Appendix 1

Supplementary methods

Treatment

Radiation treatment protocol:

The gross tumor volume (GTV) included the primary tumor volume, enlarged retropharyngeal lymph nodes (GTVnx), and involved cervical lymph nodes (GTVnd).

The high-risk clinical target volume (CTV1) was defined as the GTVnx plus a 5–10 mm margin, with a 2–3 mm margin being incorporated posteriorly if adjacent to critical structures such as the brainstem or spinal cord. This volume covers the high-risk sites of microscopic extension and the entirety of the nasopharynx.

Low dose clinical target volume (CTV2) extended from CTV1 by an additional 5 to 10-mm margin (2–3 mm posteriorly if adjacent to the brainstem or spinal cord), covering the low-risk sites of microscopic extension. This includes the foramen lacerum, sphenoid sinus, clivus, oval foramen, parapharyngeal space, pterygoid fossae, posterior parts of the nasal cavity, pterygopalatine fossae, retropharyngeal nodal regions, and the cervical levels where involved lymph nodes were located. Elective irradiation of the neck area from level II to V was performed based on the specifics of the patient's treatment group.

There were special considerations for elective irradiation. Level Ib lymph nodes (LNs) were irradiated if they were involved; meanwhile, level IIa LNs were irradiated under the following conditions: they had a diameter ≥3 cm or exhibited extracapsular extension; there was extensive nodal disease on the ipsilateral neck; and the oral cavity, soft or hard palate, or ipsilateral nasal cavity was grossly involved.

A planning target volume (PTV) was created via addition of a three-dimensional margin of 3–5 mm to each delineated target volume to account for uncertainties in treatment setup and internal organ motion. A specific 3-mm margin was added around critical organs such as the brainstem and spinal cord to form the planning organ at risk volume (PRV).

For radiation doses, 68–70 Gy was administered to GTVnx and GTVnd, 66–70 Gy to CTV1, 60–62 Gy to CTV2, and 54–56 Gy to additional elective regions. Each dose was delivered in 30–33 fractions, once per day, 5 days a week.

Chemotherapy regimens for chemotherapy or concurrent CRT (CCRT) included the administration of cisplatin at a dose of 30–40 mg/m^2 per week or 80–100 mg/m^2 for 2–3 cycles over 3 weeks. Induction chemotherapy (IC) was delivered before IMRT for 2–3 cycles within 21 days, with 80 mg/m^2 cisplatin + 1,000 mg/m^2 of 5-fluorouracil, 75 mg/m^2 cisplatin + 75 mg/m^2 of docetaxel, or 60 mg/m^2 cisplatin + 600 mg/m^2 5-fluorouracil + 60 mg/m^2 of docetaxel.

Quantification of plasma Epstein-Barr virus (EBV) DNA levels

The plasma EBV DNA levels of patients were measured using quantitative polymerase chain reaction (qPCR) at diagnosis and 3–4 months after RT. The real-time qPCR system was developed in the BamHI-W region. The system consisted of the amplification primers W-44F (5'-AGTCTCTGCCTCCAGGCA-3') and W-119R (5'-ACAGAGGGCCTGTCCACCG-3') and the dual-labeled fluorescent probe W-67T (5'-[FAM]CACTG TCTGTAAAGTCCAGCCTCC[TAMRA]-3'). The β-actin gene was used as a loading control, and the primers 5'-ACAGGCACCAGGGCGTGATGG-3' (forward) and 5'-CTC CATGTCGTCCCAGTTGGT-3' (reverse) and the dual-labeled fluorescent probe sequence 5'-[FAM]CATCCT CACCCTGAAGTACCCCATC[TAMRA]-3' were used.

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Table S1 Multivariate analysis of risk factors for 5-year OS, DMFS, and LRFS

Survival	Variable	HR (95% CI)	P value
OS	LRG-sum		
	≤2	Reference	
	≤9	1.25 (0.66–2.38)	0.493
	>9	1.88 (0.69–5.10)	0.216
	Pretreatment EBV DNA (10^3 copies/mL)		
	<1	Reference	
	<26	2.60 (0.77–8.72)	0.123
	≥26	3.93 (1.14–13.56)	0.030
	Post-RT EBV DNA (10^3 copies/mL)		
	0	Reference	
	≤2	2.37 (0.98–5.76)	0.056
	>2	7.88 (4.08–15.22)	<0.001
	Age	1.03 (1.00–1.05)	0.068
	T stage[2]		
	T1–2	Reference	
	T2–3	1.61 (0.80–3.26)	0.184
	N stage[2]		
	N1	Reference	
	N2–3	1.10 (0.60–2.01)	0.765
DMFS	LRG-sum		
	≤2	Reference	
	≤9	0.86 (0.44–1.66)	0.650
	>9	2.32 (1.06–5.08)	0.035
	Pretreatment EBV DNA (10^3 copies/mL)		
	<1	Reference	
	<26	3.33 (1.01–10.97)	0.048
	≥26	3.69 (1.07–12.74)	0.039
	Post-RT EBV DNA (10^3 copies/mL)		
	0	Reference	
	≤2	2.57 (1.14–5.80)	0.023
	>2	8.94 (4.86–16.46)	<0.001
	N stage[2]		
	N1	Reference	
	N2–3	1.26 (0.72–2.23)	4.168
LRFS	LRG-sum		
	≤2	Reference	
	≤9	2.01 (1.05–3.86)	0.035
	>9	2.86 (1.07–7.66)	0.037
	Pretreatment EBV DNA (10^3 copies/mL)		
	<1	Reference	
	<26	1.12 (0.48–2.62)	0.802
	≥26	1.33 (0.53–3.31)	0.545
	Post-RT EBV DNA (10^3 copies/mL)		
	0	Reference	
	≤2	0.73 (0.18–3.03)	0.663
	>2	1.64 (0.57–4.68)	0.356
	Sex		
	Male	Reference	
	Female	1.72 (0.93–3.17)	0.083

Hazard ratios and P values were calculated using multivariate Cox regression models. OS, overall survival; LRFS, local recurrence-free survival; DMFS, distant metastasis-free survival; HR, hazard ratio; CI, confidence interval; LRG, lymph node regression grade; EBV, Epstein-Barr virus; RT, radiotherapy.

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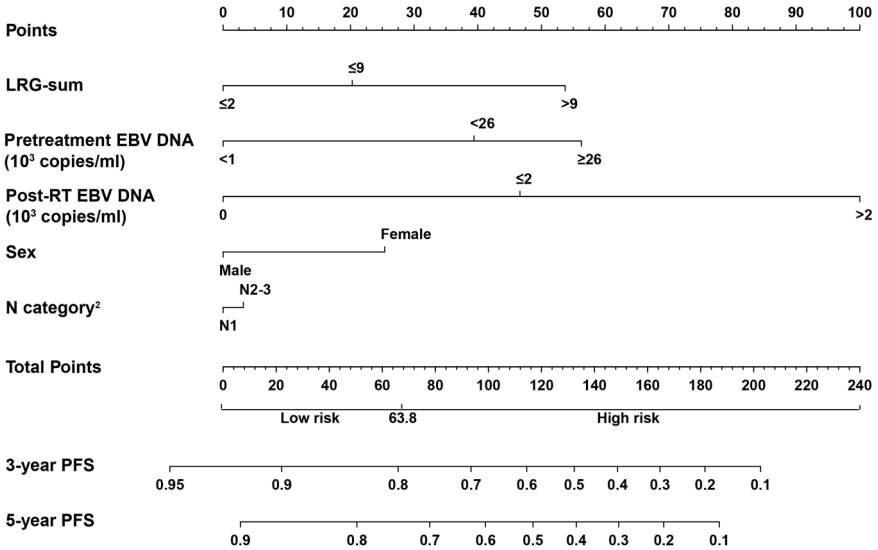

Figure 1 Nomogram of the Cox regression model A for the prediction of 3- and 5-year PFS. LRG, lymph node regression grade; EBV, Epstein-Barr virus; RT, radiotherapy; PFS, progression-free survival.

Table S2 C-indices of Models A and B and N stage for the prediction of PFS

Endpoint	Model	C-index (95% CI)	P value
PFS	N stage	0.559 (0.510–0.608)	<0.001
	Model B (N stage + sex + pretreatment EBV DNA)	0.631 (0.578–0.685)	<0.001
	Model A (N stage + sex + pretreatment EBV DNA + post-RT EBV DNA + LRG-sum)	0.696 (0.640–0.752)	Reference

The *U*-statistics test was used to compare two C-indices and to evaluate the P values, mainly via the rcorrp.cens function in the "Hmisc" package of R, and bootstraps with 1,000 resamples were used for these activities. PFS, progression-free survival; C-index; concordance index; CI, confidence interval; EBV, Epstein-Barr virus; RT, radiotherapy; LRG, lymph node regression grade.

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Table S3 Comparison of the characteristics of the groups stratified by LRG-sum

Variable	Value (N=387)	LRG-sum ≤2	LRG-sum ≤9	LRG-sum >9	P value
Age (years)	46 (13–75)	46 (38–52)	45 (39–55)	45 (34.5–52)	0.763
Sex					0.939
Male	281 (72.6%)	202 (72.7%)	63 (73.3%)	16 (69.6%)	
Female	106 (27.4%)	76 (27.3%)	23 (26.7%)	7 (30.4%)	
Histologic type					0.151
WHO type 1–2	14 (3.6%)	7 (2.5%)	6 (7.0%)	1 (4.3%)	
WHO type 3	373 (96.4%)	271 (97.5%)	80 (93.0%)	22 (95.7%)	
T stage					0.407
T1	66 (17.1%)	43 (15.5%)	21 (24.4%)	2 (8.7%)	
T2	52 (13.4%)	41 (14.7%)	8 (9.3%)	3 (13.0%)	
T3	162 (41.9%)	117 (42.1%)	35 (40.7%)	10 (43.5%)	
T4	107 (27.6%)	77 (27.7%)	22 (25.6%)	8 (34.8%)	
T stage[2]					0.531
T1–2	118 (30.5%)	84 (30.2%)	29 (33.7%)	5 (21.7%)	
T3–4	269 (69.5%)	194 (69.8%)	57 (66.3%)	18 (78.3%)	
N stage					
N1	263 (68.0%)	214 (77.0%)	43 (50.0%)	6 (26.1%)	<0.001
N2	89 (23.0%)	50 (18.0%)	29 (33.7%)	10 (43.5%)	
N3	35 (9.0%)	14 (5.0%)	14 (16.3%)	7 (30.4%)	
N stage[2]					<0.001
N1	263 (68.0%)	214 (77.0%)	43 (50.0%)	6 (26.1%)	
N2–3	124 (32.0%)	64 (23.0%)	43 (50.0%)	17 (73.9%)	
AJCC stage					0.163
II	87 (22.5%)	68 (24.5%)	18 (20.9%)	1 (4.3%)	
III	169 (43.7%)	121 (43.5%)	38 (44.2%)	10 (43.5%)	
IV	131 (33.9%)	89 (32.0%)	30 (34.9%)	12 (52.2%)	
Treatment					0.307
RT	13 (3.4%)	11 (4.0%)	1 (1.2%)	1 (4.3%)	
CCRT	126 (32.6%)	97 (34.9%)	24 (27.9%)	5 (21.7%)	
IC + CCRT	248 (64.1%)	170 (61.2%)	61 (70.9%)	17 (73.9%)	
Pretreatment EBV DNA (10^3 copies/mL)					0.005
<1	68 (17.6%)	54 (19.4%)	12 (14.0%)	2 (8.7%)	
<26	209 (54.0%)	160 (57.6%)	39 (45.3%)	10 (43.5%)	
≥26	110 (28.4%)	64 (23.0%)	35 (40.7%)	11 (47.8%)	
Post-RT EBV DNA (10^3 copies/mL)					0.083
0	330 (85.3%)	242 (87.1%)	72 (83.7%)	16 (69.6%)	
≤2	28 (7.2%)	18 (6.5%)	8 (9.3%)	2 (8.7%)	
>2	29 (7.5%)	18 (6.5%)	6 (7.0%)	5 (21.7%)	

Data are represented as median (interquartile range) or number (%). The P values were calculated using the Fishes exact test or the chi-squared test for categorical variables and the Student's *t*-test for continuous variables. LRG, lymph node regression grade; WHO, World Health Organization; AJCC, American Joint Committee on Cancer; RT, radiotherapy; CCRT, concurrent chemoradiotherapy; IC, induction chemotherapy; EBV, Epstein-Barr virus.

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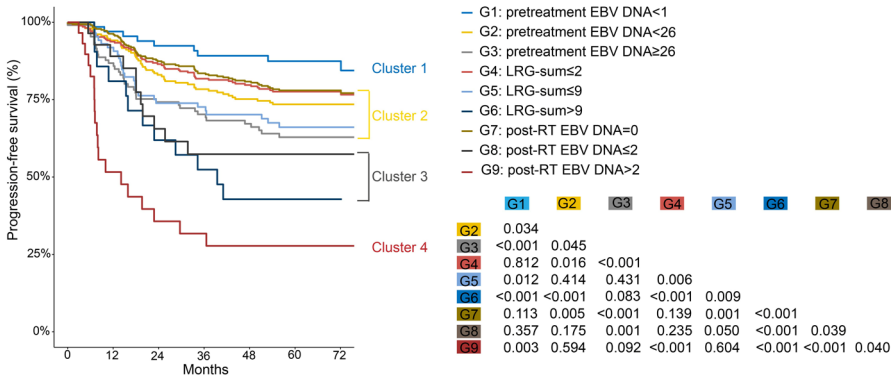

Figure S2 Survival clustering analysis. Kaplan-Meier survival plot of PFS based on pretreatment EBV DNA, LRG-sum, and post-RT EBV DNA. The nine subgroups were clustered by their intergroup HRPFS. We derived the following clusters: cluster 4–G9, due to G9 being significantly different from G1–8, except for G6 and G9; cluster 3–G6 and G8, due to G6 and G8 not being significantly different; cluster 1–G1, due to G1 being significantly different from G2–9, except for G4 and G7; and cluster 2–G2-5 and G7, due to these groups not being significantly different, except for G2 and G3, G3 and G4, G3 and G7, G4 and G5, and G5 and G7. The cluster plot was prepared using the "ggplot2" package in R. EBV, Epstein-Barr virus; LRG, lymph node regression grade; RT, radiotherapy; PFS, progression-free survival; HR, hazard ratio.

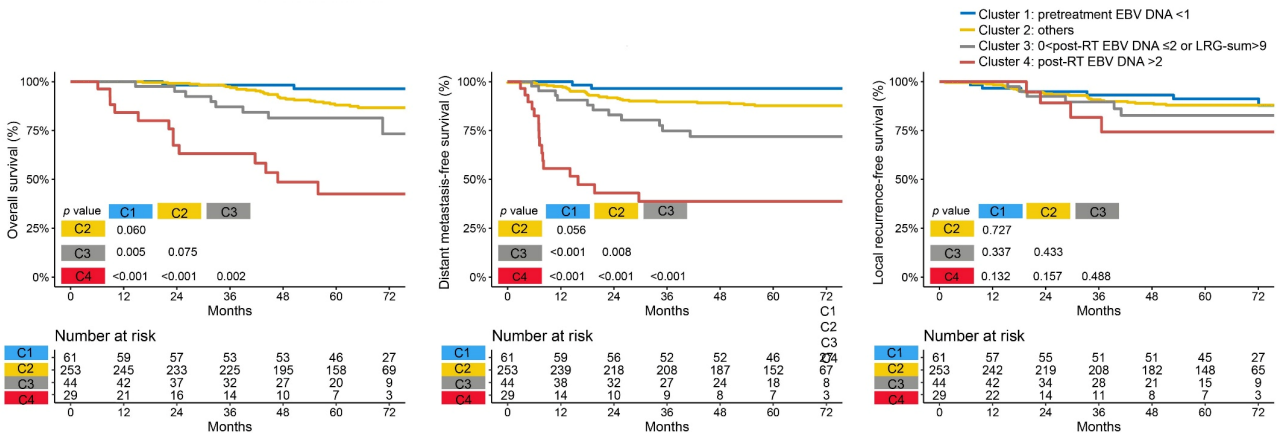

Figure S3 Kaplan-Meier plot of OS, DMFS, and LRFS for survival clustering analysis-based decision tree. EBV, Epstein-Barr virus; RT, radiotherapy; LRG, lymph node regression grade; OS, overall survival; DMFS, distant metastasis-free survival; LRFS, local recurrence-free survival.

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