The methodology of detecting plasma EBV DNA

Before therapy, peripheral blood (5 mL) was collected from each patient, placed in an ethylene diamine tetra acetic (EDTA)-coated tube, and centrifuged at 1,600×g for 15 min to isolate plasma and peripheral blood cells (PBC). DNA was extracted from plasma and PBC using the QIAamp Blood Kit (Qiagen, Hilden, Germany) and blood and body fluid protocol was used for DNA extraction per column and the final elution volume was 50 µL per column.

The concentration of EBV DNA in the plasma was measured using a real-time quantitative PCR assay targeting the BamH I-W region of the EBV genome. The sequences of the forward and reverse primers were: 5′-GCCAG AGGTA AGTGG ACTTT-3′ and 5′-TACCA CCTCC TCTTC TTGCT-3′ respectively. A dual fluorescently-labelled oligomer, 5′-(FAM) CACAC CCAGG CACAC ACTAC ACAT (TAMRA)-3′ served as the probe. Sequence data for the EBV genome were obtained from the GenBank sequence database. Amplifications were performed in an Applied Biosystems 7700 Sequence Detector and then analyzed using the Sequence Detection System software (version 1.6.3) developed by Applied Biosystems (Foster City, CA). The plasma EBV DNA concentration was calculated using the following equation: C = Q × (VDNA/VPCR) × (1/VEXT), in which C represents the target concentration in plasma (copies/mL), Q represents the target quantity (copy number) determined by PCR, VDNA represents the total volume of DNA obtained after extraction (typically 50 µL/Qiagen extraction), VPCR represents the volume of DNA solution used for PCR (typically 2 µL) and VEXT represents the volume of plasma extracted (typically 0.5 mL).

Table S1 Subgroup analyses of 5-year survival outcomes of patients with stage I/II or III/IV in the propensity score matched cohort

	TNM staging	HBsAg (–) (n=324)	HBsAg (+) (n=162)	HR (95% CI)	P
OS	I/II	97.9%	100.0%	N/A†	0.508
	III/IV	84.7%	80.2%	1.33 (0.81–2.17)	0.258
LRFS	I/II	95.8%	100.0%	N/A†	0.344
	III/IV	81.7%	73.3%	1.52 (0.98–2.34)	0.058
DMFS	I/II	93.7%	100.0%	N/A†	0.245
	III/IV	81.0%	77.7%	1.21 (0.77–1.89)	0.417
PFS	I/II	93.7%	100.0%	N/A†	0.245
	III/IV	77.4%	69.4%	1.40 (0.94–2.08)	0.096

†, N/A meant not applicable because the upper limit of 95% CI was large with the number exceeded 800. OS, overall survival; LRFS, locoregional relapse-free survival; DMFS, distant metastasis-free survival; PFS, progression-free survival; TNM, tumor, node, and metastasis; HBsAg, hepatitis B surface antigen; HBsAg (–), HBsAg-negative; HBsAg (+), HBsAg-positive; HR, hazard ratio; CI, confidence interval.

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Table S2 Subgroup analyses of 5-year survival outcomes of patients with nasopharyngeal carcinoma based on pre-treatment plasm Epstein-Barr virus DNA load in the propensity score matched cohort

		OS			
		EBV-DNA <1,500 copies/mL	EBV-DNA ≥1,500 copies/mL	HR (95% CI)	P
OS	HBsAg (–)	92.8%	80.8%	2.85 (1.43–5.67)	0.003
	HBsAg (+)	94.3%	72.8%	5.28 (1.82–15.33)	0.002
	HR (95% CI)	0.79 (0.25–2.49)	1.47 (0.85–2.54)		
	P	0.688	0.166		
			LRFS		
		EBV-DNA <1,500 copies/mL	EBV-DNA ≥1,500 copies/mL	HR (95% CI)	P
LRFS	HBsAg (–)	88.3%	79.6%	1.85 (1.04–3.29)	0.036
	HBsAg (+)	91.5%	64.3%	4.95 (2.05–11.93)	<0.001
	HR (95% CI)	0.71 (0.28–1.79)	1.87 (1.13–3.08)		
	P	0.469	0.013		
			DMFS		
		EBV-DNA <1,500 copies/mL	EBV-DNA ≥1,500 copies/mL	HR (95% CI)	P
DMFS	HBsAg (–)	89.0%	77.2%	2.23 (1.26–3.96)	0.006
	HBsAg (+)	91.4%	71.8%	3.72 (1.52–9.12)	0.004
	HR (95% CI)	0.77 (0.30–1.94)	1.29 (0.77–2.15)		
	P	0.570	0.333		
			PFS		
		EBV-DNA <1,500 copies/mL	EBV-DNA ≥1,500 copies/mL	HR (95% CI)	P
PFS	HBsAg (–)	85.1%	74.9%	1.84 (1.11–3.07)	0.019
	HBsAg (+)	88.6%	60.9%	4.18 (1.93–9.06)	<0.001
	HR (95% CI)	0.74 (0.33–1.66)	1.65 (1.04–2.60)		
	P	0.464	0.031		

OS, overall survival; LRFS, locoregional relapse-free survival; DMFS, distant metastasis-free survival; PFS, progression-free survival; HBsAg, hepatitis B surface antigen; HBsAg (–), HBsAg-negative; HBsAg (+), HBsAg-positive; EBV-DNA, pre-treatment plasm Epstein-Barr virus DNA load; HR, hazard ratio; CI, confidence interval.

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