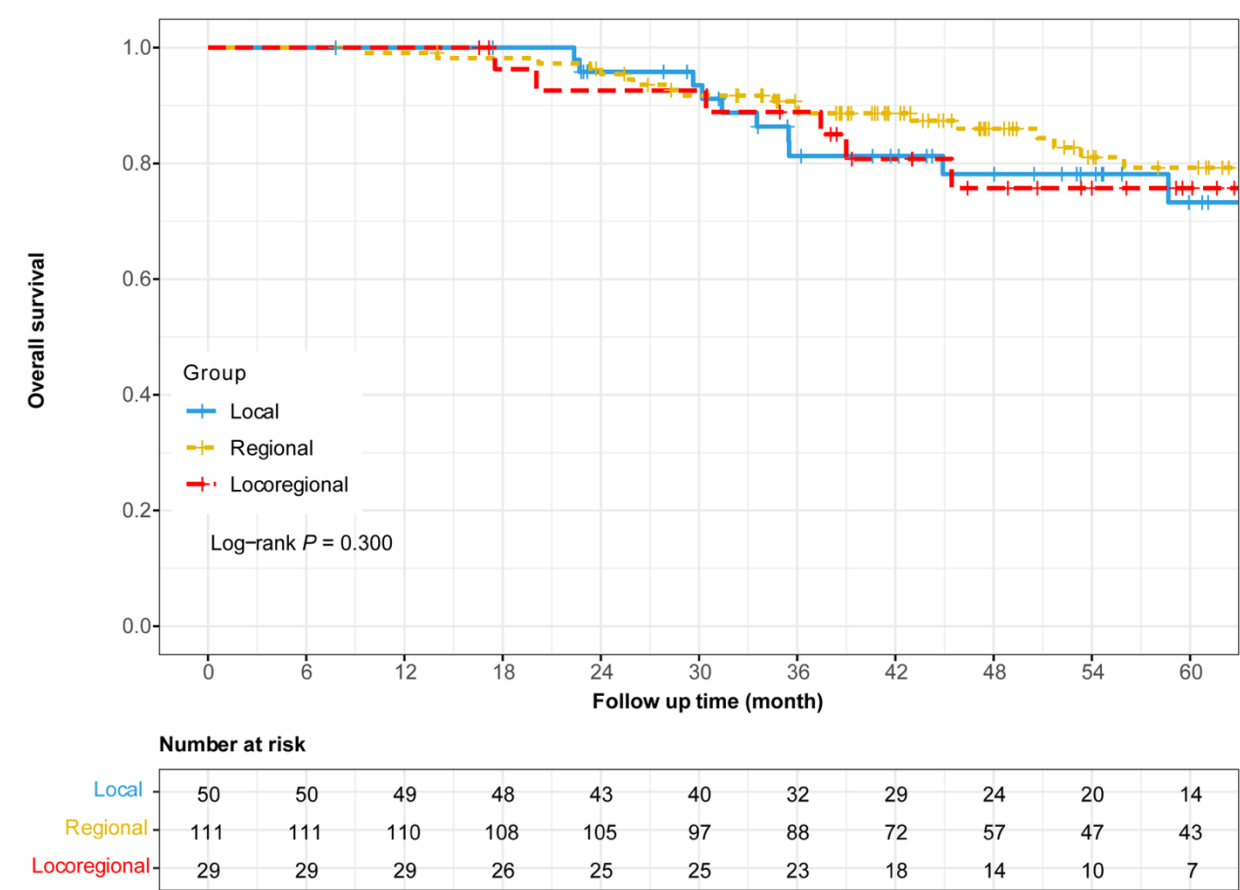
sTable 6. Pretreatment and postradiotherapy levels of EBV DNA in all patients (n=1050)

Pretreatment EBV DNA, copies/ml	Postradiotherapy EBV DNA, copies/ml			
	0	1-499	≥500	Total (%)
0	262 (24.9)	8 (0.8)	5 (0.5)	275 (26.2)
1-4999	360 (34.3)	23 (2.2)	17 (1.6)	400 (38.1)
≥5000	317 (30.2)	18 (1.7)	40 (3.8)	375 (35.7)
Total	939 (89.4)	49 (4.7)	62 (5.9)	1050 (100.0)

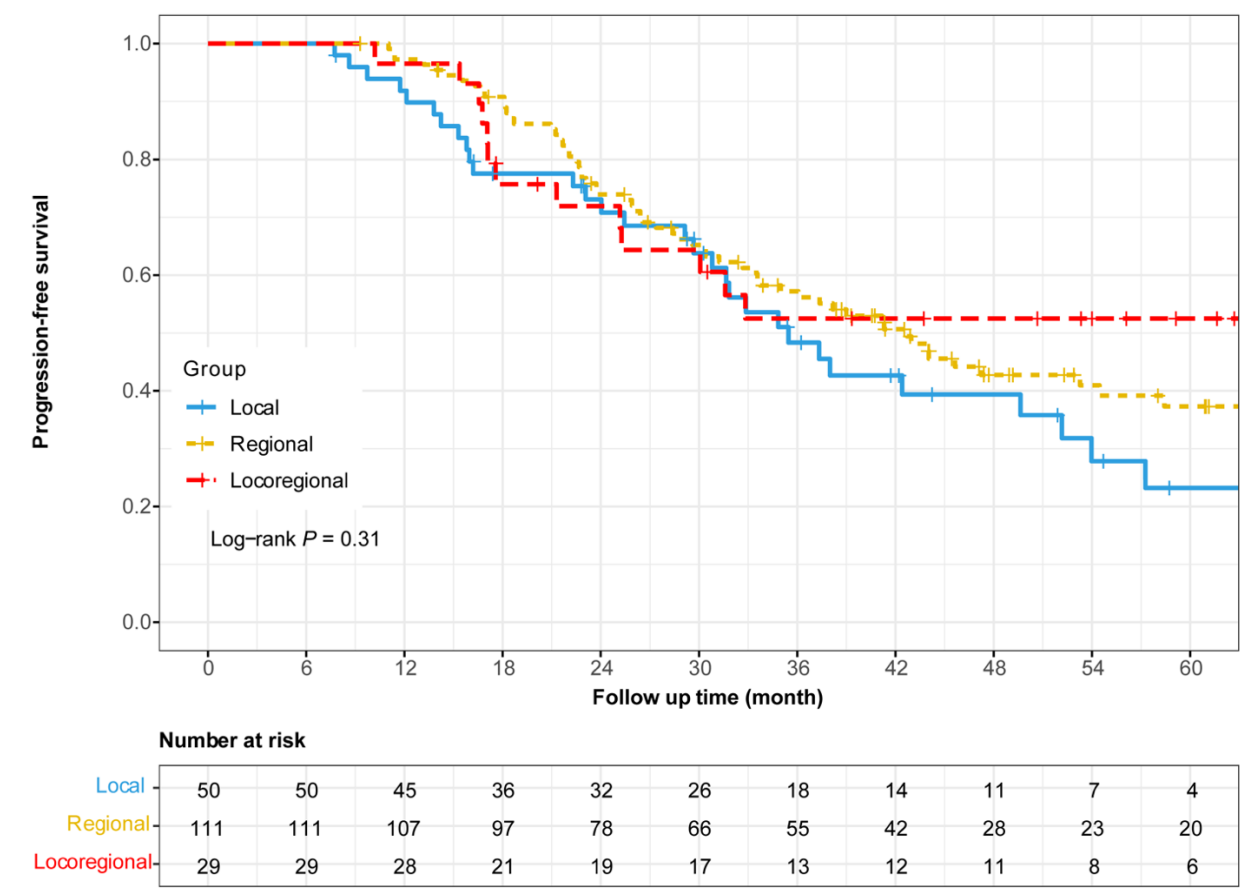
Abbreviations: EBV, Epstein-Barr virus; DNA, deoxyribonucleic acid

sFigure 1. Kaplan-Meier curves for overall survival (A), progression-free survival (B), locoregional recurrence-free survival (C) and distant metastasis-free survival (D) in patients stratified by residual tumor type (n=190)

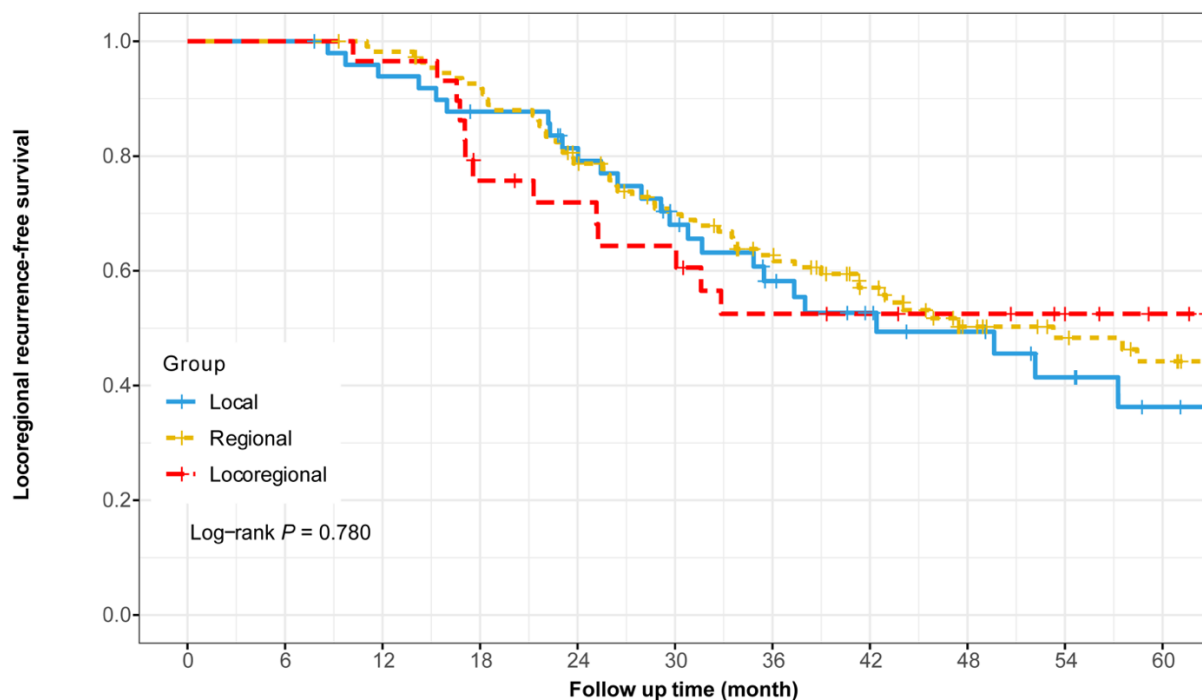
A.



B.



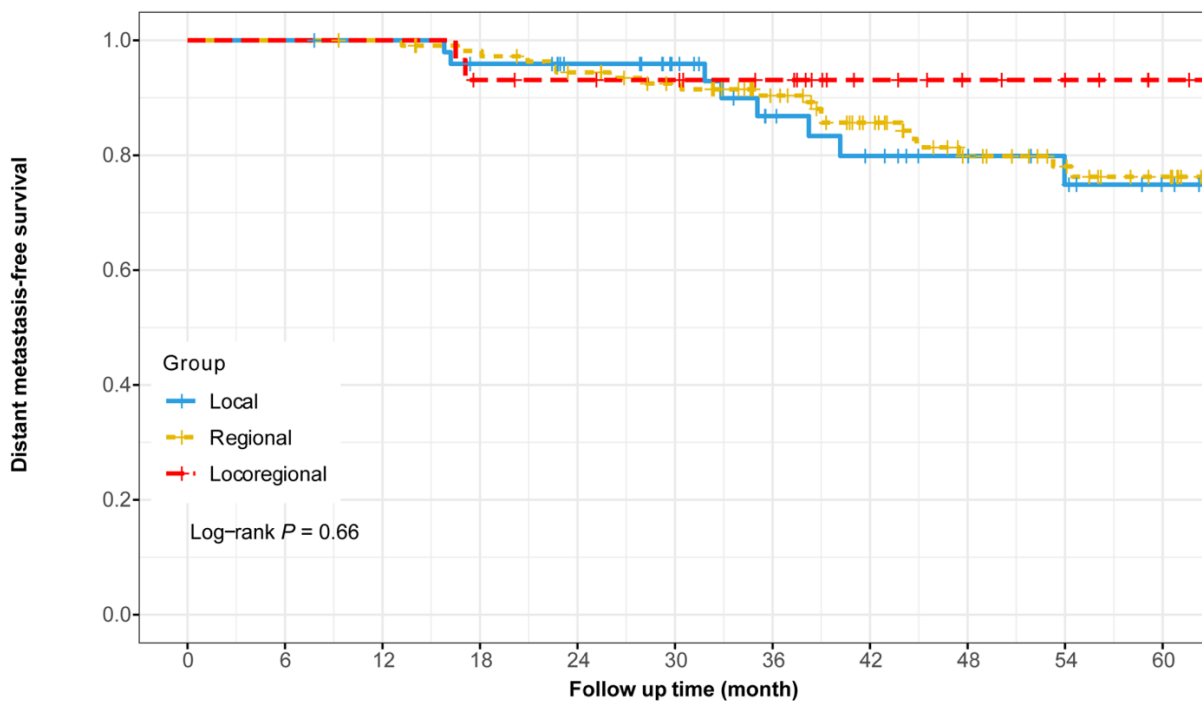
C.



Number at risk

Local	50	50	46	42	37	29	22	17	14	10	6
Regional	111	111	108	100	83	70	59	46	31	25	21
Locoregional	29	29	28	21	19	17	13	12	11	8	6

D.



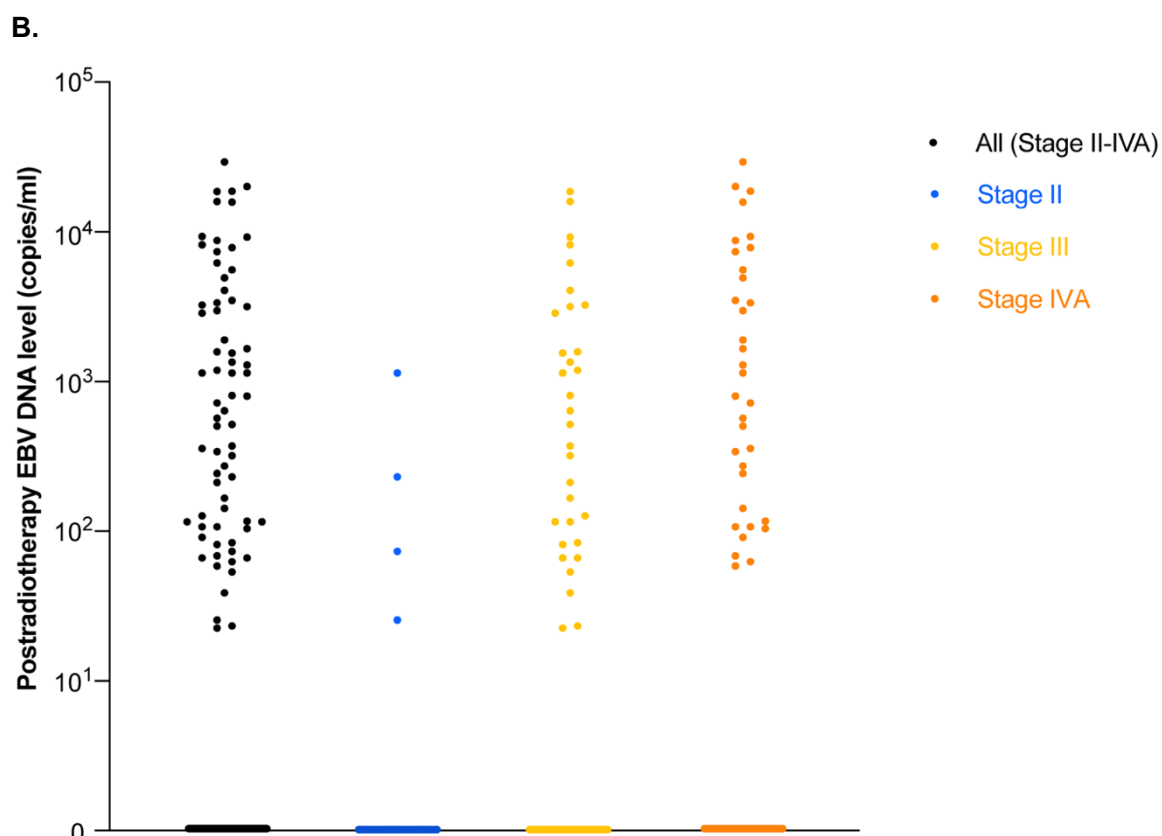
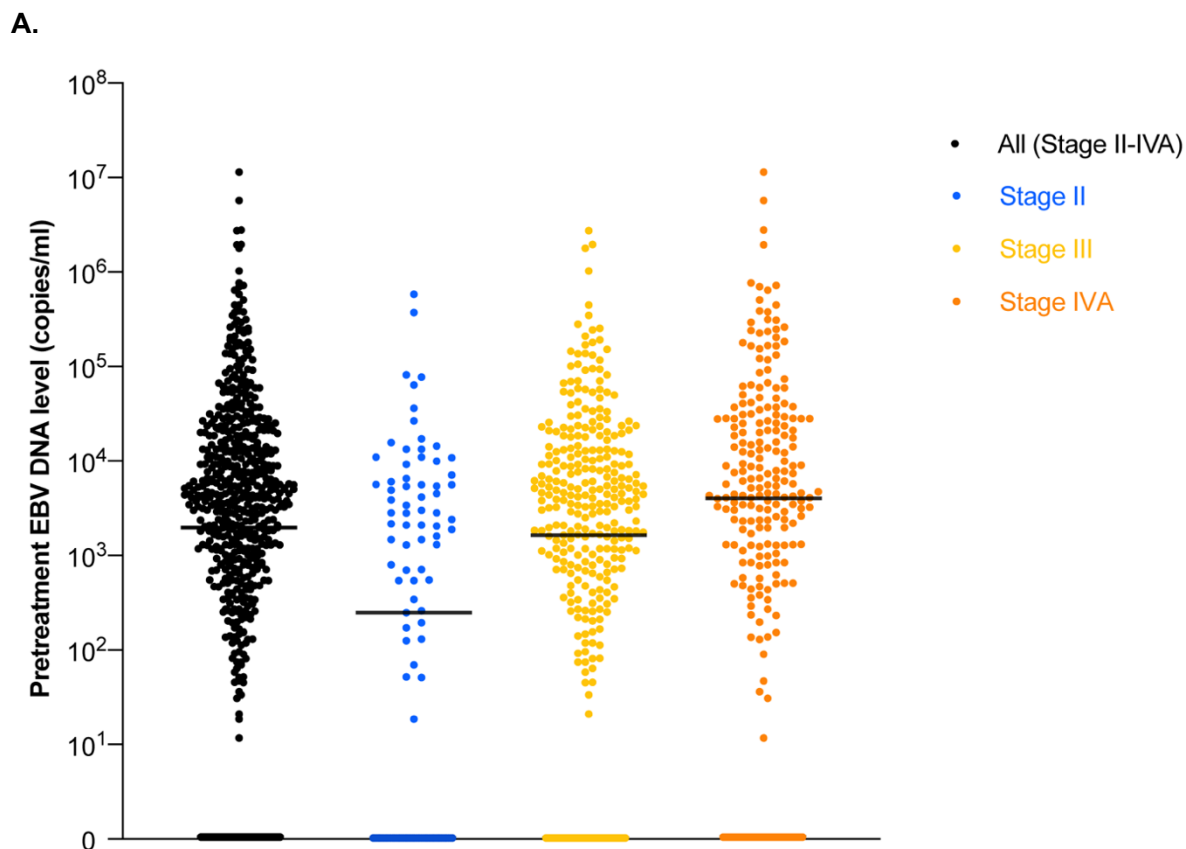
Number at risk

Local	50	50	49	46	41	35	26	22	17	15	11
Regional	111	111	110	106	100	93	81	65	51	44	37
Locoregional	29	29	29	26	25	24	21	14	11	9	6

sFigure 2. Pretreatment (A) and postradiotherapy (B) plasma EBV DNA levels by TNM stage (AJCC 8th edition) in the development cohort (n=736)

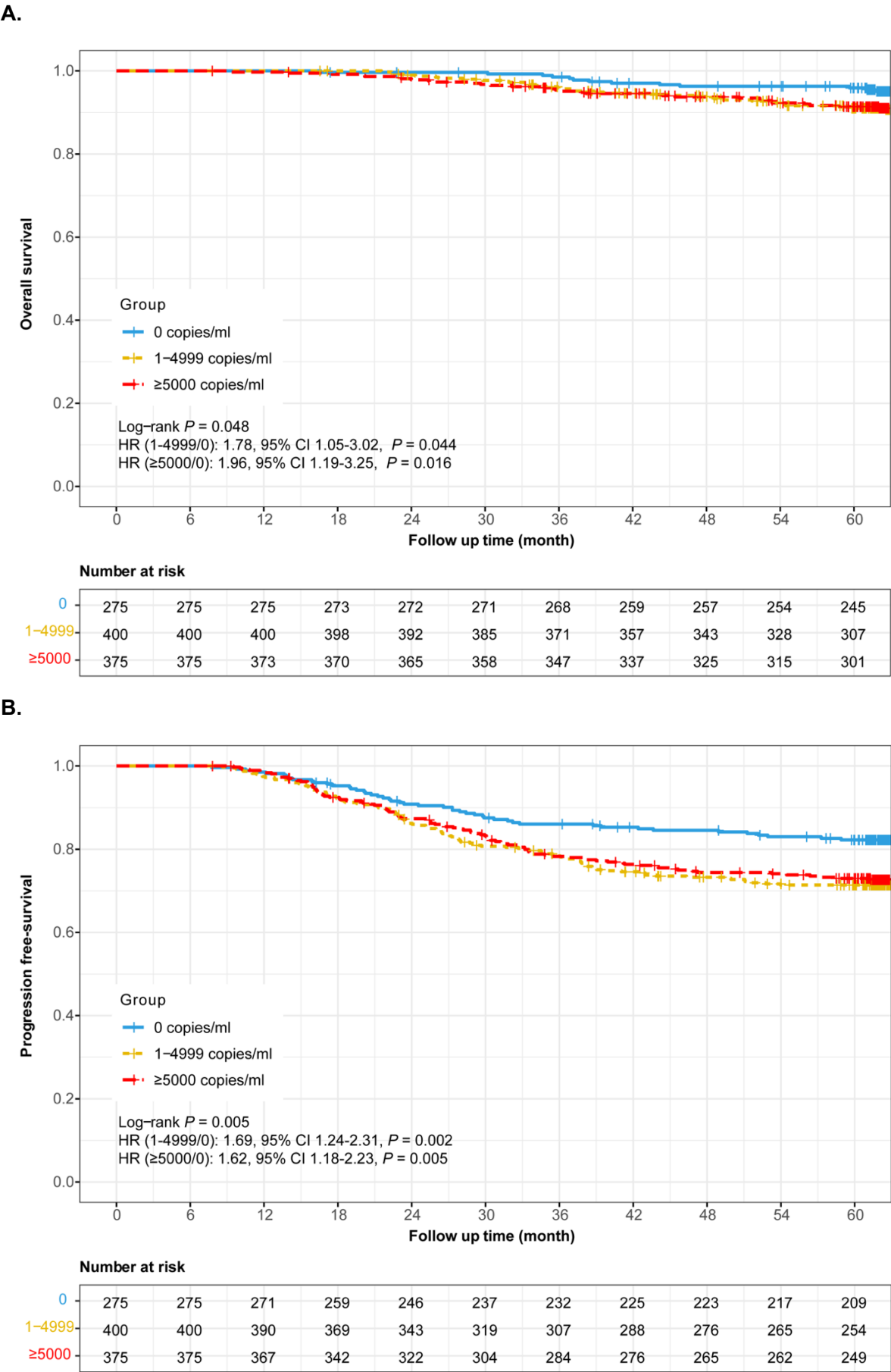
Abbreviations: EBV, Epstein-Barr virus; DNA, deoxyribonucleic acid

The black line represents the median value. The bottom and top of the dots are the 25th and 75th percentiles (interquartile range).

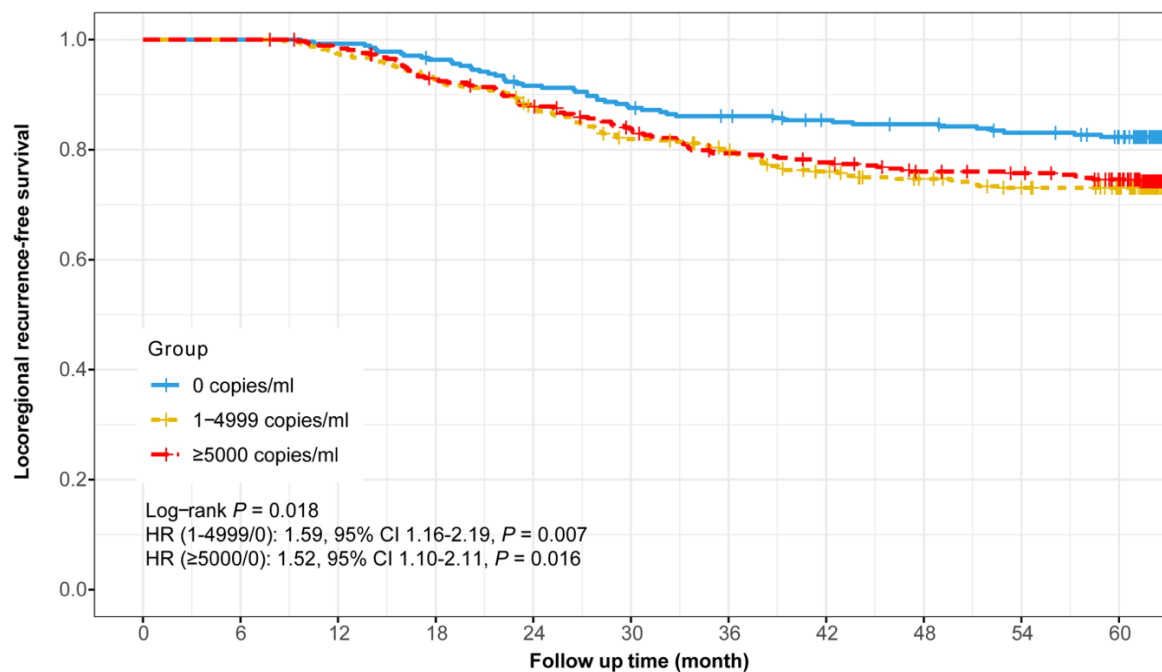


sFigure 3. Kaplan-Meier curves for overall survival (A), progression-free survival (B), locoregional recurrence-free survival (C) and distant metastasis-free survival (D) in all patients stratified by pretreatment plasma EBV DNA level (n=1050)

Abbreviations: EBV, Epstein-Barr virus; DNA, deoxyribonucleic acid



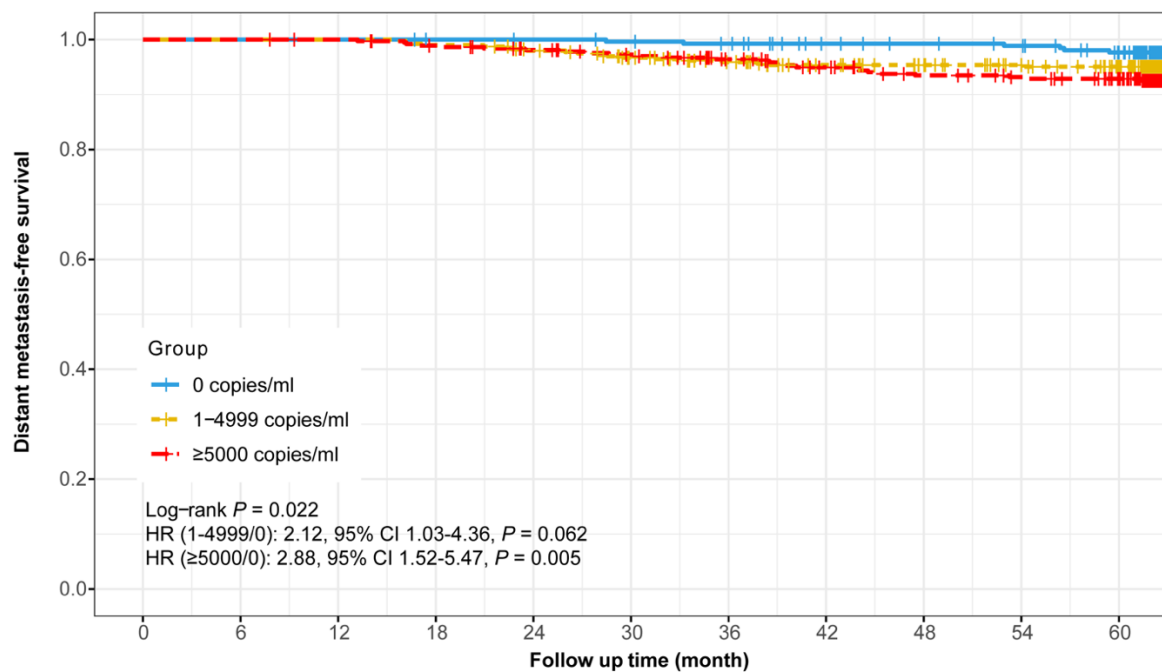
C.



Number at risk

0	275	275	273	264	250	239	233	226	223	217	209
1-4999	400	400	390	371	347	323	311	290	278	267	255
≥5000	375	375	367	344	324	305	287	280	269	265	251

D.

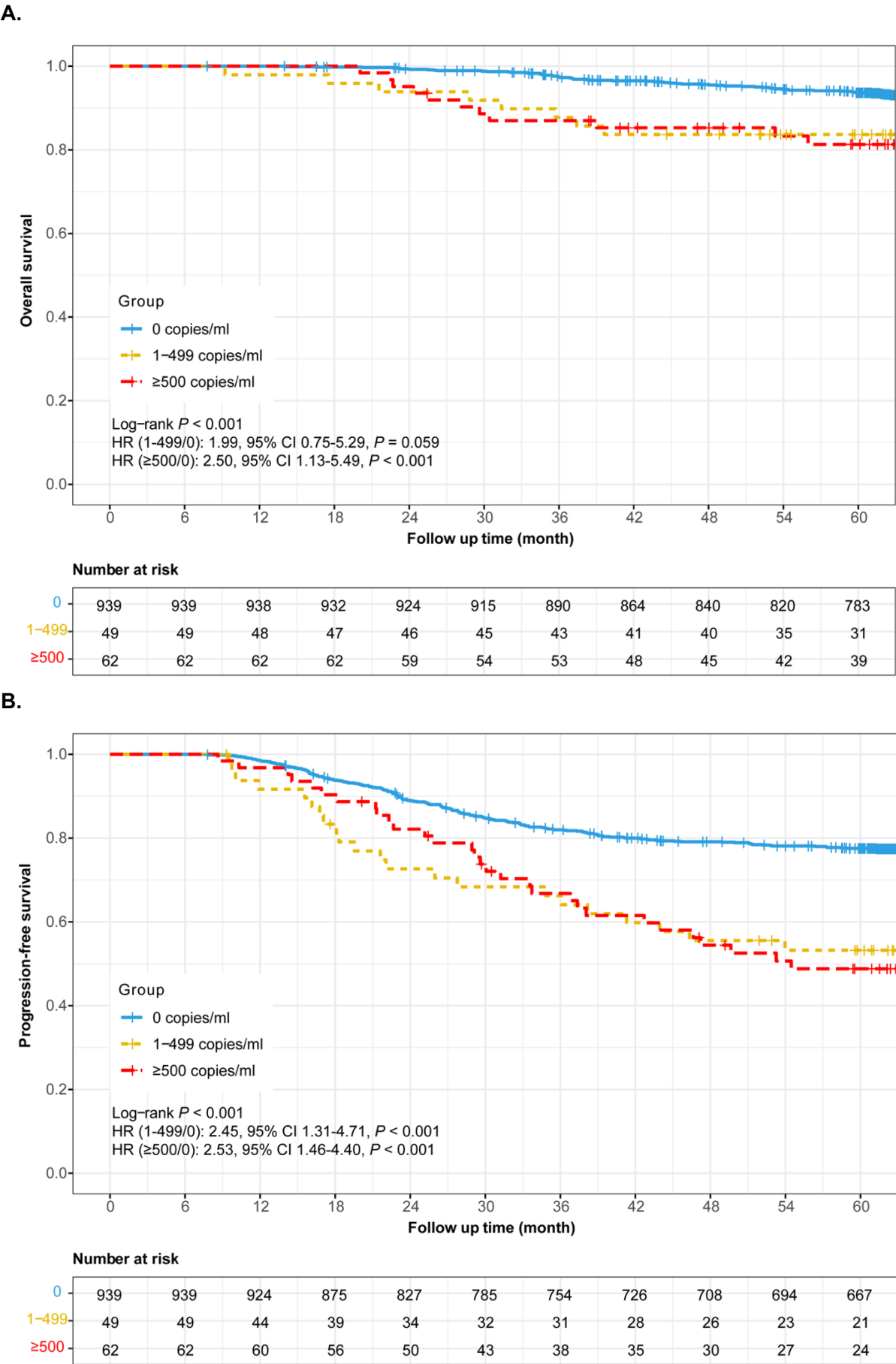


Number at risk

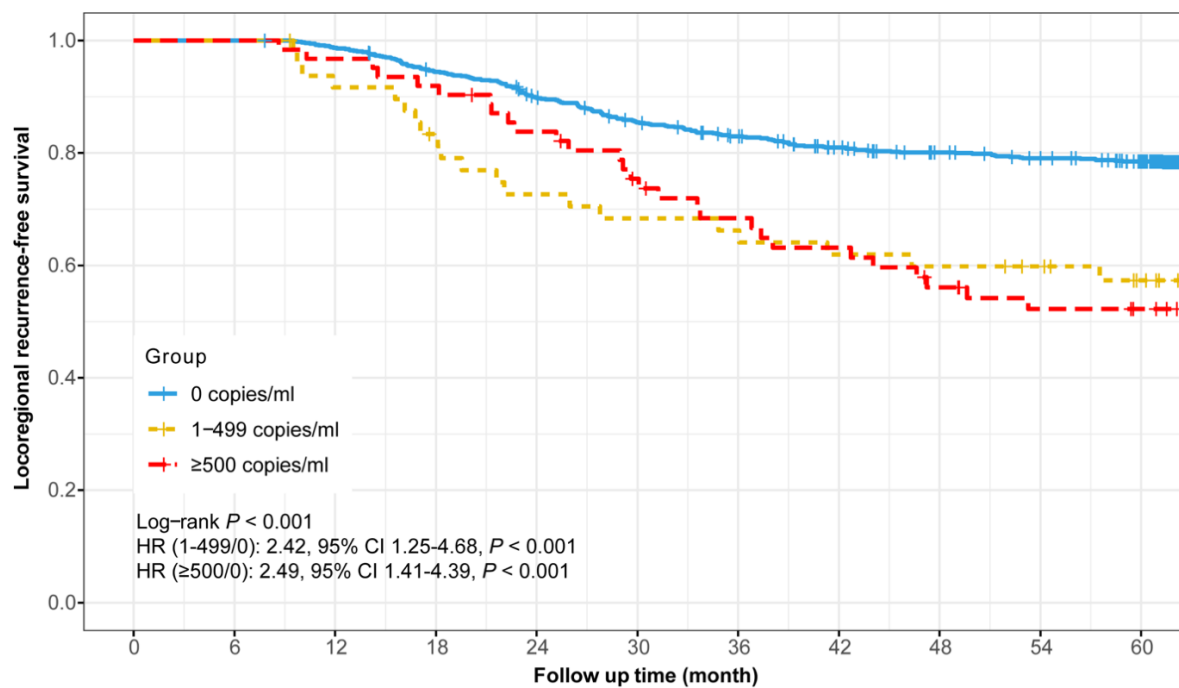
0	275	275	275	273	272	270	267	258	255	252	241
1-4999	400	400	400	396	387	373	359	344	332	320	301
≥5000	375	375	373	366	358	349	335	322	311	305	290

sFigure 4. Kaplan-Meier curves for overall survival (A), progression-free survival (B), locoregional recurrence-free survival (C) and distant metastasis-free survival (D) in all patients stratified by postradiotherapy plasma EBV DNA level (n=1050)

Abbreviations: EBV, Epstein-Barr virus; DNA, deoxyribonucleic acid



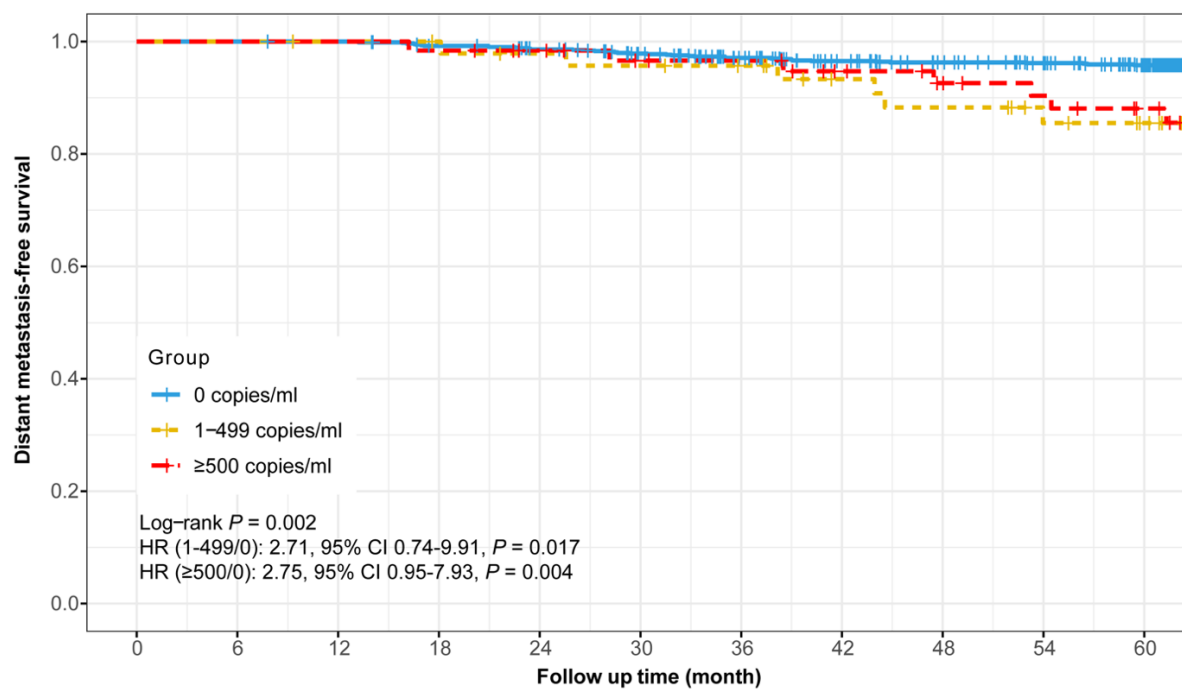
C.



Number at risk

0	939	939	926	883	836	791	761	731	711	696	669
1-499	49	49	44	39	34	32	31	29	28	26	21
≥500	62	62	60	57	51	44	39	36	31	27	25

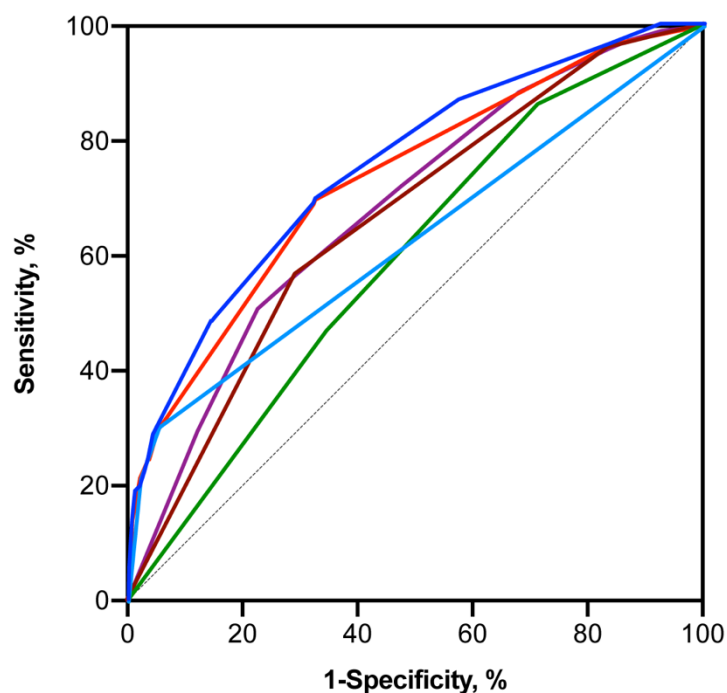
D.



Number at risk

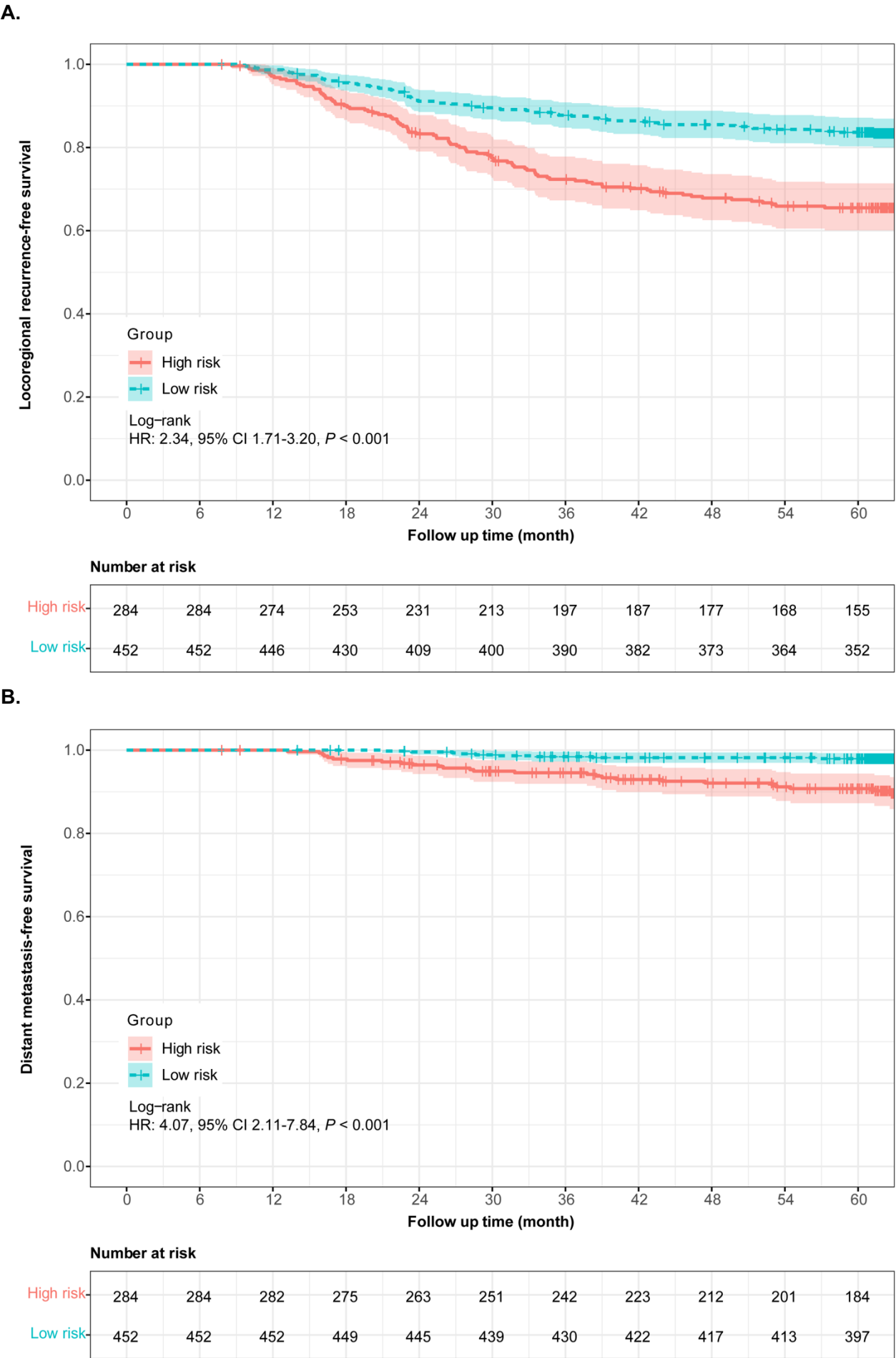
0	939	939	938	927	914	895	867	840	821	806	768
1-499	49	49	48	47	45	44	42	37	35	31	28
≥500	62	62	62	61	58	53	52	47	42	40	36

sFigure 5. Comparison of six models using area under the receiver operating characteristic curve calculated for the development cohort (n=736)

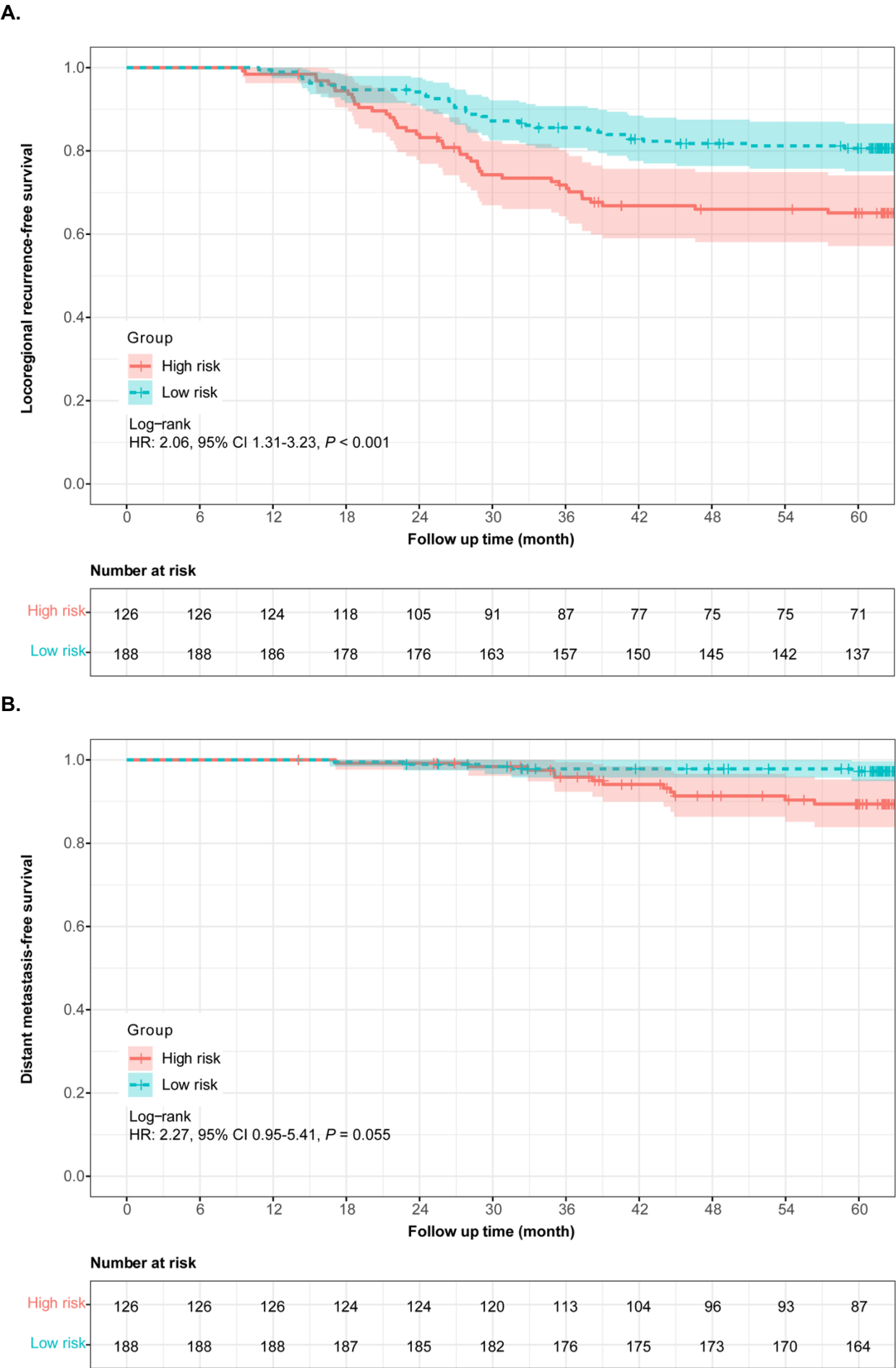


Model	AUC (95% CI)
Pretreatment EBV DNA	0.593 (0.541-0.646)
Postradiotherapy EBV DNA	0.627 (0.567-0.688)
Clinical stage	0.659 (0.607-0.710)
Clinical stage+Pretreatment EBV DNA	0.683 (0.633-0.733)
Clinical stage+Postradiotherapy EBV DNA	0.733 (0.682-0.783)
Prediction nomogram	0.752 (0.704-0.800)

sFigure 6. Kaplan-Meier curves for locoregional recurrence-free survival (A) and distant metastasis-free survival (B) in development cohort stratified by predicted risk in tumor residue (n=736)

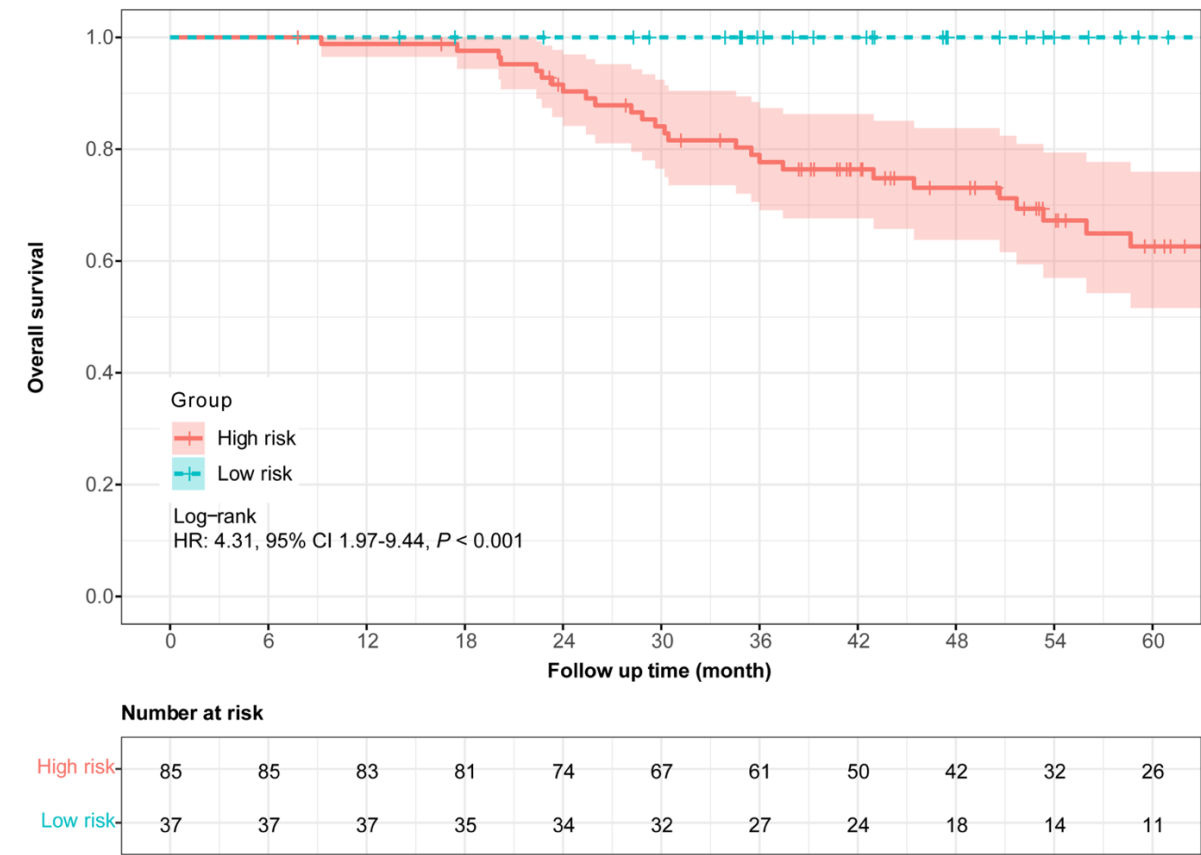


sFigure 7. Kaplan-Meier curves for locoregional recurrence-free survival (A) and distant metastasis-free survival (B) in validation cohort stratified by predicted risk in tumor residue (n=314)

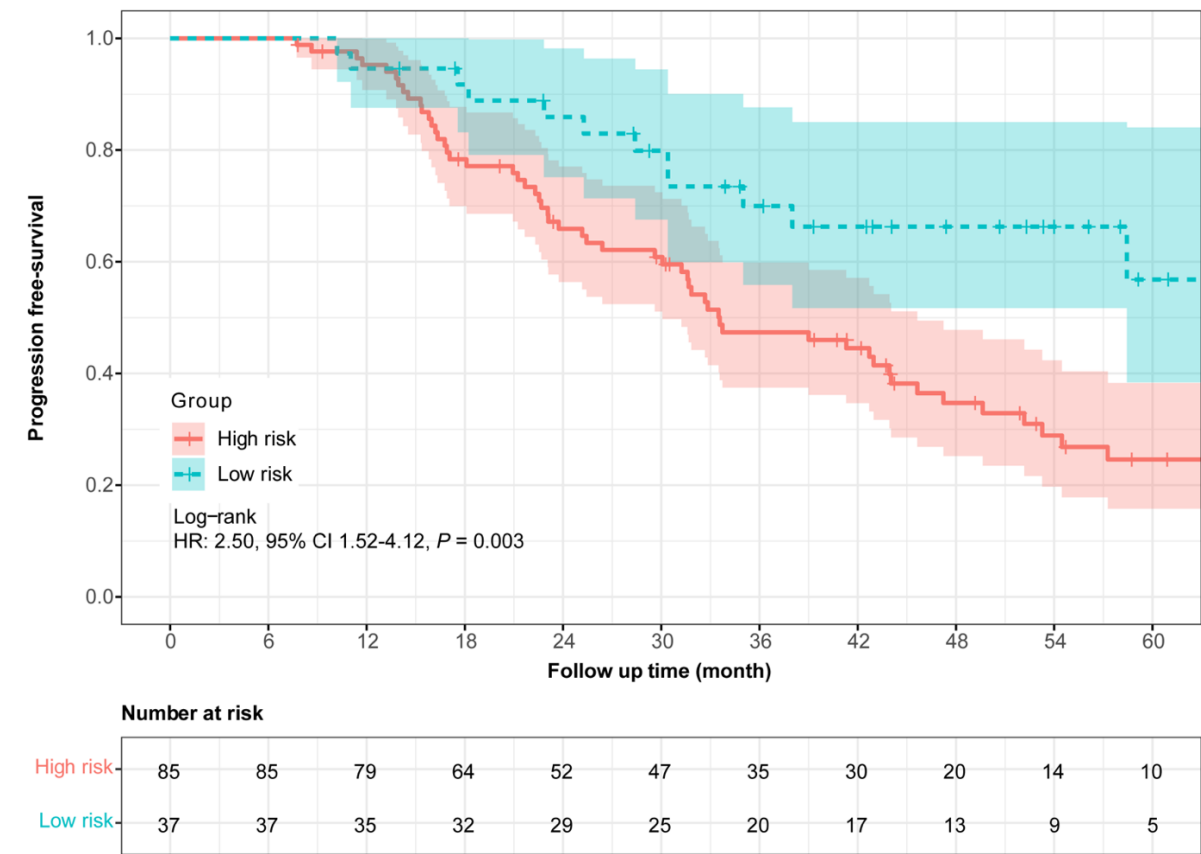


sFigure 8. Kaplan-Meier curves for overall survival (A), progression free-survival (B), locoregional recurrence-free survival (C) and distant metastasis-free survival (D) in patients with residue from development cohort stratified by predicted risk in tumor residue (n=122)

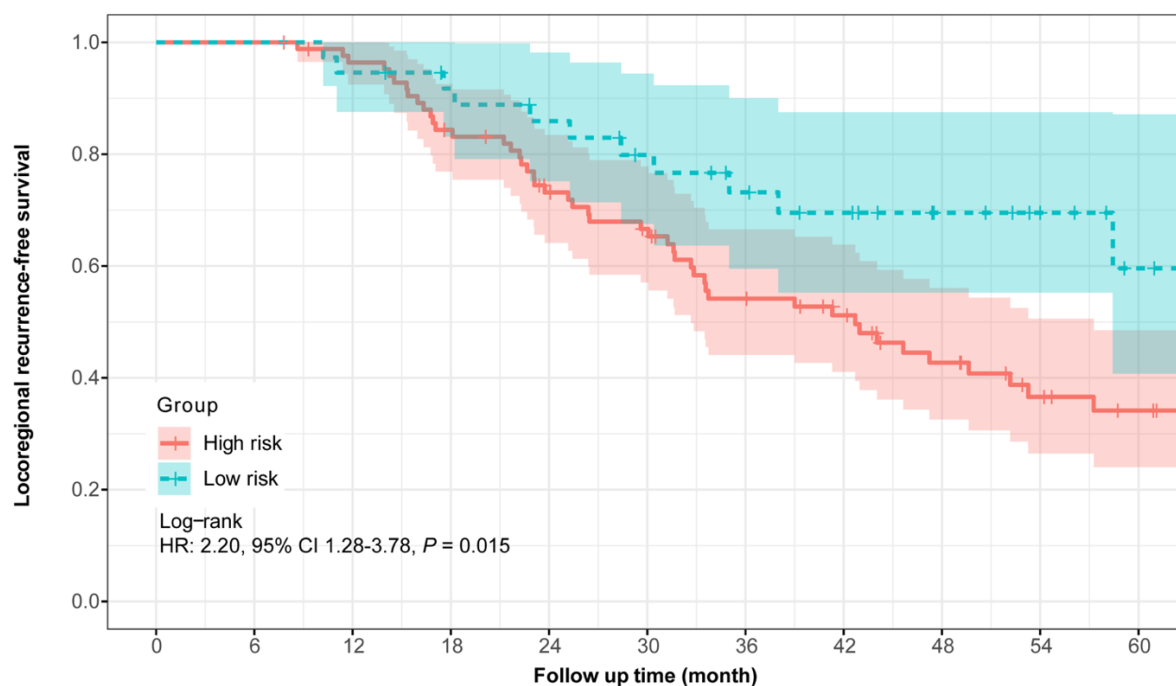
A.



B.



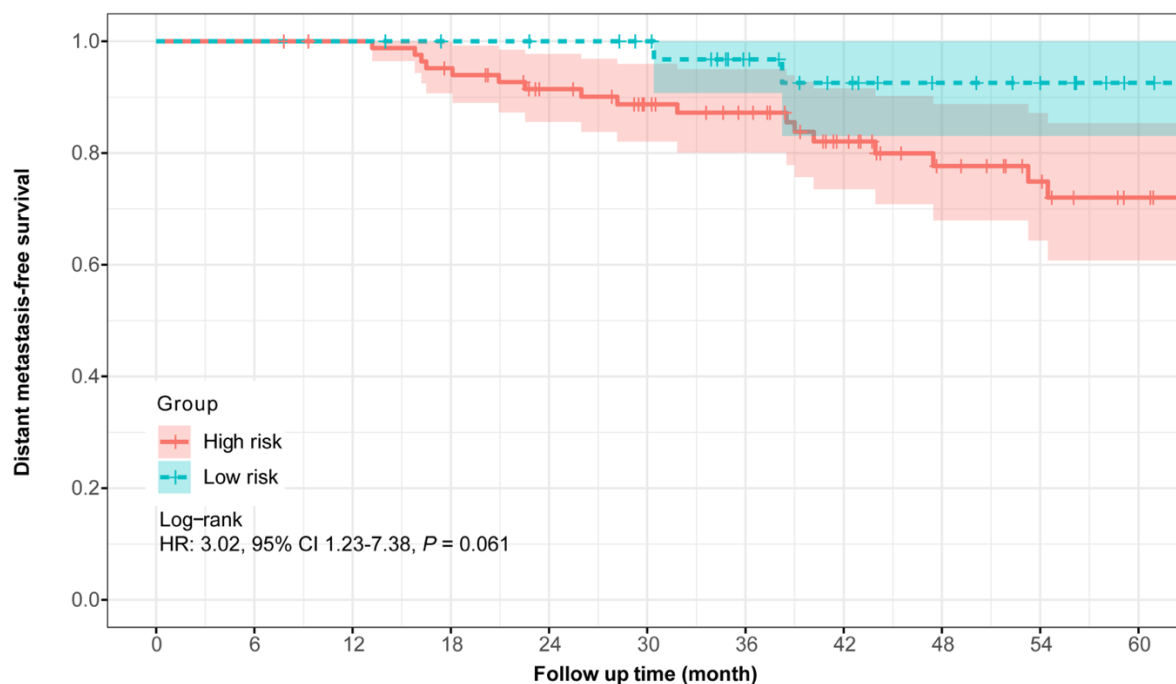
C.



Number at risk

High risk	85	85	80	69	57	50	39	33	24	17	13
Low risk	37	37	35	32	29	25	21	18	13	9	5

D.

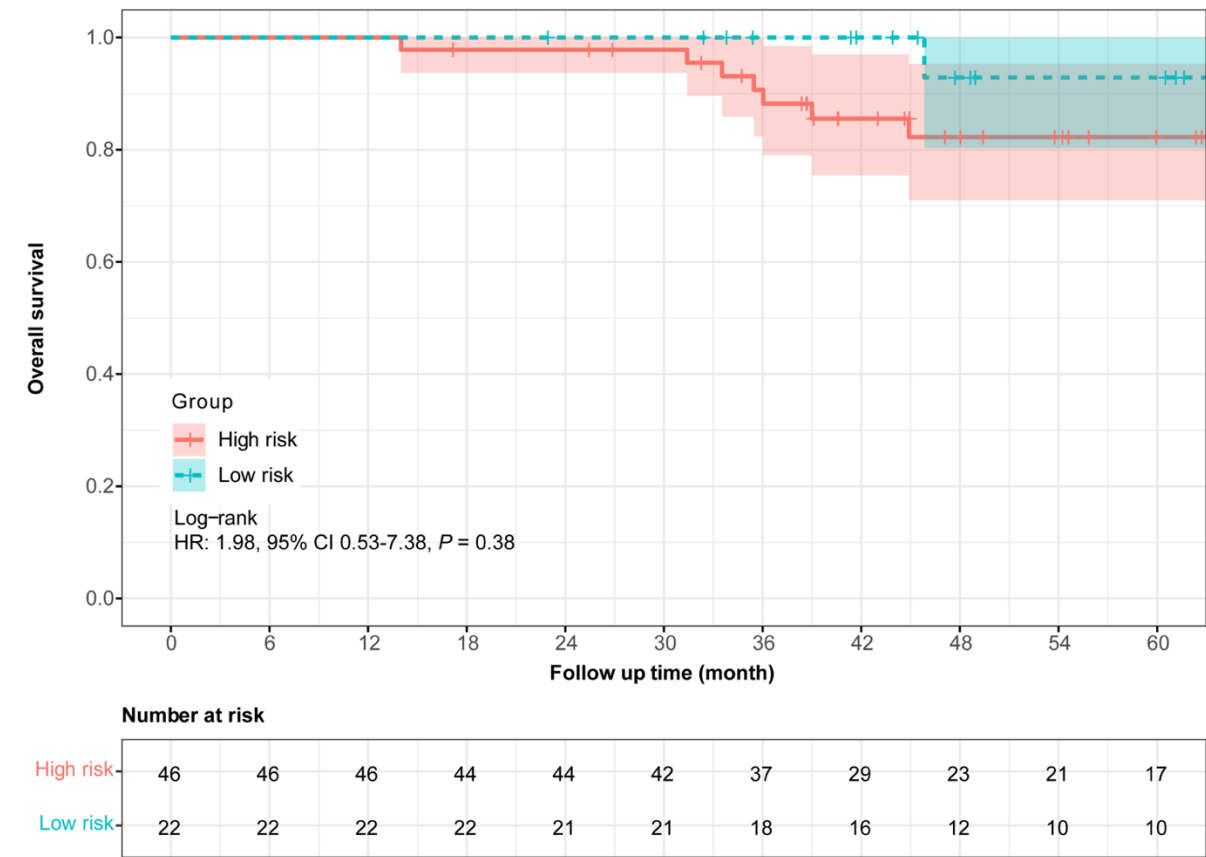


Number at risk

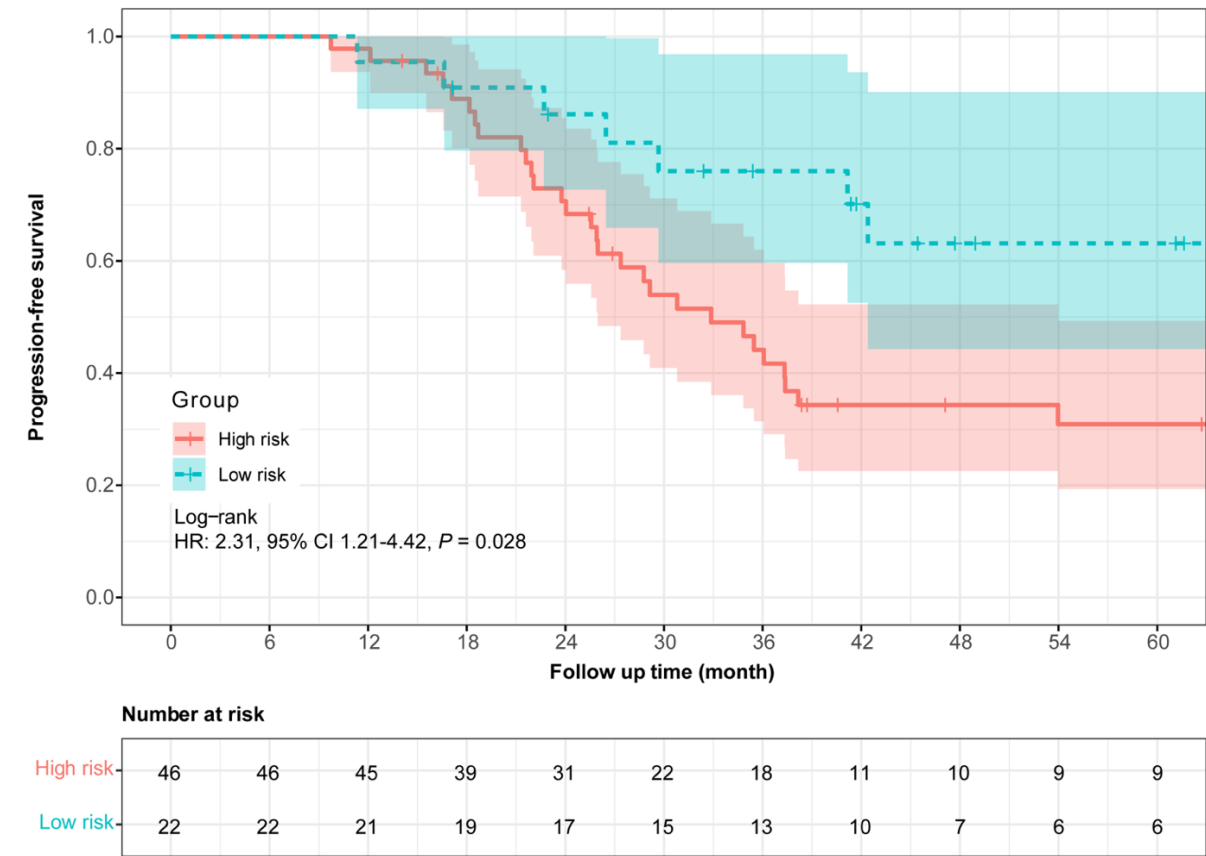
High risk	85	85	83	78	69	61	55	43	33	27	21
Low risk	37	37	37	35	34	32	25	20	16	13	8

sFigure 9. Kaplan-Meier curves for overall survival (A), progression free-survival (B), locoregional recurrence-free survival (C) and distant metastasis-free survival (D) in patients with residue from validation cohort stratified by predicted risk in tumor residue (n=68)

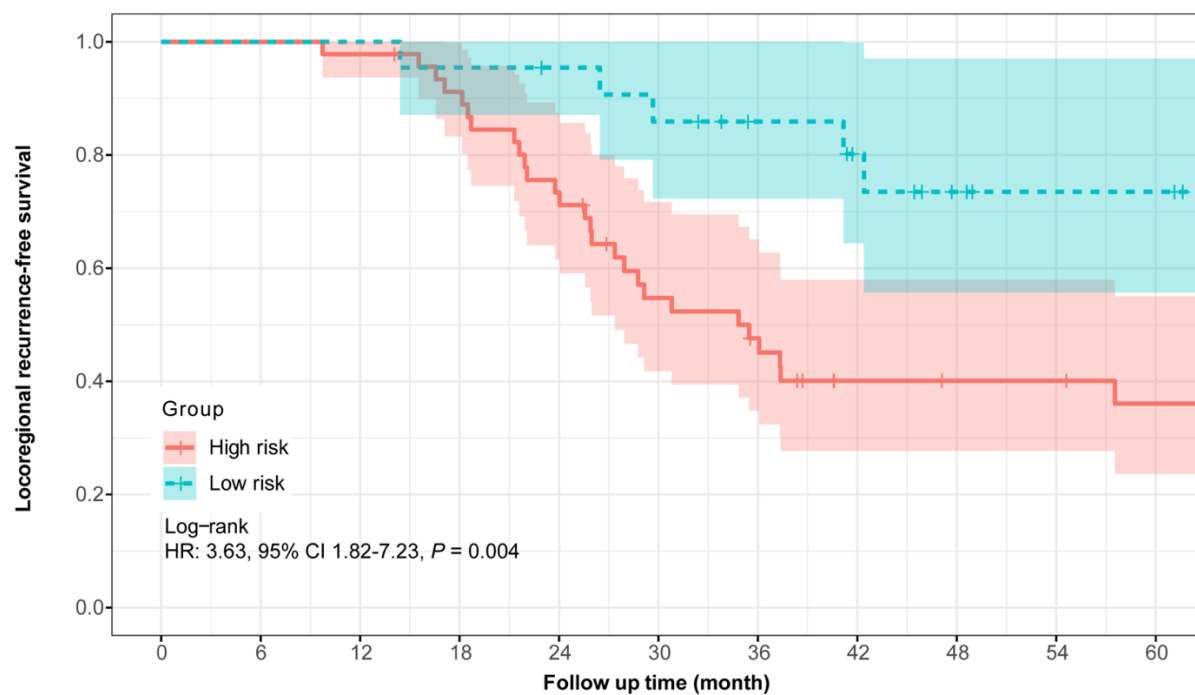
A.



B.



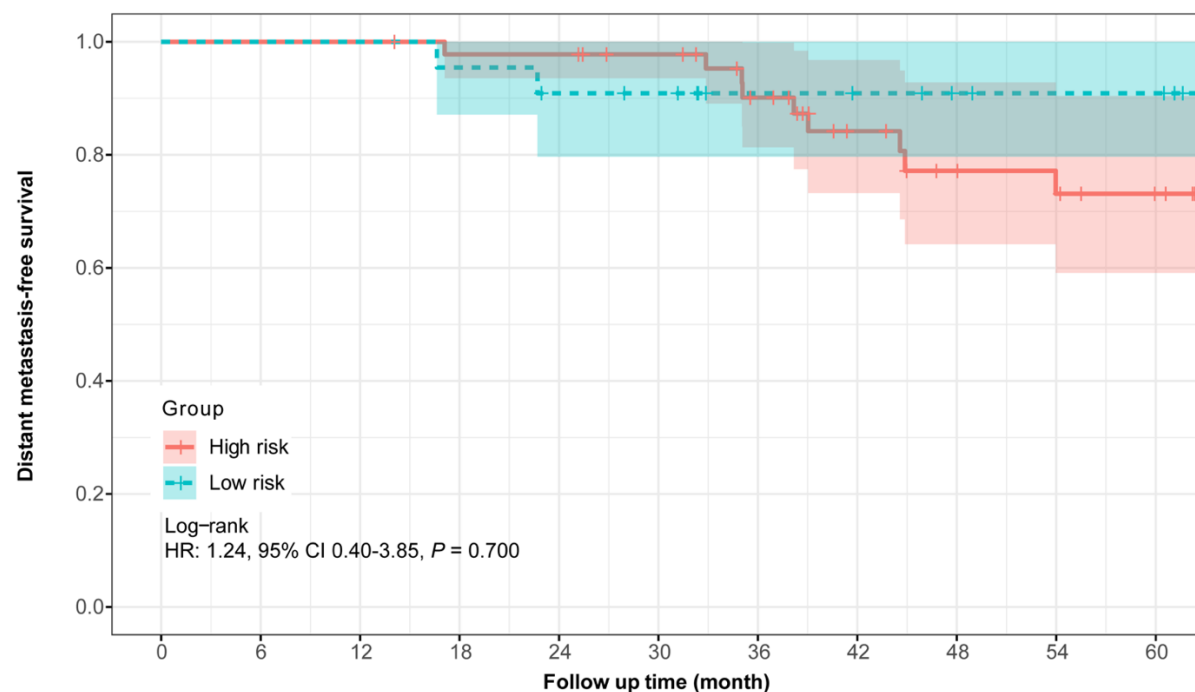
C.



Number at risk

High risk	46	46	45	41	33	23	19	12	11	11	9
Low risk	22	22	22	21	20	18	15	12	8	6	6

D.



Number at risk

High risk	46	46	46	44	44	41	34	25	19	18	15
Low risk	22	22	22	21	19	18	14	13	11	10	10