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On-treatment and early post-radiotherapy EBV-DNA dynamics in locoregionally advanced nasopharyngeal carcinoma: a systematic review

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Abstract

Background: Plasma Epstein–Barr virus DNA (EBV-DNA) is an established prognostic biomarker in nasopharyngeal carcinoma (NPC). While baseline EBV-DNA reflects tumour burden, increasing interest has focused on on-treatment EBV-DNA dynamics as a marker of early molecular response (EMR), but heterogeneity in timepoints, EMR definitions and study designs limits clinical translation.

Objective: To systematically review and qualitatively synthesise the prognostic significance of plasma EBV-DNA dynamics measured during curative-intent therapy in locoregionally advanced, non-metastatic NPC.

Methods: This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2020 statement. PubMed/Medical Literature Analysis and Retrieval System Online, Embase, Scopus and Web of Science were searched (January 2004 to December 4, 2025). Eligible studies included prospective and observational designs reporting serial plasma EBV-DNA measurements during treatment and survival outcomes. Owing to heterogeneity in sampling timepoints, EMR constructs and EBV-DNA assays, no quantitative meta-analysis was performed. Risk of bias was assessed using design-appropriate tools.

Results: Twenty-one eligible reports, representing 20 analytically distinct study cohorts, met the inclusion criteria. Across treatment strategies and analytical approaches, unfavourable on-treatment EBV-DNA dynamics (persistent detectability, delayed clearance or adverse kinetic patterns) were consistently associated with inferior survival outcomes, particularly distant metastasis-free and event-free survival. In reports providing hazard ratios for distant metastasis-related endpoints, effect sizes were generally greater than 2. EBV-DNA rebound after initial clearance was also associated with unfavourable prognosis, although prospective validation remains required.

Conclusion: On-treatment and early post-radiotherapy EBV-DNA dynamics show consistent prognostic associations in locoregionally advanced NPC, but heterogeneous assays, sampling windows and response definitions preclude validated clinical decision thresholds. These measures should not guide treatment adaptation outside clinical trials; prospective standardisation and validation are required.

Keywords: *nasopharyngeal carcinoma, Epstein–Barr virus DNA, early molecular response, circulating tumour DNA, prognostic biomarkers, treatment response, chemoradiotherapy*

Review

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Introduction

Nasopharyngeal carcinoma (NPC) is an epithelial malignancy strongly linked to Epstein–Barr virus (EBV), particularly in endemic regions. Most patients present with locoregionally advanced disease and receive curative-intent multimodality therapy, commonly incorporating induction chemotherapy (IC) and concurrent chemoradiotherapy (CCRT) [1–3]. Despite major gains in locoregional control with intensity-modulated radiotherapy (IMRT) and optimised systemic treatment, distant relapse remains clinically important, motivating prognostic biomarkers that can refine on-treatment risk stratification [4, 5].

Plasma EBV DNA (EBV-DNA) is an established biomarker in NPC: baseline levels correlate with tumour burden and have consistent prognostic value [4–6]. Baseline assessment is nevertheless static and does not capture real-time tumour response. Increasing attention has therefore focused on serial on-treatment and early post-radiotherapy dynamics as indicators of treatment sensitivity and residual disease [7–10].

Across reports, early molecular response (EMR) has been operationalised using several non-equivalent constructs [2, 7–16]. In this review, detectability denotes qualitative EBV-DNA status at a specified timepoint; clearance denotes conversion from detectable to undetectable; kinetics denotes the rate or magnitude of change; trajectory denotes a sequence of values across multiple timepoints; and rebound denotes re-detectability after documented clearance. The terms rapid and delayed clearance were retained only in relation to the windows defined by the original reports. Early post-treatment was defined as occurring within 3 months after radiotherapy. These constructs were categorised separately and synthesised within post-induction/pre-radiotherapy, mid-radiotherapy, end-radiotherapy and early post-treatment windows.

Previous systematic reviews and meta-analyses evaluated plasma EBV-DNA across broad clinical applications, including screening, pre-treatment prognostication, monitoring and surveillance or pooled pre-treatment, mid-treatment and post-treatment measurements [17–19]. The present review addresses a narrower question: serial on-treatment and early post-radiotherapy dynamics during curative-intent therapy for predominantly locoregionally advanced NPC. Its distinctive contribution is the use of explicit temporal windows together with construct-level differentiation among detectability, quantitative thresholds, kinetics, trajectories and rebound, rather than pooling biologically non-equivalent measures. It also incorporates studies published through 4 December 2025 and examines how assay and methodological heterogeneity constrain interpretation and clinical translation.

Methods

Study design and reporting standards

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement [20]. The review was not prospectively registered, and no standalone dated protocol was archived before screening. The review question, eligibility criteria, temporal categories, data-extraction domains, risk-of-bias approach and qualitative synthesis framework were established within the internal working methodology before completion of screening and data extraction. A retrospectively compiled methodological framework, clearly identified as having been prepared during peer review rather than as a contemporaneously archived protocol, is provided in Supplementary Material S1. Given the anticipated heterogeneity in EBV-DNA sampling schedules, constructs and effect measures, no quantitative meta-analysis was undertaken.

Eligibility criteria

Eligibility was defined using a population, intervention/exposure, comparator, outcomes (PICO)-based framework with additional methodological considerations.

We included original studies (randomised trials, prospective or retrospective cohort studies) involving adults (≥18 years) with non-metastatic NPC treated with curative intent, primarily those with locoregionally advanced disease corresponding to American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) stages III–IVA [21, 22]. Studies including earlier-stage disease were eligible if ≥50% of patients had locoregionally advanced disease or if relevant subgroups were extractable.

Eligible studies were required to report serial plasma EBV-DNA measurements with at least one on-treatment timepoint beyond baseline (post-induction, mid-radiotherapy, end-of-radiotherapy or early post-treatment). Comparisons included detectability versus non-detectability, quantitative thresholds, kinetic parameters (e.g., slope, half-life) or trajectory-based classifications. At least one time-to-event outcome (event-free, disease-free, distant metastasis–free, progression-free or overall survival) had to be reported. Hazard ratios (HRs) with 95% confidence intervals were extracted when available; otherwise, studies providing comparative survival analyses were included for qualitative synthesis. Studies were restricted to English-language publications between January 2004 and December 2025.

Exclusion criteria included paediatric-only cohorts, metastatic disease at diagnosis, recurrent/metastatic-only populations (unless non-metastatic subgroups were extractable), studies reporting baseline EBV-DNA only, technical assay-validation reports without clinical outcomes and non-original publications (reviews, editorials, letters, abstracts without full peer-reviewed data). In cases of overlapping cohorts, consistent report-selection rules were applied to retain the most comprehensive eligible analysis.

Management of overlapping cohorts

Potential cohort overlap was assessed by comparing recruitment centres, enrolment periods, eligibility criteria, treatment regimens, sample sizes and patient characteristics. Reports arising from the same underlying cohort were linked and counted once at the study level; complementary reports could contribute non-duplicative timepoint analyses, but their participant counts were not summed. When reports were judged to describe duplicate populations, selection favoured the most complete serial EBV-DNA and survival assessment. Potential partial overlap among large institutional series with different eligibility criteria or sampling schedules was acknowledged rather than assumed to represent complete duplication.

Information sources and search strategy

A systematic search was conducted in PubMed/Medical Literature Analysis and Retrieval System Online (MEDLINE), Embase, Scopus and Web of Science for English-language publications from 1 January 2004 through 4 December 2025; the final search was run on 4 December 2025. Reference lists and forward citations were screened manually. ClinicalTrials.gov was reviewed, and no additional eligible studies were identified.

Search strategies combined controlled vocabulary (MeSH/Emtree, where applicable) and free-text terms across four domains: NPC, EBV, plasma/circulating EBV-DNA and dynamic or on-treatment assessment. The complete database-specific strings, platforms, search date, language restriction, date limits and absence of design filters are reported in Supplementary Material S2.

Study selection

Records were imported into a reference management system and deduplicated automatically and manually. Screening was performed in two stages (title/abstract, then full text) by three reviewers (I.B., S.N. and H.M.) independently. Discrepancies were resolved by consensus or majority decision. No automation tools were used. The selection process is summarised in a PRISMA 2020 flow diagram.

Data collection and items

Two reviewers (I.B. and S.N.) independently extracted data using a standardised, piloted form; a third reviewer (H.M.) verified accuracy. Disagreements were resolved by consensus.

Primary outcomes were time-to-event endpoints (event-free, disease-free and distant metastasis-free survival). Overall survival was a key secondary endpoint. When multiple analyses were reported, multivariable models with the most comprehensive covariate adjustment were prioritised.

Extracted variables included study characteristics, patient and tumour features, treatment modalities, EBV-DNA assay characteristics, sampling timepoints and EMR definitions. Missing data were recorded as not reported.

Risk-of-bias assessment

Risk-of-bias tools were assigned according to the primary analytical objective of each report. The Newcastle–Ottawa Scale (NOS) was used for general observational cohort evidence [23]; Risk of Bias in Non-randomised Studies of Interventions (ROBINS-I) for non-randomised intervention or exposure comparisons [24]; Quality in Prognosis Studies (QUIPS) for prognostic-factor analyses in which EBV-DNA dynamics were the principal prognostic exposure [25]; and Prediction Model Risk-of-Bias Assessment Tool (PROBAST) for studies developing or validating multivariable prediction models [26]. Each report was assessed with one primary tool. Tool-specific domain judgments were retained, and ratings were not converted into numerical scores or pooled across instruments.

Assessment was performed independently by I.B. and S.N., with adjudication by H.M. Overall risk was categorised as low, moderate, serious or high based on predefined rules, and domain-level judgments are reported to support interpretation.

Certainty of evidence

The Grading of Recommendations Assessment, Development and Evaluation (GRADE) framework was not formally applied. Relative confidence in the evidence was summarised qualitatively for each temporal window using four pre-specified considerations: consistency of effect direction, risk-of-bias patterns, directness of the biomarker timepoint and construct and precision or replication across independent cohorts. This approach does not constitute a formal GRADE rating. Assay variability, sampling schedules, EMR definitions and the predominance of observational evidence were treated as factors that lowered confidence.

Effect measures and synthesis

Extracted effect measures included HRs with 95% confidence intervals. When HRs were unavailable, findings were described narratively based on reported survival analyses. No transformation or estimation from survival curves was performed.

Studies were grouped according to predefined temporal EBV-DNA categories and EMR constructs. Given heterogeneity in definitions and timing, synthesis was qualitative. Constructs were not pooled across non-comparable definitions. Tabular summaries present key characteristics and findings. No meta-analysis, subgroup analysis, sensitivity analysis or formal reporting bias assessment was conducted.

Reporting bias and certainty assessment

Formal assessment of reporting bias (e.g., funnel plots) and certainty of evidence (e.g., GRADE) was not conducted, as quantitative synthesis was not undertaken. Instead, confidence in the body of evidence was interpreted qualitatively, based on consistency of findings, biological plausibility and risk-of-bias assessments.

Results

Study selection

The database search identified 2,354 records, with no additional records from other sources. After removal of 612 duplicates, 1,742 records underwent title and abstract screening, of which 1,601 were excluded as clearly irrelevant.

Full texts were sought for 141 reports; seven could not be retrieved. A total of 134 full-text reports were assessed for eligibility, and 113 were excluded: metastatic or recurrent-only populations (n = 31), baseline EBV-DNA assessment without eligible on-treatment dynamics (n = 39), absence of survival outcomes (n = 21), overlapping cohorts or duplicate populations (n = 14) and non-original publications (n = 8). The screening archive retained the aggregate exclusions by reason but not a report-level appendix suitable for reliable retrospective publication; no speculative post hoc list was generated.

Twenty-one eligible reports, representing 20 analytically distinct study cohorts, were included in the qualitative synthesis (Figure 1).

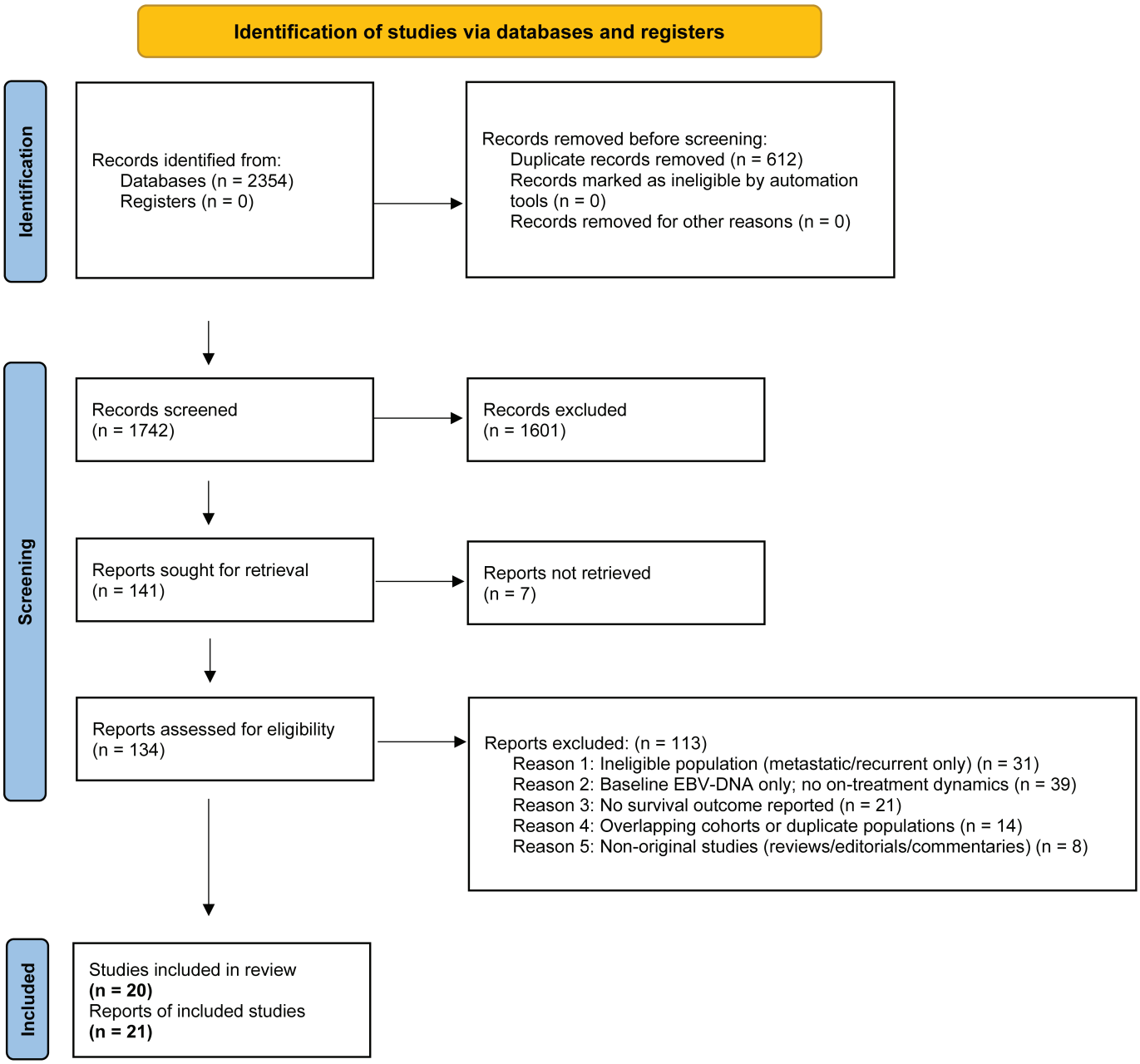

Figure 1. PRISMA 2020 flow diagram. Twenty-one eligible reports representing 20 analytically distinct study cohorts were included in the qualitative synthesis.

Characteristics of included studies

The 21 eligible reports represented 20 analytically distinct study cohorts (Table 1). Nominal sample sizes across reports exceeded 12,000; because linked reports and potential partial overlap among large institutional series could not be fully quantified, this value is not interpreted as a unique-patient total. Most evidence originated from endemic regions in mainland China, Hong Kong, Taiwan and Thailand.

Study designs were predominantly observational cohorts (retrospective and prospective) (Table 1), with one multicentre single-arm phase II trial [27]. Sample sizes ranged from fewer than 50 patients to over 2,000 [12, 28]. Most cohorts consisted primarily of locoregionally advanced disease (AJCC/UICC II–IVA) [2, 12, 14, 27].

Treatment strategies mainly involved CCRT using IMRT, either alone or following (IC; IC → CCRT) [2–3, 12, 15, 29–31]. A small subset evaluated non-standard strategies, including induction chemo-immunotherapy followed by radiotherapy without concurrent cisplatin [27].

Plasma EBV-DNA was quantified using polymerase chain reaction (PCR)-based assays, most commonly quantitative PCR (qPCR) targeting BamHI-W [2, 9, 11–13, 15, 29]. Thirteen reports used copies/mL exclusively, one used international units per millilitre (IU/mL), one allowed either IU/mL or copies/mL and six did not clearly report the unit in extractable text. Detectability definitions ranged from 0 copies/mL or greater than 0 IU/mL to assay-specific limits or thresholds of 316, 400, 600, 1,000, 2,000 or 4,000 copies/mL. These categories were not treated as interchangeable. Sampling schedules were categorised as baseline [2, 9, 12], post-induction/pre-radiotherapy [3, 12, 27, 31], mid-radiotherapy [9, 11, 13, 29] and end-radiotherapy/early post-radiotherapy [12–13, 15–16].

Table 1. Concise characteristics and principal findings of 21 eligible reports representing 20 analytically distinct study cohorts. The two linked Thai reports contribute complementary analyses from one underlying cohort and are not treated as independent replications. Full extraction fields are provided in Supplementary Material S3.

Study	Setting/design/*N*	Treatment	Sampling windows	EMR construct	Principal finding
Alfieri *et al* [30]	Italy (non-endemic); Retrospective single-centre observational cohort; *N* = 167	Curative intent: IC + CCRT (52%), CCRT alone (43%), RT alone (1%), CCRT + adjuvant CT (4%)	T1 pre-treatment; T4 post-induction (if IC); T2a ≤6 weeks post-RT; T2b ≤14 weeks post-RT; T3 longitudinal follow-up	EBV-DNA classified as negative versus positive (quantifiable or unquantifiable); no validated quantitative cut-off; kinetics assessed descriptively (Detectable versus Undetectable; Kinetics (longitudinal descriptive))	Associations were assessed descriptively; no Cox HR was reported. Full results are provided in S3.
Huang *et al* [32]	China; endemic region; single cancer centre (Sun Yat-sen University Cancer Centre); Retrospective cohort; *N* = 233	On-treatment	Baseline and serial during treatment (between adjacent chemo cycles and/or during RT)	'Elevation' is defined as ≥5% increase versus prior value (to account for fluctuation/variation) between adjacent chemo cycles or during RT (this is a kinetic change definition, not clearance/undetectable) (Increase/decrease (% change))	Unfavourable EBV-DNA status or kinetics was associated with poorer 1-year DFS; EBV-DNA elevation rate. Full estimates are provided in S3.

Continued

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Study	Setting/design/*N*	Treatment	Sampling windows	EMR construct	Principal finding
Chan *et al* [9]	Hong Kong (endemic region implied); single institution (Queen Mary Hospital); Prospective observational cohort; *N* = 45 recruited; 44 analysed for half-life (one excluded from half-life analysis)	Radical IMRT; concurrent cisplatin-based chemoradiation ± induction or adjuvant chemotherapy (distribution reported)	Baseline immediately before treatment, then weekly during treatment until undetectable in two consecutive measurements	Kinetics modelled assuming exponential decay: log-transformed weekly ratios versus baseline; half-life = ln2/k. Cut-off for 'slow' versus 'fast' clearance derived by ROC to predict progression; half-life >15 days used as main discriminator (Kinetics (half-life / clearance slope))	Unfavourable EBV-DNA status or kinetics was associated with poorer pre-specified: PFS, DMFS and OS. Full estimates are provided in S3.
Huang *et al* [12]	China (Fuzhou, Fujian); Retrospective cohort (observational); *N* = 2203 (from 2,613 M0 NPC screened)	IC followed by radical IMRT (69.3–72.0 Gy), with concurrent cisplatin in 69.8%; IMRT alone in 30.2%; adjuvant chemotherapy in 9.2%	T0 baseline (≤28 days pre-IC); T1 end-IC / around RT start (24 days pre-RT to 1 week after RT start); T2 end-RT (≤21 days post-RT)	Binary D/U at each timepoint (T0/T1/T2) → trajectory types: I (U-U-U), II (D-U-U), III (D-D-U), IV (D-D-D), V 'resurgence' (D-U-D / U-D-U / U-D-D / U-U-D) (Trajectory (T0/T1/T2 patterns))	Unfavourable EBV-DNA status or kinetics was associated with poorer PFS, OS, LRFS and DMFS. Full estimates are provided in S3.
Lan *et al* [2]	China (Sun Yat-sen University Cancer Center, Guangzhou); Retrospective cohort; *N* = 775	IMRT + concurrent cisplatin 100 mg/m² q3w; ± concurrent cetuximab/nimotuzumab; radiation dose schema described	Pre-DNA: within 7 days before CCRT start. Mid-DNA: within 7 days before the 2nd cycle of cisplatin during RT	Pre-DNA high versus low using 4,000 copies/mL cut-off; Mid-DNA detectable (>0) versus undetectable (=0) (Quantitative threshold (cut-off); Detectable versus Undetectable)	Unfavourable EBV-DNA status or kinetics was associated with poorer Primary: PFS. Secondary: DMFS, LRRFS, OS; definitions provided. Full estimates are provided in S3.
Xu *et al* [27]	China (seven academic hospitals across regions); Multicentre, single-arm phase two clinical trial (PLATINUM; NCT03984357); *N* = 152	Three cycles induction chemo-immunotherapy: gemcitabine + cisplatin + nivolumab → IMRT + three cycles nivolumab (no concurrent cisplatin) → adjuvant nivolumab (six cycles) [Non-standard curative regimen: induction chemo-immunotherapy + IMRT without concurrent cisplatin]	Serial blood samples 'pre-treatment' and during treatment; reported EBV-DNA status after induction, after radiotherapy and after adjuvant phases	'Early clearance' operationalised as undetectable EBV-DNA after induction-phase treatment (versus detectable), evaluated for association with FFS (Detectable versus Undetectable)	Unfavourable EBV-DNA status or kinetics was associated with poorer Primary: Failure-free survival (FFS). Secondary: OS, locoregional recurrence-free survival, DMFS, safety/QoL. Full estimates are provided in S3.

Table 1. Concise characteristics and principal findings of 21 eligible reports representing 20 analytically distinct study cohorts. The two linked Thai reports contribute complementary analyses from one underlying cohort and are not treated as independent replications. Full extraction fields are provided in Supplementary Material S3. *Continued*

Study	Setting/design/*N*	Treatment	Sampling windows	EMR construct	Principal finding
Yang *et al* [29]	China; Observational cohort (NR if prospective/ retrospective not explicitly stated); *N* = 1,881	IMRT with or without concurrent chemotherapy; excluded induction and adjuvant chemotherapy; mid-IMRT sampling occurred before cumulative cisplatin 200 mg/m²	Three timepoints: pre-treatment, middle of IMRT, post-treatment / end of IMRT	Primary 'on-treatment' definition: plasma EBV-DNA >0 copies/mL in the middle of IMRT; risk-grouping reported as EBV-DNA = 0 / EBV-DNA decrease / EBV-DNA increase (operational definition of decrease/ increase NR) (Increase/ decrease (% change); Detectable versus Undetectable)	Unfavourable EBV-DNA status or kinetics was associated with poorer OS; PFS; LRFS; DMFS. Full estimates are provided in S3.
Zheng *et al* [31]	China (Xiamen); Retrospective observational cohort; *N* = 172	Three cycles IC → IMRT + two cycles platinum-based CCRT	Blood collected before IC and after completion of IC (prior to radiotherapy)	Post-IC EBV-DNA detectable (>0 IU/mL) versus undetectable (0 IU/ mL) (Detectable versus Undetectable)	Unfavourable EBV-DNA status or kinetics was associated with poorer LRFS; DMFS; DFS; OS. Full estimates are provided in S3.
Le *et al* [33]	USA (Stanford) + Hong Kong (Queen Mary Hospital); Prospective observational cohort (assay comparison + prognostic biomarker); *N* = 58	Definitive RT (median 70 Gy); RT alone 12; concurrent CT 46 (cisplatin-based); neoadjuvant PF 14; adjuvant CT 21	Blood: pre-treatment, halfway into RT, completion of RT, then every 3–6 months after completion	EBV-DNA detectable versus undetectable at the end of RT and post-treatment (≤3 months) (clearance = response); timepoint mid-RT explored (Detectable versus Undetectable)	Unfavourable EBV-DNA status or kinetics was associated with poorer Freedom-from-relapse (FFR), OS. Full estimates are provided in S3.
Zhang *et al* [34]	China (Sun Yat-sen University Cancer Center, Guangzhou); Retrospective comparative cohort; propensity score matching (1:1); *N* = 256 (Routine 150; New 106); PSM: 192 (96 pairs)	Induction TPF q3w (1–4 cycles) → IMRT + concurrent cisplatin q3w ×2. Routine: standard 3 IC cycles. New: IC cycles stage + post-IC cfEBV-DNA–guided (stop IC if cfEBV-DNA becomes undetectable)	≤2 weeks pre-IC; after each IC cycle; within/ during 1 week of CCRT completion	Complete disappearance to 0 copies/mL after a given IC cycle (used as 'clearance' and to adapt IC cycle number) (Detectable versus Undetectable; Trajectory)	Unfavourable EBV-DNA status or kinetics was associated with poorer DFS (primary), OS; LRFS, DMFS. Full estimates are provided in S3.

Table 1. Concise characteristics and principal findings of 21 eligible reports representing 20 analytically distinct study cohorts. The two linked Thai reports contribute complementary analyses from one underlying cohort and are not treated as independent replications. Full extraction fields are provided in Supplementary Material S3. *Continued*

Study	Setting/design/*N*	Treatment	Sampling windows	EMR construct	Principal finding
He *et al* [13]	China (Guangzhou; Sun Yat-sen University Cancer Center); Retrospective cohort; *N* = 949	IMRT ± chemotherapy (stage-adapted): IMRT alone (I); IMRT + concurrent chemo (II); CCRT ± neoadjuvant/adjuvant chemo (III–IVAB). Neoadjuvant/adjuvant: cisplatin+5FU and/or taxane (2–3 cycles). Concurrent cisplatin weekly/triweekly. Some received adjuvant cetuximab/ nimotuzumab.	Baseline (pre-treatment), mid-treatment (after ~4 weeks RT), end of treatment, 3 months post-treatment	Timepoint-specific ROC cut-offs for 'low versus high': pre-EBV 2,500; mid-EBV 871; end-EBV 721; 3-month EBV 211 copies/ mL. Dynamic grouping was also assessed (e.g., Group 2 = detectable end-EBV + detectable 3-month EBV). (Quantitative threshold (cut-off); Trajectory)	Unfavourable EBV-DNA status or kinetics was associated with poorer OS (primary), DMFS, PFS. Full estimates are provided in S3.
Lertbutsayanukul *et al* [11]	Thailand; Secondary analysis of a prospective randomised trial cohort (prognostic biomarker analysis); *N* = 105	IMRT 70 Gy/33–35 fx + concurrent weekly cisplatin 40 mg/m² (up to 7 cycles) for >T1 or N+; adjuvant cisplatin 80 mg/m² + 5-FU 1,000 mg/m²/day continuous infusion (96 hours) q4w ×3	Pre-IMRT (baseline), week 5 of RT (mid-treatment) and 3 months post-completion	Mid-EBV: undetectable (<316 copies/mL) versus detectable at week 5; Post-EBV: undetectable versus detectable at 3 months (Detectable versus Undetectable)	Unfavourable EBV-DNA status or kinetics was associated with poorer OS, PFS, DMFS (also LPFS, RPFS reported). Full estimates are provided in S3.
Lv *et al* [14]	China (Sun Yat-sen University Cancer Center, Guangzhou); Prospective observational cohort (EP-SEASON; per-protocol ctDNA assessments); *N* = 1,000	Sequential chemo-radiotherapy: no-NAC CCRT ± AC (24.8%) versus NAC→CCRT±AC (75.2%); NAC regimens GP/TPF/ TP/PF	11 timepoints: baseline (T0); post-NAC cycles (T1–T3); weekly during RT (T4–T9); within 1 week post-RTend (T10); 3 months post-RT (T11)	Early response defined by undetectable cfEBV-DNA (0 copies/mL) at early on-treatment points (e.g., post-NAC1 T1 = 0; also refined to include post-RT2w T5 = 0) (Detectable versus Undetectable; Trajectory)	Unfavourable EBV-DNA status or kinetics was associated with poorer DFS, OS (also DMFS, LRFS referenced as survival endpoints). Full estimates are provided in S3.

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Study	Setting/design/*N*	Treatment	Sampling windows	EMR construct	Principal finding
Lu *et al* [8]	China (single center); Retrospective cohort; *N* = 173	IC followed by CCRT; targeted therapy/ICIs used in a subset (NR for exact criteria)	Baseline and pre-each-chemotherapy-cycle (21–28-day intervals) during both IC and CCRT	Early EBV-DNA status conversion (ESC): undetectable cEBV-DNA before the 3rd chemotherapy cycle; Late EBV-DNA status conversion (LSC): conversion from the 3rd cycle to the end of CCRT; No EBV-DNA status conversion (NSC): persistently positive throughout treatment (Trajectory (EBV-DNA status conversion patterns))	Unfavourable EBV-DNA status or kinetics was associated with poorer OS; PFS (NR for DFS/DMFS/EFS). Full estimates are provided in S3.
Liu *et al* [35]	China (Guangxi); Retrospective cohort; model development + internal split validation (7:3); *N* = 581	Induction chemo (2–3 cycles; TP/PF/GP/TPF) + CCRT with IMRT + concurrent cisplatin	Before treatment; after completion of IC (post-IC); follow-up testing includes EBV-DNA (routine)	EBV-DNA after IC ≤5,660 versus >5,660 copies/mL (risk strata); IC response CR/PR versus SD/PD (Quantitative threshold (cut-off))	Unfavourable EBV-DNA status or kinetics was associated with poorer OS (primary), DMFS, LRFS, PFS. Full estimates are provided in S3.
Prayongrat *et al* [15]	Thailand (Bangkok); Retrospective cohort (medical records); *N* = 204	IMRT; definitive 70–74 Gy (PTV-HR) + 50–56 Gy (PTV-LR), 33–35 fractions; with/without CT; concurrent CT (most commonly cisplatin) + adjuvant platinum/5-FU CT in 93.1%	Pre-IMRT; mid-IMRT (S5); post-IMRT (3 months); then q6 months until relapse	Disappearance/clearance to 'undetectable' (<600 copies/mL) at mid-IMRT and/or post-IMRT; dynamic categories (Upre–Umid/post, Dpre–Umid/post, etc.) (Detectable versus Undetectable; Trajectory)	Unfavourable EBV-DNA status or kinetics was associated with poorer DMFS, PFS, OS. Full estimates are provided in S3.
Liu *et al* [3]	NR; Prospective observational cohort; *N* = 185	NACT → CCRT (curative intent)	Before and after NACT; also reported after CCRT	Post-NACT EBV-DNA undetectable versus detectable (cut-off 0 copies/mL) (Detectable versus Undetectable)	Unfavourable EBV-DNA status or kinetics was associated with poorer PFS, DMFS, LRFS. Full estimates are provided in S3.
Li *et al* [28]	Endemic area (NR); Retrospective cohort; *N* = 2,942	Radical IMRT-based curative treatment	Pre-treatment; pre-RT; post-RT EBV-DNA	EBV-DNA negative = 0 copies/mL at all three timepoints; positive otherwise (Detectable versus Undetectable)	Unfavourable EBV-DNA status or kinetics was associated with poorer OS; LRFFS. Full estimates are provided in S3.

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Study	Setting/design/*N*	Treatment	Sampling windows	EMR construct	Principal finding
Lin *et al* [36]	Taiwan; Observational cohort (serial plasma sampling); *N* = 99	10 weekly chemo → RT; dose 70 Gy (T1–3) / 74 Gy (T4)	Pre-treatment (D-1); D35 and D64 during chemo; 1 week post-RT; then q6mo follow-up	Detectable versus undetectable EBV-DNA after completion of treatment (1 week post-RT); baseline cutpoint reported (≥1,500 copies/mL) (Detectable versus Undetectable)	Unfavourable EBV-DNA status or kinetics was associated with poorer Relapse-free survival; Overall survival. Full estimates are provided in S3.
Zhang *et al* [16]	China; Retrospective cohort; *N* = 273	Radical IMRT; stage I RT only, stage II–IVB CCRT ± neoadj/adjuvant	Pre-treatment; end of treatment (±1 week); 3 months post-treatment (±1 week)	Post-treatment change pattern: Group 1–4 by detectability at end-DNA and 3-month-DNA (Trajectory (post-treatment detectability patterns))	Unfavourable EBV-DNA status or kinetics was associated with poorer DFS; DMFS; OS (3-year rates reported). Full estimates are provided in S3.
Chen *et al* [37]	Taiwan; Prospective cohort (consecutive enrolment); *N* = 60	Definitive RT (≥64.8 Gy) + concurrent cisplatin-based chemo (cisplatin 50 mg/m² D1 + tegafur 800 mg/day + leucovorin 60 mg/day D1–14 q2w during RT); no induction chemo	Pre-treatment + during CCRT while receiving 35–40 Gy of RT	Interim EBV-DNA dichotomised: detectable versus undetectable (undetectable became dominant; thus binary) (Detectable versus Undetectable)	Unfavourable EBV-DNA status or kinetics was associated with poorer OS; RFS; response at 3 months. Full estimates are provided in S3.

NR indicates not reported

Risk-of-bias assessment

Risk-of-bias assessments are summarised in Table 2. Most observational studies were judged at moderate risk of bias due to confounding and selection limitations. Biomarker-focused prognostic analyses frequently showed moderate-to-serious risk across domains, particularly in retrospective single-centre studies with limited covariate adjustment.

The single-arm phase II trial was considered at serious risk of bias because of the absence of a comparator. Across the evidence base, objective survival endpoints and generally adequate follow-up supported confidence in outcome ascertainment, but recurrent confounding, complete-case biomarker availability and selection of patients who reached later sampling timepoints reduced confidence in causal or treatment-adaptive interpretations. Risk-of-bias judgments, therefore, informed the strength of wording: consistent associations were described as prognostic signals, whereas clinical utility and treatment-selection claims were considered unproven.

Results of individual studies and qualitative synthesis

Given the heterogeneity in timing, definitions of EMR and effect measures, no meta-analysis was conducted. Findings are synthesised qualitatively according to standardised temporal categories and EMR constructs.

Baseline EBV-DNA and dynamic context

Although baseline EBV-DNA retained independent prognostic value in multivariable models, baseline measurements alone did not capture the prognostic information provided by dynamic on-treatment changes and were not the primary focus of this review.

Table 2. Report-level risk-of-bias assessment for the 21 eligible reports. Linked reports from one underlying cohort were assessed separately because their analytical objectives differed.

Study (Author, Year)	Type of study	Risk of bias tool used	Risk by domain (summary)	Overall risk of bias
Alfieri *et al* (2025) [30]	Retrospective observational cohort	ROBINS-I	Confounding: Moderate (retrospective design; treatment heterogeneity). Selection of participants: Low (consecutive curatively treated EBER+ NPC). Classification of interventions: Low (treatment strategies clearly defined). Deviations from intended interventions: Low (observational, guideline-based care). Missing data: Moderate (exclusion of patients lacking key timepoints). Measurement of outcomes: Low (objective recurrence and survival outcomes). Selection of reported results: Moderate (no time-to-event Cox models; reliance on descriptive and logistic analyses).	Moderate
Huang *et al* 2022 [32]	Retrospective cohort (treatment delay exposure)	ROBINS-I	Confounding: Moderate (treatment delays may correlate with baseline risk, logistics, disease burden; multivariable modelling stated but covariate set not fully extractable from the published report). Selection of participants: retrospective inclusion of consecutive treated patients within a defined window; some selection risk remains. Classification of interventions/exposures: delays defined operationally and cut-offs derived by ROC; measurement should be reliable, but data source is not fully detailed. Deviations from intended interventions: not applicable in the same way (observational); supportive care adjustments not reported. Missing data: not described in extracted pages. Measurement of outcomes: DFS definition provided; EBV-DNA 'elevation' operationalised (≥5%); assay details partly by reference. Selection of reported result: unclear without a protocol/analysis plan in the extracted text.	Moderate
Chan *et al* [9]	Prospective observational cohort (single arm)	ROBINS-I	Confounding: Moderate (non-randomised; prognosis influenced by stage/tumour burden and treatment selection; authors adjusted for key covariates including T category, N category and stage and GTVs). Selection of participants: Low (prospective recruitment of consecutive eligible non-metastatic NPC under institutional care; metastatic disease excluded). Classification of interventions/exposures: Low (weekly plasma EBV-DNA protocol pre-specified; assay described; 'undetectable' definition provided). Deviations from intended interventions: Unclear (treatment delivered as standard-of-care with permitted IC/adjuvant; no protocolised treatment modification based on EBV kinetics described in extracted text). Missing data: Unclear (one patient was excluded from half-life calculation because clearance was not achieved; otherwise, follow-up/events reported, but completeness of EBV series per patient not fully detailed in extracted text). Measurement of outcomes: Low (PFS/DMFS/OS are objective time-to-event outcomes; standard survival methods are used). Selection of reported result: Unclear (pre-specified endpoints listed; protocol/analysis plan details beyond registration not fully shown in extracted text)	Moderate

Continued

Table 2. Report-level risk-of-bias assessment for the 21 eligible reports. Linked reports from one underlying cohort were assessed separately because their analytical objectives differed. *Continued*

Study (Author, Year)	Type of study	Risk of bias tool used	Risk by domain (summary)	Overall risk of bias
Huang *et al* (2025) [12]	Observational, retrospective cohort (prognostic biomarker kinetics)	ROBINS-I	Confounding: Moderate (disease burden/stage, baseline EBV-DNA, treatment intensity); authors adjusted for key clinical factors and AC. Selection bias: inclusion required completion of IC + radical IMRT and availability of all three EBV-DNA timepoints (may exclude early events/interruptions). Classification of exposure: low (pre-specified timepoints; D/U dichotomisation; assay described). Missing data: limited details; histologic subtype missingness addressed via imputation, but broader missingness not fully described. Outcome measurement: low concern (time-to-event endpoints). Selective reporting: protocol/analysis plan not specified (unclear risk).	Moderate
Lan *et al* (2024) [2]	Retrospective cohort	ROBINS-I	Confounding: partially addressed via multivariable Cox, including key clinical factors (age/sex/T category, N category and CCD, etc.), but residual confounding likely (e.g., treatment intensity/ selection for targeted agents). Selection of participants: retrospective design with acknowledged selection bias (and inclusion depends on availability of specified EBV-DNA measurements). Classification of interventions: CCRT/IMRT and cisplatin schedule described; some heterogeneity with targeted therapy. Deviations from intended interventions: not clearly reported as controlled; observational setting. (Not explicitly detailed.) Missing data: handling is not clearly specified in the extracted text; unclear extent beyond eligibility-based inclusion. (Not explicitly detailed.) Measurement of outcomes: time-to-event outcomes with explicit definitions; likely objective. Selection of reported results: multiple models/subgroups presented; no preregistered analysis plan reported in extracted text.	Serious
Xu *et al* [27]	Multicentre single-arm phase 2 interventional study	ROBINS-I	Confounding: Serious (single-arm, non-comparative design without randomised or concurrent control; historical benchmarks only). Selection of participants: Moderate (trial eligibility criteria; biomarker analyses restricted to a subset with complete data, n = 121). Classification of interventions: Low (protocol-defined induction immunochemotherapy and radiotherapy). Deviations from intended interventions: Moderate (treatment delays or interruptions reported). Missing data: Moderate (loss to follow-up and incomplete biomarker availability). Measurement of outcomes: Low (objective time-to-event endpoints). Selection of reported result: Unclear/NR (statistical analysis plan not fully detailed in available excerpts).	Serious

Table 2. Report-level risk-of-bias assessment for the 21 eligible reports. Linked reports from one underlying cohort were assessed separately because their analytical objectives differed. *Continued*

Study (Author, Year)	Type of study	Risk of bias tool used	Risk by domain (summary)	Overall risk of bias
Yang *et al* (2022) [29]	Observational cohort	ROBINS-I	Confounding: Moderate (non-randomised prognostic study; multivariable Cox models adjusting for key clinical covariates). Selection of participants: Moderate (eligibility required complete EBV-DNA measurements at three predefined timepoints; exclusions applied, including induction/adjuvant chemotherapy). Classification of exposure: Low (objective plasma EBV-DNA PCR; predefined detectability thresholds). Deviations from intended interventions: Low (prognostic exposure study). Missing data: Moderate (complete-case analysis implied; extent of missingness not fully reported). Measurement of outcomes: Low (objective time-to-event endpoints). Selection of reported result: Unclear/NR (protocol or statistical analysis plan not explicitly reported).	Moderate
Zheng *et al* [31]	Retrospective observational cohort	ROBINS-I	Confounding: Serious (limited covariate adjustment; non-randomised). Selection of participants: Serious (single-institution retrospective cohort; selection bias acknowledged). Classification of exposure: Low (ddPCR-based EBV-DNA; explicit detectability threshold >0 IU/mL). Deviations from intended interventions: Low (exposure/prognostic study). Missing data: Moderate (handling and extent of missing data NR). Measurement of outcomes: Low (standard survival endpoints). Selection of reported result: Unclear (analysis plan/ protocol NR).	Serious
Le *et al* (2005) [33]	Prospective observational cohort (prognostic biomarker; nonrandomised)	ROBINS-I	Confounding: Moderate (heterogeneity in RT/CT strategies, partial adjustment); Selection: Moderate (recruitment not clearly consecutive); Classification of interventions: Low; Deviations from intended interventions: Unclear; Missing data: Moderate (serial samples possibly incomplete); Measurement of outcomes: Low; Selective reporting: Unclear	Moderate
Zhang *et al* (2023) [34]	Non-randomised retrospective comparative cohort (with PSM)	ROBINS-I	Confounding: Serious (non-randomised; PSM used but residual confounding likely). Selection of participants: Moderate (retrospective single-centre inclusion; eligibility defined but recruitment flow not fully controlled prospectively). Classification of interventions: Low (routine versus new method clearly defined). Deviations from intended interventions: Moderate (no blinding; treatment delivered per strategy; protocol adherence details NR). Missing data: Unclear (extent/handling of missing baseline or EBV-DNA data NR). Measurement of outcomes: Low (time-to-event outcomes (DFS/ OS/LRFS/DMFS) are objective; ascertainment methods not fully detailed). Selection of reported result: Unclear (protocol/ statistical analysis plan not referenced).	Serious

Table 2. Report-level risk-of-bias assessment for the 21 eligible reports. Linked reports from one underlying cohort were assessed separately because their analytical objectives differed. *Continued*

Study (Author, Year)	Type of study	Risk of bias tool used	Risk by domain (summary)	Overall risk of bias
He *et al* (2018) [13]	Retrospective cohort	NOS	Selection: single-centre retrospective cohort; representativeness/consecutive enrolment NR; exposure (plasma EBV-DNA) measured with standardised qPCR (low concern). Comparability: Moderate (treatment heterogeneity and residual confounding). Outcome: survival endpoints clearly defined; follow-up duration reported and generally adequate; completeness of follow-up NR.	Moderate
Lertbutsayanukul *et al* (2018) [11]	Prognostic biomarker study (secondary analysis of prospective randomised trial cohort)	ROBINS-I	Confounding: Moderate (non-randomised comparison of biomarker-defined groups; adjustment described but residual confounding plausible). Selection of participants: Moderate (restricted to detectable pre-EBV and excluded patients with missing serial samples). Classification of interventions: Low (treatment protocol specified; IMRT technique in parent RCT). Deviations from intended interventions: Unclear (protocol-driven; limited detail on deviations). Missing data: Moderate (missing serial EBV samples led to exclusions; handling beyond exclusion NR). Measurement of outcomes: Low (time-to-event outcomes objective). Measurement of exposure: Low (standardised RT-qPCR; LOD specified; blinding of lab/outcome assessors NR). Selection of reported result: Moderate (multiple endpoints/timepoints; analysis approach stated, but risk of selective emphasis cannot be excluded).	Moderate
Lv *et al* (2024) [14]	Prospective cohort (prognostic biomarker study)	QUIPS	Study participation: Low; Study attrition: Moderate; Prognostic factor measurement: Low; Outcome measurement: Low; Confounding: Moderate; Statistical analysis/reporting: Moderate	Moderate
Lu *et al* (2025) [8]	Retrospective cohort (observational)	ROBINS-I	Confounding: Serious (non-randomised; treatment heterogeneity incl. targeted therapy/ICIs; adjustment incompletely reported). Selection of participants: Moderate (single-centre retrospective; inclusion/exclusion details partially NR). Classification of interventions/exposure: Low (exposure = measured EBV-DNA kinetics; clearly defined). Deviations from intended interventions: Moderate (non-protocolised supportive/adjunct treatments possible; NR). Missing data: Unclear (handling of missing EBV-DNA or follow-up data not clearly reported). Measurement of outcomes: Low (OS/PFS standard). Selection of reported result: Moderate (analysis plan not pre-specified; multiple groupings possible).	Serious
Liu *et al* (2025) [35]	Prognostic model/nomogram development with internal validation (retrospective cohort)	PROBAST	Participants: Moderate; Predictors: Moderate; Outcome: Low; Analysis: High (single-centre retrospective, internal validation only, potential overfitting/thresholding)	High

Table 2. Report-level risk-of-bias assessment for the 21 eligible reports. Linked reports from one underlying cohort were assessed separately because their analytical objectives differed. *Continued*

Study (Author, Year)	Type of study	Risk of bias tool used	Risk by domain (summary)	Overall risk of bias
Prayongrat *et al* (2017) [15]	Retrospective cohort	NOS (cohort)	Selection: moderate (single-centre, retrospective design, exclusions); Comparability: moderate (partial adjustment, residual confounding risk); Outcome: Moderate (survival endpoints, median follow-up 35.1 months).	Moderate
Liu *et al* (2015) [3]	Prospective observational cohort	QUIPS	Participation: Moderate; Attrition: Unclear; Prognostic factor measurement: Moderate; Outcome measurement: Low; Confounding: Moderate (adjusted); Analysis/reporting: Moderate	Moderate
Li *et al* (2025) [28]	Retrospective cohort	ROBINS-I	Confounding: Moderate; Selection: Moderate; Exposure classification: Moderate (EBER+EBV-DNA groups); Missing data: Moderate; Outcome measurement: Low; Reporting: Moderate	Moderate
Lin *et al* (2004) [36]	Observational cohort	QUIPS	Participation: Moderate; Attrition: Moderate; Prognostic factor measurement: Low; Outcome measurement: Low; Confounding: Moderate; Analysis/reporting: Moderate	Moderate
Zhang *et al* [16]	Retrospective cohort	ROBINS-I	Confounding: Moderate; Selection: Moderate; Exposure classification: Low; Missing data: NR; Outcome measurement: Low; Reporting: Moderate	Moderate
Chen *et al* [37]	Prospective cohort (observational prognostic biomarker)	QUIPS	Participation: moderate (single-centre; selection details limited); Attrition: moderate (missing baseline/interim EBV in a few); Prognostic factor measurement: moderate (assay per prior method; details limited); Outcome measurement: low; Confounding: moderate (multivariable/score but limited events); Analysis/reporting: Serious (data-driven baseline cut-off; small event counts)	Moderate

Post-induction/pre-radiotherapy EBV-DNA dynamics

In patients receiving IC, post-induction EBV-DNA status consistently emerged as a strong prognostic marker. Studies uniformly showed that detectable EBV-DNA after induction was associated with inferior progression-free, distant metastasis-free or overall survival, even after adjustment for baseline EBV-DNA and clinical stage. Trajectory-based analyses further refined risk stratification, identifying patients with persistent detectability across induction and radiotherapy as the highest-risk subgroup.

Across studies, early clearance to an undetectable state after induction was associated with a favourable prognosis, whereas persistence or re-emergence of EBV-DNA signalled increased the risk of relapse.

Mid-radiotherapy (on-treatment) EBV-DNA dynamics

A substantial body of evidence evaluated mid-radiotherapy EBV-DNA, typically measured around weeks 4–5 of IMRT. The dominant EMR construct at this timepoint was detectable versus undetectable EBV-DNA, although some studies also examined kinetic changes, such as percentage increase or decrease relative to prior measurements.

Patients with detectable mid-radiotherapy EBV-DNA consistently experienced worse survival outcomes compared with those achieving clearance. In several cohorts, mid-treatment detectability retained independent prognostic significance after multivariable adjustment. Studies using kinetic definitions demonstrated that on-treatment elevation or lack of decline was associated with substantially increased risk of progression or death, underscoring the biological relevance of real-time tumour response during radiotherapy.

End-radiotherapy and early post-radiotherapy EBV-DNA

Strong and directionally consistent prognostic associations were observed at end-radiotherapy and during the early post-radiotherapy window. Across multiple cohorts, persistent detectability at treatment completion or within the first 3 months after treatment was associated with inferior progression-free, distant metastasis-free and overall survival. The apparent strength of later measurements must nevertheless be interpreted in light of time-dependent selection and heterogeneity in the exact post-treatment sampling interval.

Trajectory-based models integrating end-treatment and early post-treatment measurements further identified high-risk groups characterised by persistent or recurrent detectability, with HRs for adverse outcomes often exceeding those observed at earlier timepoints. Conversely, durable clearance at treatment completion was associated with favourable long-term control.

Qualitative confidence by temporal window

Relative confidence was judged moderate for post-induction/pre-radiotherapy evidence (replicated, directionally consistent associations, limited mainly by retrospective confounding); low-to-moderate for mid-radiotherapy evidence (fewer cohorts and variable timing and kinetic definitions); moderate for end-radiotherapy evidence (consistent associations across several cohorts but assay and selection heterogeneity); and moderate for early post-treatment evidence (strong, replicated prognostic separation offset by variable sampling windows and guarantee-time selection). These descriptors are qualitative and are not formal GRADE ratings (Supplementary Material S1).

Therapeutic context and subgroup considerations

Most evidence pertained to standard CCRT or IC → CCRT strategies. Within these contexts, the prognostic value of on-treatment EBV-DNA dynamics was highly consistent. Data on innovative strategies, including induction chemo-immunotherapy, were limited but suggested that early EBV-DNA clearance remained prognostically informative even in non-standard regimens. However, due to the small number of such studies and their higher risk of bias, these findings should be interpreted cautiously.

Magnitude of effect estimates

Across the included studies, effect estimates were not uniformly reported, and several studies provided prognostic associations without extractable HRs. Reported HRs are presented descriptively to illustrate the order of magnitude and directional consistency of associations, rather than to provide exhaustive or pooled estimates. Among studies reporting HRs, unfavourable on-treatment EBV-DNA dynamics were consistently associated with inferior time-to-event outcomes, with effect magnitudes generally increasing as assessments approached treatment completion and the early post-treatment window.

At the post-induction/pre-radiotherapy timepoint, detectable EBV-DNA or adverse dynamic constructs were associated with substantially higher risks across survival endpoints in multivariable models, and trajectory-based approaches similarly discriminated prognostic subgroups [3, 31]. Mid-radiotherapy assessments showed comparable directional consistency, with persistent detectability or unfavourable kinetic patterns independently associated with increased risks of progression-related outcomes [2, 9, 29]. The strongest and most consistent

associations were observed at end-radiotherapy and within the early post-radiotherapy period (≤3 months), where persistent detectability or high-risk trajectories conferred markedly increased hazards across multiple survival endpoints [11, 13, 15–16, 33, 36].

Discussion

Interpretation of the findings in the context of existing evidence

This systematic review provides a timepoint- and construct-specific qualitative synthesis of on-treatment plasma EBV-DNA dynamics in patients with locoregionally advanced NPC treated with curative intent. Earlier reviews evaluated EBV-DNA broadly across clinical settings or pooled pre-treatment, mid-treatment and post-treatment values [17–19]. By separating detectability, thresholds, kinetics, trajectories and rebound within standardised treatment windows, the present review shows that on-treatment EMR carries prognostic information beyond baseline assessment while also making explicit why the available evidence cannot yet support standardised treatment decisions.

Baseline plasma EBV-DNA is an established prognostic biomarker in NPC, reflecting tumour burden and metastatic potential, but it remains a static measure that does not capture treatment sensitivity [4–6]. Serial assessment after IC, during radiotherapy or at treatment completion provides dynamic information that is consistently associated with survival outcomes, particularly distant metastasis-free and event-free survival.

This prognostic signal was observed across multiple EMR constructs. Reports using binary detectability consistently described poorer outcomes with persistent detectability [9, 11, 13, 15–16, 36], while quantitative thresholds, kinetic parameters and trajectory classifications similarly identified unfavourable dynamics [2, 8, 12, 14, 31]. These constructs are not interchangeable, but they interrogate a related biological signal: the extent to which therapy achieves early reduction of EBV-associated tumour DNA.

These findings align with broader oncology paradigms emphasising dynamic response biomarkers rather than baseline risk alone, including minimal residual disease monitoring and circulating tumour DNA kinetics in other malignancies [38–40]. In NPC, plasma EBV-DNA is particularly attractive because it is disease-specific and biologically grounded.

Biological and clinical interpretation of on-treatment EBV-DNA dynamics

The convergence of findings across heterogeneous studies suggests that on-treatment EBV-DNA dynamics reflect more than residual tumour volume. Early clearance likely integrates tumour radiosensitivity, systemic treatment efficacy, micrometastatic control and possibly host–tumour immune interactions. Conversely, persistent or slowly declining EBV-DNA may indicate resistant disease biology, suboptimal systemic control or ongoing dissemination from occult metastatic deposits.

The conceptual framework shown in Figure 2 summarises these observations into favourable, intermediate and unfavourable EMR profiles along a standardised treatment timeline. As highlighted by EP-SEASON, favourable prognosis may still be observed in patients who clear EBV-DNA during early radiotherapy despite persistence after IC, supporting a prognostic gradient rather than a strictly binary model. In this context, the literature generally considers ‘rapid clearance’ as undetectability during induction or within the first 4 weeks of radiotherapy, and ‘delayed clearance’ as clearance between mid-radiotherapy and treatment completion. These profiles are not proposed as new formal definitions but as an interpretative framework for contextualising heterogeneous evidence.

Clinically, when both distant and locoregional outcomes were reported, unfavourable on-treatment EBV-DNA dynamics appeared more strongly associated with distant failure than isolated locoregional relapse, supporting the hypothesis that persistent circulating EBV-DNA reflects ongoing systemic disease activity and may be particularly relevant for systemic treatment stratification.

The figure illustrates a unified therapeutic timeline encompassing baseline, post-induction (when applicable), mid-radiotherapy and end-radiotherapy or early post-treatment assessments. Distinct on-treatment plasma EBV-DNA dynamic patterns, early clearance, delayed clearance and persistent detectability are derived from the present qualitative synthesis and integrated into favourable, intermediate and unfavourable EMR profiles. These profiles are associated with differential prognostic risk across included studies and are presented to facilitate interpretative synthesis rather than clinical prescription. Proposed treatment-adaptive implications are shown as hypothesis-generating concepts and require prospective validation in dedicated clinical trials.

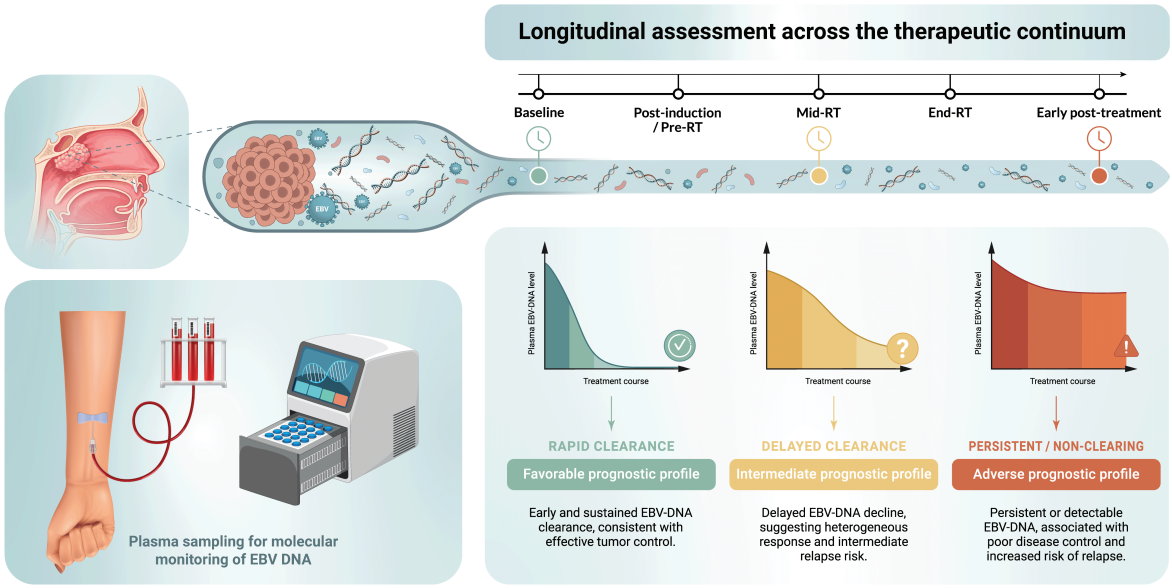

Figure 2. Hypothesis-generating conceptual framework integrating on-treatment plasma EBV-DNA dynamics with prognostic stratification in locoregionally advanced NPC. This interpretative model is not a validated clinical pathway or treatment-decision algorithm.

Methodological heterogeneity and its implications

Methodological heterogeneity was a defining feature of the evidence base. Included studies varied in treatment backbone (CCRT alone, IC followed by CCRT, and more recent investigational approaches including immunotherapy), assay methods, sampling schedules and analytical definitions.

Despite this diversity, the directionality of associations between unfavourable EMR and poorer outcomes was remarkably consistent, strengthening confidence in the biological relevance of the signal. However, some of this consistency may also reflect shared methodological limitations, including publication bias, selective reporting and cohort enrichment for patients completing multimodal therapy. For this reason, quantitative pooling across biologically distinct constructs and timepoints would likely have produced misleading summary estimates.

Some reports assessing both windows suggested stronger prognostic discrimination for early post-radiotherapy detectability [13, 15], but this requires prospective validation. Kinetic parameters such as half-life and slope remain promising but inconsistently defined and infrequently externally validated.

Limitations of the evidence base

Several limitations of the included studies warrant caution. Firstly, most studies were retrospective and single-centre, increasing risks of selection bias, residual confounding and heterogeneous follow-up. Although multivariable adjustment was common, covariate sets varied widely and unmeasured confounding remains likely.

Secondly, the availability of on-treatment or early post-treatment sampling may introduce time-dependent selection (guarantee-time) bias, because patients must remain event-free and on treatment long enough to reach the biomarker timepoint. This may inflate the apparent prognostic value of clearance assessed at later timepoints.

Thirdly, assay heterogeneity remains a major barrier to translation. Studies varied in plasma volume, extraction method, PCR platform, genomic target, calibration standard, reporting unit and limit of detection. Most used copies/mL, one used IU/mL, one allowed either unit and several did not report units or analytical limits clearly. Even among studies using BamHI-W qPCR, detectability thresholds ranged from complete non-detection to assay-specific cut-offs between 316 and 4,000 copies/mL. Absolute thresholds and kinetic estimates are therefore not directly interchangeable or readily generalisable across laboratories (Supplementary Material S3).

Fourthly, the evidence is geographically concentrated in endemic East and Southeast Asian populations. Although epidemiologically appropriate, this may limit generalisability to non-endemic settings with different tumour biology, host genetics and treatment patterns. In addition, some cohorts included earlier-stage disease; although eligibility criteria prioritised locoregionally advanced NPC, this may still have introduced heterogeneity affecting the precision of estimates for strictly locoregionally advanced populations.

Finally, patient-centred outcomes such as treatment-related toxicity, quality of life and functional outcomes were rarely integrated with EBV-DNA dynamics, limiting broader clinical contextualisation.

Limitations of the review process

This review also has methodological limitations. It was not prospectively registered, and no standalone dated protocol was archived before screening. The methodological framework was documented retrospectively in Supplementary Material S1 and cannot provide the same auditability as a prospectively registered protocol. In addition, the screening archive retained aggregate full-text exclusion categories but not a report-level appendix for historical cohort-overlap decisions; individual exclusions were therefore not reconstructed retrospectively. Potential partial overlap among large single-centre series may remain despite report-linkage checks and is considered when interpreting replication and nominal sample size.

In addition, despite a comprehensive multi-database search, unpublished and non-peer-reviewed data were not systematically included, which may contribute to publication bias. Formal statistical assessment of reporting bias was not performed because no quantitative synthesis was undertaken. Nevertheless, the consistency of findings across study designs and time periods partly mitigates this concern.

Implications for clinical practice and policy

Current evidence does not support escalation, de-escalation or other routine treatment modification based solely on on-treatment EBV-DNA dynamics outside a clinical trial. Serial measurements may provide supplementary prognostic information in centres with validated assays, but thresholds, sampling windows and treatment consequences remain insufficiently standardised. Figure 2 should therefore be read only as an interpretative research model [9–10, 14].

From a policy and research-standardisation perspective, these findings underscore the need for consensus-based harmonisation of EBV-DNA measurement and reporting, including sampling timepoints, assay characteristics, analytical constructs and reporting standards [6–8, 41–43].

Directions for future research

Future studies should prioritise prospective, multicentre validation of standardised EMR definitions across predefined timepoints [9, 14, 27]. Response-adaptive escalation or de-escalation should be evaluated only in rigorously controlled trials.

Integration of EBV-DNA dynamics with imaging response metrics and immune profiling may enable composite risk models that better capture tumour–host interactions [37, 44–46]. Development and external validation using robust prognostic-modelling frameworks are required before clinical implementation.

Conclusion

Across 21 eligible reports representing 20 analytically distinct cohorts, a consistent but methodologically heterogeneous signal emerged: favourable EBV-DNA clearance or kinetics during therapy was associated with improved survival outcomes, particularly distant metastasis-free and event-free survival. These findings extend the established prognostic role of baseline EBV-DNA while remaining insufficient for routine treatment adaptation.

Although methodological heterogeneity precluded quantitative meta-analysis, the direction of association was broadly consistent across timepoints and EMR constructs. Persistent detectability or delayed clearance identified patients at higher risk of treatment failure, and trajectory patterns, including rebound after initial clearance, may offer additional prognostic refinement. The observational design and variable assay methods nevertheless limit certainty regarding the magnitude and transportability of these associations.

Current evidence remains insufficient to support routine treatment modification based solely on on-treatment EBV-DNA outside clinical trials. Prospective studies should validate standardised assays, sampling windows and EMR definitions before response-adaptive strategies are tested. Until such evidence is available, EBV-DNA dynamics should be regarded as a promising prognostic research marker rather than a validated prescriptive biomarker.

List of abbreviations

5-FU, 5-Fluorouracil; AC, Adjuvant chemotherapy; AJCC/UICC, American Joint Committee on Cancer/Union for International Cancer Control; CCD, Cumulative cisplatin dose; CCRT, Concurrent chemoradiotherapy; cEBV-DNA, Circulating Epstein–Barr virus DNA; cfEBV-DNA, Circulating cell-free Epstein–Barr virus DNA; CR, Complete response; CT, Chemotherapy; ddPCR, Droplet digital polymerase chain reaction; DFS, Disease-free survival; DMFS, Distant metastasis-free survival; EBER, Epstein–Barr virus-encoded RNA; EBV, Epstein–Barr virus; EBV-DNA, Epstein–Barr virus DNA; EFS, Event-free survival; EMR, Early molecular response; ESC, Early EBV-DNA status conversion; FFR, Freedom-from-relapse; FFS, Failure-free survival; GP, Gemcitabine + Cisplatin; GRADE, Grading of Recommendations Assessment, Development and Evaluation; GTV, Gross tumour volume; HR, Hazard ratio; IC, Induction chemotherapy; ICI, Immune checkpoint inhibitor; IMRT, Intensity-modulated radiotherapy; IU/mL, International units per millilitre; LOD, Limit of detection; LPFS, Local progression-free survival; LRFFS, Locoregional failure-free survival; LRFS, Locoregional failure-free survival; LRRFS, Locoregional relapse-free survival; LSC, Late EBV-DNA status conversion; MEDLINE, Medical Literature Analysis and Retrieval System Online; MeSH, Medical Subject Headings; NACT/NAC, Neoadjuvant chemotherapy; NOS, Newcastle–Ottawa Scale; NR, Not reported; NSC, No EBV-DNA status conversion; NPC, Nasopharyngeal carcinoma; OS, Overall survival; PCR, Polymerase chain reaction; PD, Progressive disease; PF, Cisplatin + 5-fluorouracil; PFS, Progression-free survival; PICO, Population, intervention/exposure, comparator, outcomes; PR, Partial response; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; PROBAST, Prediction model risk-of-bias assessment tool; PSM, Propensity score matching; PTV-HR, Planning target volume-high risk; PTV-LR, Planning target volume-low risk; qPCR, Quantitative polymerase chain reaction; QoL, Quality of life; QUIPS, Quality in prognosis studies; RCT, Randomised controlled trial; RFS, Recurrence-free survival; ROBINS-I, Risk of Bias in Non-randomised Studies of Interventions; ROC, Receiver operating characteristic; RPFS, Regional progression-free survival; RT, Radiotherapy; SD, Stable disease; TP, Taxane + Cisplatin; TPF, Taxane + Cisplatin + 5-fluorouracil.

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Conflicts of interest

The authors declare that they have no competing interests.

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Consent for publication

Not applicable.

Human ethics and consent to participate

Not applicable.

Ethics approval and consent to participate

Not applicable. This study is a systematic review based exclusively on previously published literature and did not involve any new research with human participants or animals.

Data availability

All data analysed in this systematic review were derived from publicly available, peer-reviewed original studies. The study selection process and PRISMA 2020 flow diagram are reported in the manuscript. Complete database-specific search strategies are provided in Supplementary Material S2; the retrospective methodological framework and extraction template in Supplementary Material S1; and detailed study- and assay-level extraction tables in Supplementary Material S3.

Originality and prior publication

This manuscript is original, has not been published previously and is not under consideration for publication elsewhere.

Declaration of artificial intelligence-assisted editorial support.

The study conception, literature screening, data extraction, risk-of-bias assessment, evidence synthesis, interpretation and initial manuscript drafting were completed by the authors without generative artificial intelligence. During revision, an artificial intelligence-assisted tool was used for editorial and organisational support, including language refinement. It did not generate or independently determine the study data or scientific conclusions. All revisions were critically reviewed, verified and approved by the authors, who take full responsibility for the final content.

References

1. Li XY, Luo DH, and Guo L, *et al* (2022) **Deintensified chemoradiotherapy for pretreatment Epstein-Barr Virus DNA-selected low-risk locoregionally advanced nasopharyngeal carcinoma: a phase II randomized noninferiority trial** *J Clin Oncol* **40** 1163–1173 https://doi.org/10.1200/JCO.21.01467 PMID: 34990291
2. Lan K, Mao J, and Sun X, *et al* (2024) **Combined pre-treatment and middle-treatment Epstein–Barr virus DNA load contributes to prognostication and treatment modification in nasopharyngeal carcinoma patients** *Therapeutic Adv Med Oncol* **16** 17588359231221343 https://doi.org/10.1177/17588359231221343
3. Liu LT, Tang LQ, and Chen QY, *et al* (2015) **The prognostic value of plasma Epstein-Barr Viral DNA and tumor response to neoadjuvant chemotherapy in advanced-stage nasopharyngeal carcinoma** *Int J Radiat Oncol Biol Phys* **93** 862–869 https://doi.org/10.1016/j.ijrobp.2015.08.003 PMID: 26530755
4. Liang H, Lv X, and Wang L, *et al* (2018) **The plasma Epstein–Barr virus DNA level guides precision treatment for nasopharyngeal carcinoma in the intensity-modulated radiotherapy era: a large population-based cohort study from an endemic area** *Ther Adv Med Oncol* **10** 1758835918782331 [https://doi.org/10.1177/1758835918782331]
5. Lu L, Li J, and Zhao C, *et al* (2016) **Prognostic efficacy of combining tumor volume with Epstein-Barr virus DNA in patients treated with intensity-modulated radiotherapy for nasopharyngeal carcinoma** *Oral Oncol* **60** 18–24 https://doi.org/10.1016/j.oraloncology.2016.06.013 PMID: 27531868
6. Allen Chan KC and Dennis Lo YM (2002) **Circulating EBV DNA as a tumor marker for nasopharyngeal carcinoma** *Semin Cancer Biol* **12** 489–496 https://doi.org/10.1016/S1044579X02000913
7. Li W, Chen J, and Liang B, *et al* (2021) **Long-term monitoring of dynamic changes in plasma EBV DNA for improved prognosis prediction of nasopharyngeal carcinoma** *Cancer Med* **10** 883–894 https://doi.org/10.1002/cam4.3669 PMCID: 7897970
8. Lu X, Zhu L, and Tan B, *et al* (2025) **Early circulating EBV-DNA clearance predicts enhanced short-term treatment response and survival benefit in locally advanced nasopharyngeal carcinoma** *BMC Cancer* **25** 1499 https://doi.org/10.1186/s12885-025-14888-1 PMID: 41039233 PMCID: 12492512
9. Chan SK, Chan SY, and Choi HCW, *et al* (2020) **Prognostication of half-life clearance of plasma EBV DNA in previously untreated non-metastatic nasopharyngeal carcinoma treated with radical intensity-modulated radiation therapy** *Front Oncol* **10** 1417 https://doi.org/10.3389/fonc.2020.01417 PMID: 32974150 PMCID: 7472777
10. Ho-Fun Lee V, Lai-wan Kwong D, and Leung TW, *et al* (2017) **Prognostication of serial post-intensity-modulated radiation therapy undetectable plasma EBV DNA for nasopharyngeal carcinoma** *Oncotarget* **8** 5292–5308 https://doi.org/10.18632/oncotarget.14137
11. Lertbutsayanukul C, Kannarunimit D, and Prayongrat A, *et al* (2018) **Prognostic value of plasma EBV DNA for nasopharyngeal cancer patients during treatment with intensity-modulated radiation therapy and concurrent chemotherapy** *Radiol Oncol* **52** 195–203 https://doi.org/10.2478/raon-2018-0016 PMID: 30018524 PMCID: 6043877
12. Huang Z, Li Y, and Wu W, *et al* (2025) **Plasma Epstein-Barr Virus DNA temporal clearance pattern during induction-concurrent (chemo) radiation therapy for risk stratification in nasopharyngeal carcinoma** *Int J Radiat Oncol Biol Phys* **123** 129–140 https://doi.org/10.1016/j.ijrobp.2025.03.076 PMID: 40204246
13. He SS, Wang Y, and Bao Y, *et al* (2018) **Dynamic changes in plasma Epstein–Barr virus DNA load during treatment have prognostic value in nasopharyngeal carcinoma: a retrospective study** *Cancer Med* **7** 1110–1117 https://doi.org/10.1002/cam4.1381 PMID: 29493874 PMCID: 5911595
14. Lv J, Xu LX, and Li ZX, *et al* (2024) **Longitudinal on-treatment circulating tumor DNA as a biomarker for real-time dynamic risk monitoring in cancer patients: the EP-SEASON study** *Cancer Cell* **42** 1401–1414 https://doi.org/10.1016/j.ccell.2024.07.001 PMID: 39059389

15. Prayongrat A, Chakkabat C, and Kannarunimit D, *et al* (2017) **Prevalence and significance of plasma Epstein-Barr Virus DNA level in nasopharyngeal carcinoma** *J Radiat Res* **58** 509–516 https://doi.org/10.1093/jrr/rrw128 PMID: 28204596 PMCID: 5570077

16. Zhang Y, Li WF, and Mao YP, *et al* (2016) **Risk stratification based on change in plasma Epstein-Barr virus DNA load after treatment in nasopharyngeal carcinoma** *Oncotarget* **7** 9576–9585 https://doi.org/10.18632/oncotarget.7083 PMID: 26840023 PMCID: 4891061

17. Lee AWM, Lee VHF, and Ng WT, *et al* (2021) **A systematic review and recommendations on the use of plasma EBV DNA for nasopharyngeal carcinoma** *Eur J Cancer* **153** 109–122 https://doi.org/10.1016/j.ejca.2021.05.022 PMID: 34153713

18. Qu H, Huang Y, and Zhao S, *et al* (2020) **Prognostic value of Epstein–Barr virus DNA level for nasopharyngeal carcinoma: a meta-analysis of 8128 cases** *Eur Arch Oto-Rhino-Laryngol* **277** 9–18 https://doi.org/10.1007/s00405-019-05699-9

19. Xie X, Ren Y, and Wang K, *et al* (2019) **Molecular prognostic value of circulating Epstein–Barr Viral DNA in nasopharyngeal carcinoma: a meta-analysis of 27,235 cases in the endemic area of Southeast Asia** *Genetic Test Mol Biomarkers* **23** 448–459 https://doi.org/10.1089/gtmb.2018.0304

20. Page MJ, McKenzie JE, and Bossuyt PM, *et al* (2021) **The PRISMA 2020 statement: an updated guideline for reporting systematic reviews** *BMJ* **372**(71) [https://doi.org/10.1136/bmj.n71] PMID: 33782057 PMCID: 8005924

21. Edge SB and Compton CC (2010) **The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM** *Ann Surg Oncol* **17** 1471–1474 https://doi.org/10.1245/s10434-010-0985-4 PMID: 20180029

22. Amin MB, Greene FL, and Edge SB, *et al* (2017) **The eighth edition AJCC cancer staging manual: continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging** *CA A Cancer J For Clin* **67** 93–99 [10.3322/caac.21388]

23. Stang A (2010) **Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses** *Eur J Epidemiol* **25** 603–605 https://doi.org/10.1007/s10654-010-9491-z PMID: 20652370

24. Schünemann HJ, Cuello C, and Akl EA, *et al* (2019) **GRADE guidelines: 18. How ROBINS-I and other tools to assess risk of bias in non-randomized studies should be used to rate the certainty of a body of evidence** *J Clin Epidemiol* **111** 105–114 https://doi.org/10.1016/j.jclinepi.2018.01.012 PMCID: 6692166

25. Hayden JA, Van Der Windt DA, and Cartwright JL, *et al* (2013) **Assessing bias in studies of prognostic factors** *Ann Internal Med* **158** 280–286 https://doi.org/10.7326/0003-4819-158-4-201302190-00009

26. Moons KGM, Wolff RF, and Riley RD, *et al* (2019) **PROBAST: a tool to assess risk of bias and applicability of prediction model studies: explanation and elaboration** *Ann Internal Med* **170** W1–W33 https://doi.org/10.7326/M18-1377

27. Xu C, Zhou GQ, and Li WF, *et al* (2025) **Nivolumab combined with induction chemotherapy and radiotherapy in nasopharyngeal carcinoma: a multicenter phase 2 PLATINUM trial** *Cancer Cell* **43** 925–936 https://doi.org/10.1016/j.ccell.2025.01.014 PMID: 40020668

28. Li Y, Wu L, and Huang Z, *et al* (2025) **Prognostic implications of EBER and EBV DNA combinations in nasopharyngeal carcinoma in endemic areas** *BMC Oral Health* **25** 1361 https://doi.org/10.1186/s12903-025-06733-5 PMID: 40849665 PMCID: 12374416

29. Yang ZC, Du CC, and Liu LT, *et al* (2022**) The prognostic role of plasma Epstein-Barr Virus DNA levels in the middle of intensity modulated radiation therapy to guide cisplatin dose recommendation in concurrent chemoradiation therapy in patients with locally advanced nasopharyngeal carcinoma: a large cohort study** *Adv Radiat Oncol* **7** 100908 [https://doi.org/10.1016/j.adro.2022.100908]

30. Alfieri S, Romanò R, and Marceglia S, *et al* (2025) **Longitudinal assessment of plasma EBV-DNA in non-endemic EBV-related nasopharyngeal cancer (NPC): the LEA study** *Eur J Cancer* **226** 115625 https://doi.org/10.1016/j.ejca.2025.115625 PMID: 40729842

31. Zheng H, Zhou P, and Wang J, *et al* (2023) **Prognostic effect of residual plasma Epstein–Barr viral DNA after induction chemotherapy for locoregionally advanced nasopharyngeal carcinoma** *Cancer Med* **12** 14979–14987 https://doi.org/10.1002/cam4.6132 PMID: 37212447 PMCID: 10417187

32. Huang CL, Fang XL, and Mao YP, *et al* (2022) **Association of delayed chemoradiotherapy with elevated Epstein-Barr virus DNA load and adverse clinical outcome in nasopharyngeal carcinoma treatment during the COVID-19 pandemic: a retrospective study** *Cancer Cell Int* **22** 331 https://doi.org/10.1186/s12935-022-02748-y PMID: 36316696 PMCID: 9623943

33. Le QT, Jones CD, and Yau TK, *et al* (2005) **A comparison study of different PCR assays in measuring circulating plasma Epstein-Barr Virus DNA levels in patients with nasopharyngeal carcinoma** *Clin Cancer Res* **11** 5700–5707 https://doi.org/10.1158/1078-0432.CCR-05-0648 PMID: 16115906

34. Zhang Q, Peng ZW, and Gu ZS, *et al* (2023) **Can Epstein–Barr virus-deoxyribonucleic acid load after induction chemotherapy combined with American Joint Committee on Cancer stage determine the chemotherapy intensity of locally advanced nasopharyngeal carcinoma?** *Cancer Med* **12** 223–235 https://doi.org/10.1002/cam4.4899 PMCID: 9844613

35. Liu F, Ma C, and Chen M, *et al* (2025) **A Nomogram based on tumor response to induction chemotherapy and plasma Epstein–Barr Virus DNA level after induction chemotherapy to explore individualized treatment of patients with locally advanced nasopharyngeal carcinoma** *J Inflammation Res* **18** 3677–3693 https://doi.org/10.2147/JIR.S507926

36. Lin JC, Wang WY, and Chen KY, *et al* (2004) **Quantification of plasma Epstein–Barr Virus DNA in patients with advanced nasopharyngeal carcinoma** *New England J Med* **350** 2461–2470 https://doi.org/10.1056/NEJMoa032260

37. Chen YH, Chang KP, and Chu SC, *et al* (2019) **Value of early evaluation of treatment response using 18F-FDG PET/CT parameters and the Epstein-Barr virus DNA load for prediction of outcome in patients with primary nasopharyngeal carcinoma** *Eur J Nucl Med Mol Imag* **46** 650–660 https://doi.org/10.1007/s00259-018-4172-3

38. Colmenares R, Álvarez N, and Barrio S, *et al* (2022) **The minimal residual disease using liquid biopsies in hematological malignancies** *Cancers* **14** 1310 https://doi.org/10.3390/cancers14051310 PMID: 35267616 PMCID: 8909350

39. Zhong R, Gao R, and Fu W, *et al* (2023) **Accuracy of minimal residual disease detection by circulating tumor DNA profiling in lung cancer: a meta-analysis** *BMC Med* **21** 180 https://doi.org/10.1186/s12916-023-02849-z PMID: 37173789 PMCID: 10176776

40. Negro S, Pulvirenti A, and Trento C, *et al* (2025) **Circulating tumor DNA as a real-time biomarker for minimal residual disease and recurrence prediction in stage II colorectal cancer: a systematic review and meta-analysis** *Int J Mol Sci* **26** 2486 https://doi.org/10.3390/ijms26062486 PMID: 40141130 PMCID: 11942625

41. Lee VH, Kwong DL, and Leung TW, *et al* (2018) **5-year results of the prognostic roles of serial post-intensity-modulated radiation therapy undetectable plasma EBV DNA for non-metastatic nasopharyngeal carcinoma** *Int J Radiat Oncol Biol Phys* **102** S126–S127 https://doi.org/10.1016/j.ijrobp.2018.06.315

42. Chan KCA, Leung SF, and Yeung SW, *et al* (2008) **Quantitative analysis of the transrenal excretion of circulating EBV DNA in nasopharyngeal carcinoma patients** *Clin Cancer Res* **14** 4809–4813 https://doi.org/10.1158/1078-0432.CCR-08-1112 PMID: 18676752

43. Chan KC, Zhang J, and Chan AT, *et al* (2003) **Molecular characterization of circulating EBV DNA in the plasma of nasopharyngeal carcinoma and lymphoma patients** *Cancer Res* **63** 2028–2032 PMID: 12727814

44. Yang Y, Shang N, and Lu S, *et al* (2025) **Exploring the prognostic value of EBV DNA in advanced nasopharyngeal carcinoma treated with chemoradiotherapy using AI-based modeling** *Front Oncol* **15** 1650377 https://doi.org/10.3389/fonc.2025.1650377 PMID: 41018095 PMCID: 12463603

45. Guo SS, Chen YZ, and Liu LT, *et al* (2022) **Prognostic significance of AKR1C4 and the advantage of combining EBV DNA to stratify patients at high risk of locoregional recurrence of nasopharyngeal carcinoma** *BMC Cancer* **22** 880 https://doi.org/10.1186/s12885-022-09924-3 PMID: 35953777 PMCID: 9373296

46. Ghose S, Roy S, and Ghosh V, *et al* (2024) **The plasma EBV DNA load with IL-6 and VEGF levels as predictive and prognostic biomarker in nasopharyngeal carcinoma** *Virol J* **21** 224 https://doi.org/10.1186/s12985-024-02473-0 PMID: 39304953 PMCID: 11414088

Supplementary Materials

Supplementary Material S1

Supplementary methodological framework

Retrospectively compiled for transparency during peer review (June 2026)

Document status: This document was prepared during peer review to describe, in a consolidated form, the methodological framework used for the systematic review. It is a retrospective transparency document, not a prospectively registered or contemporaneously archived protocol. Where the archival record does not permit reliable retrospective reconstruction, this is stated explicitly.

Parent study: On-treatment and early post-radiotherapy EBV-DNA dynamics in locoregionally advanced NPC: a systematic review.

Investigators: Imad Barjij, Sarah Naciri, Sihame Lkhoyaali, Saoussane Kharmoum, Hanane Inrhaouen, Ibrahim Elghissassi, Saber Boutayeb, Hind Mrabti, Hassan Errihani

Affiliations: Department of Medical Oncology, National Institute of Oncology, Ibn Sina University Hospital, Rabat, Morocco; Faculty of Medicine and Pharmacy of Rabat, Mohammed V University of Rabat, Morocco; Department of Medical Oncology, Tangier Regional Hospital Center, Tangier, Morocco.

Background and rationale

Plasma EBV DNA is an established prognostic biomarker in NPC. While baseline levels reflect tumour burden, increasing interest has focused on on-treatment and early post-treatment EBV-DNA dynamics as markers of EMR. However, definitions of EMR, sampling timepoints, analytical constructs and assay methodologies vary substantially across studies, precluding direct comparison or quantitative pooling.

This supplementary document formalises the methodological framework that guided the systematic review, including objectives, eligibility criteria, search strategy, data extraction plan, quality assessment methods and synthesis approach.

Review objectives

Primary objective: To systematically review and qualitatively synthesise the prognostic significance of plasma EBV-DNA dynamics measured during curative-intent therapy (on-treatment and early post-treatment timepoints) in patients with locoregionally advanced, non-metastatic NPC.

Secondary objectives:

- To characterise the heterogeneity in EMR definitions (binary detectability, quantitative thresholds, kinetic parameters, trajectory-based constructs) across the literature.
- To identify which temporal assessment windows (post-induction, mid-radiotherapy, end-radiotherapy, early post-treatment) yield the most consistent prognostic associations.
- To describe the magnitude and direction of reported prognostic associations (HRs) across treatment strategies.
- To identify methodological gaps and formulate recommendations for future standardisation and prospective validation.

Eligibility criteria (PICO framework)

PICO element	Criteria
Population	Adults (≥18 years) with non-metastatic NPC treated with curative intent, primarily locoregionally advanced disease corresponding to AJCC/UICC stages III–IVA. Studies including earlier-stage disease were eligible if ≥50% of patients had locoregionally advanced disease or if relevant subgroups were extractable.
Intervention/Exposure	Serial plasma EBV-DNA measurements with at least one on-treatment timepoint beyond baseline (post-induction, mid-radiotherapy, end-of-radiotherapy, or early post-treatment). Comparisons included detectability versus non-detectability, quantitative thresholds, kinetic parameters (e.g., slope, half-life), or trajectory-based classifications.
Comparator	Within-cohort comparison of patients with favourable versus unfavourable EBV-DNA dynamics.
Outcomes	Primary: Time-to-event survival endpoints (event-free survival [EFS], disease-free survival [DFS], distant metastasis-free survival [DMFS], progression-free survival [PFS]). Secondary: Overall survival (OS). HRs with 95% confidence intervals were extracted when available; otherwise, studies providing comparative survival analyses were included for qualitative synthesis.

Eligible study designs: Randomised controlled trials, prospective cohort studies and retrospective cohort studies. Studies were restricted to English-language publications between January 2004 and December 2025

Exclusion criteria

- Paediatric-only cohorts
- Metastatic disease at diagnosis (unless non-metastatic subgroups were independently extractable)
- Recurrent or metastatic-only populations
- Studies reporting only baseline EBV-DNA without on-treatment dynamics
- Technical assay-validation reports without clinical outcome data
- Non-original publications (reviews, editorials, letters, abstracts without full peer-reviewed data)
- Studies where EBV-DNA results could not be separated from composite biomarker panels

Management of overlapping cohorts

Potential cohort overlap was assessed using recruitment centre, enrolment period, eligibility criteria, treatment regimen, sample size and patient characteristics. Reports from the same underlying cohort were linked and counted once at the study level; complementary analyses could be retained without summing participants. The screening archive retained aggregate exclusion counts by reason but not a report-level appendix suitable for reliable retrospective publication, so no speculative list of historical overlap exclusions was generated.

Information sources and search strategy

Databases: PubMed/MEDLINE, Embase, Scopus and Web of Science. Coverage: 1 January 2004 through 4 December 2025. The final search was performed on 4 December 2025.

Supplementary sources: Reference lists and forward citations were screened manually. Trial registries (ClinicalTrials.gov) were reviewed.

Search strategies combined controlled vocabulary (MeSH/Emtree) and free-text terms across four conceptual domains: (1) NPC, (2) EBV, (3) plasma/circulating EBV DNA and (4) dynamic or on-treatment assessment. No design filters were applied.

Complete database-specific search strategies are provided in Supplementary Material S2.

Study selection process

Records were imported into a reference management system and deduplicated automatically and manually. Screening was performed in two stages:

- Stage 1 (Title/Abstract): Three reviewers (I.B., S.N. and H.M.) independently screened all records. Discrepancies were resolved by consensus or majority decision.
- Stage 2 (Full-text): All potentially eligible records underwent full-text review against the predefined eligibility criteria.
- No automation tools were used.
- The study selection process was documented using a PRISMA 2020 flow diagram.

Data collection and extraction

Two reviewers (I.B. and S.N.) independently extracted data using a standardised, piloted extraction form. A third reviewer (H.M.) verified accuracy. Disagreements were resolved by consensus.

Extracted variables included:

- Study characteristics: first author, year, country, design, sample size, follow-up duration
- Patient and tumour features: age, sex, TNM staging system and distribution, histological subtype
- Treatment modalities: IC regimens, CCRT details, adjuvant therapy
- EBV-DNA assay characteristics: platform (qPCR, ddPCR), target gene (BamHI-W), sample type (plasma/serum), detection limit, reporting units
- Sampling timepoints and schedule: post-induction, mid-RT (weeks 4–5), end-RT, early post-treatment (≤3 months)
- EMR definitions: binary detectability, quantitative thresholds (copies/mL), kinetic parameters (half-life, decay slope), trajectory-based constructs
- Primary outcomes: HRs with 95% CI for EFS, DFS, DMFS, PFS from multivariable models (preferred) or univariable analyses
- Secondary outcomes: HR with 95% CI for overall survival (OS)
- When multiple analyses were reported, multivariable models with the most comprehensive covariate adjustment were prioritised
- Missing data were recorded as not reported

A template of the standardised extraction form is provided in Appendix A.

Risk-of-bias assessment

Risk of bias was assessed according to study design and research objective:

Study type/Objective	Assessment tool
Observational cohorts (general)	NOS
Non-randomised intervention/exposure studies	ROBINS-I
Prognostic factor studies	QUIPS
Prediction model studies	PROBAST

Assessment was performed independently by I.B. and S.N., with adjudication by H.M. Overall risk was categorised as low, moderate, serious or high based on predefined rules, and domain-level judgments are reported to support interpretation.

Effect measures and data synthesis

Extracted effect measures included HRs with 95% confidence intervals. When HRs were unavailable, findings were described narratively based on reported survival analyses. No transformation or estimation from survival curves was performed.

Given the heterogeneity in EMR definitions, sampling timepoints, assay platforms and treatment contexts, quantitative meta-analysis was not undertaken. Synthesis was qualitative.

Synthesis approach:

- Studies were grouped according to predefined temporal EBV-DNA categories: (a) post-induction/pre-radiotherapy, (b) mid-radiotherapy, (c) end-radiotherapy and (d) early post-treatment.
- Within each temporal category, findings were further grouped by EMR construct type (binary detectability, quantitative threshold, kinetic parameters and trajectory-based models).
- Tabular summaries present study-level effect estimates (HR with 95% CI where available).
- Constructs were not pooled across non-comparable definitions.
- Direction, consistency and magnitude of associations were described narratively.

Certainty of evidence

The GRADE framework was not formally applied. Given the predominance of heterogeneous observational prognostic studies, variability in EBV-DNA assays (platforms, units, detection limits), sampling schedules and EMR definitions (detectability, thresholds, kinetics, trajectories), formal GRADE rating would have been highly model-dependent and of limited interpretability. Certainty was therefore discussed qualitatively, based on consistency of effect direction, biological plausibility, magnitude of associations and risk-of-bias patterns.

Reporting bias assessment

Formal assessment of reporting bias (e.g., funnel plots) was not conducted, as quantitative synthesis was not undertaken. The potential for publication bias is discussed qualitatively in the manuscript, noting that studies with null results for EMR may be less likely to be published or to report EBV-DNA dynamics as a primary analysis.

Protocol registration and transparency statement

This review was not prospectively registered in PROSPERO or another registry, and no standalone dated protocol was archived before screening. The review question, eligibility criteria, temporal categories, data-extraction domains, risk-of-bias approach and qualitative synthesis framework were established in the internal working methodology before completion of screening and data extraction.

This supplementary document was compiled during peer review (June 2026) to improve transparency. It must not be interpreted as evidence that a standalone protocol existed before the review. The absence of prospective registration and of a contemporaneously archived protocol is acknowledged as a methodological limitation in the revised manuscript.

Qualitative confidence by temporal window

Temporal window	Relative confidence	Main considerations
Post-induction/pre-radiotherapy	Moderate	Replicated, directionally consistent associations; limited by retrospective confounding and assay variability.
Mid-radiotherapy	Low-to-moderate	Fewer cohorts, variable week of sampling and heterogeneous detectability or kinetic constructs.
End-radiotherapy	Moderate	Consistent associations across several cohorts; reduced by the selection of patients reaching treatment completion and assay heterogeneity.
Early post-treatment (≤3 months)	Moderate	Strong prognostic separation replicated across cohorts; reduced by variable sampling windows and guarantee-time selection.

The descriptors below summarise relative confidence within this review and are not formal GRADE ratings

Assay reporting summary

The final synthesis comprised 21 eligible reports representing 20 analytically distinct study cohorts. Thirteen reports used copies/mL exclusively, one used IU/mL, one allowed either IU/mL or copies/mL and six did not clearly report the unit in extractable text. One report used ddPCR, and the remainder used qPCR or RT-qPCR. Detectability definitions ranged from complete non-detection to assay-specific thresholds between 316 and 4,000 copies/mL. Study-level details are provided in Supplementary Material S3.

Authors' responsibilities

Role	Investigators
Conception and methodological design	I.B., H.E.
Screening and study selection	I.B., S.N., H.M.
Data extraction	I.B., S.N. (verified by H.M.)
Risk-of-bias assessment	I.B., S.N. (adjudicated by H.M.)

Appendix A: Standardised data extraction form (template).

Field	Description/Response
Study ID	[First author, Year]
Country/Region	
Study design	[RCT/Prospective cohort/Retrospective cohort]
Sample size (N)	
Median follow-up (months)	
TNM staging system	[AJCC 7th/8th]
Stage distribution	[III/IVA/IVB] and proportion LANPC
Proportion with locoregionally advanced disease	[%] (for studies including early stages)
Treatment strategy	[CCRT/IC+CCRT/IC+CCRT+AC/Other]
EBV-DNA assay platform	[qPCR/ddPCR/Other]
Target gene	[BamHI-W/Other]
Detection limit	[copies/mL or IU/mL]
Reporting units	[copies/mL/IU/mL/Other]
Sampling timepoints	[Post-IC/Mid-RT/End-RT/Early post-RT]
EMR definition	[Detectable versus undetectable/Threshold/Kinetic/Trajectory]
EMR threshold (if applicable)	[value and units]
Primary endpoint	[EFS/DFS/DMFS/PFS]
HR (95% CI) – Primary endpoint	
Analysis type (primary)	[Multivariable/Univariable]
Covariates adjusted for	
OS HR (95% CI)	
Analysis type (OS)	[Multivariable/Univariable]
Risk-of-bias tool used	[NOS/ROBINS-I/QUIPS/PROBAST]
Overall risk-of-bias rating	[Low/Moderate/Serious/High]
Notes/Comments	

Supplementary Material S2

Complete database-specific search strategies

Parent study: On-treatment and early post-radiotherapy EBV-DNA dynamics in locoregionally advanced NPC: a systematic review

Search date: 4 December 2025

This supplement reports the complete database-specific strategies retained in the review files. Searches combined controlled vocabulary, where applicable, with free-text terms across four domains: NPC, EBV, circulating or plasma EBV-DNA and dynamic or on-treatment assessment.

Search parameters

Databases and platforms: PubMed/MEDLINE (NLM), Embase (Elsevier), Web of Science (Clarivate) and Scopus (Elsevier)

Coverage: 1 January 2004 through 4 December 2025

Language restriction: English-language publications

Study-design filters: None applied

Supplementary searching: Reference lists and forward citations were screened manually; ClinicalTrials.gov was reviewed

Database-specific strategies

a. **PubMed/MEDLINE (NLM)**

((nasopharyngeal carcinoma OR nasopharyngeal neoplasms OR NPC) AND (EBV OR Epstein-Barr virus OR EBV DNA OR EBV-DNA) AND (liquid biopsy OR plasma OR serum OR circulating) AND (on-treatment OR monitoring OR early response OR clearance OR kinetics OR during radiotherapy))

b. **Embase (Elsevier)**

('nasopharyngeal carcinoma'/exp OR 'nasopharynx tumor' OR npc) AND ('epstein barr virus'/exp OR 'ebv dna' OR 'ebv-dna') AND ('liquid biopsy'/exp OR 'circulating tumor dna'/exp OR plasma OR serum) AND ('treatment response'/exp OR 'on-treatment' OR 'early response' OR monitoring OR kinetics)

c. **Web of Science (Clarivate)**

TS=((nasopharyngeal carcinoma OR npc) AND (EBV OR Epstein-Barr virus OR EBV-DNA) AND (plasma OR serum OR circulating) AND (on-treatment OR monitoring OR kinetics OR clearance))

d. **Scopus (Elsevier)**

(TITLE-ABS-KEY ({nasopharyngeal carcinoma} OR npc) AND TITLE-ABS-KEY ({Epstein-Barr virus} OR {EBV-DNA}) AND TITLE-ABS-KEY (plasma OR serum) AND TITLE-ABS-KEY ({on-treatment} OR kinetics OR clearance))

— End of Supplementary Material S2 —

Supplementary Material S3

Detailed study characteristics and EBV-DNA assay reporting

Parent study: On-treatment and early post-radiotherapy EBV-DNA dynamics in locoregionally advanced NPC: a systematic review

S3.1. Cross-study assay summary.

Domain	Summary across included studies
Assay platform	20 qPCR/RT-qPCR reports; 1 ddPCR report
Reporting units	13 copies/mL only; 1 IU/mL only; 1 mixed IU/mL or copies/mL; 6 not clearly reported
Common target	BamHI-W was the dominant reported target; several publications referred to prior methods or did not report the target
Detectability/ cut-offs	0 copies/mL or >0 IU/mL in some studies; other study-specific thresholds included 316, 400, 600, 1,000, 2,000 and 4,000 copies/mL
Interpretation	Units, analytical limits and thresholds were not treated as interchangeable; no universal clinical cut-off was inferred

S3.2. Study design, population, treatment and sampling.

Study	Country/design	*N*	Population/stage	Treatment	Sampling timepoints	Timing category
Alfieri *et al* [30]	Italy (non-endemic); Retrospective single-centre observational cohort	167	EBER+ NPC; non-metastatic; AJCC VIII: Stage III–IVA 84%	Curative intent: IC + CCRT (52%), CCRT alone (43%), RT alone (1%), CCRT + adjuvant CT (4%)	T1 pre-treatment; T4 post-induction (if IC); T2a ≤6 weeks post-RT; T2b ≤14 weeks post-RT; T3 longitudinal follow-up	Dynamic (≥2 timepoints including on-treatment)
Huang *et al* [32]	China; endemic region; single cancer centre (Sun Yat-sen University Cancer Centre); Retrospective cohort	233	Newly diagnosed non-keratinising NPC, stage II–IVa (AJCC/UICC 8th ed.); majority stage III–IVa	On-treatment	Baseline and serial during treatment (between adjacent chemo cycles and/or during RT)	Mid-RT (weeks 4–5/mid-IMRT)
Chan *et al* [9]	Hong Kong (endemic region implied); single institution (Queen Mary Hospital); Prospective observational cohort	45 recruited; 44 analysed for half-life (one excluded from half-life analysis)	Non-metastatic, previously untreated NPC; restaged for analyses using AJCC/UICC 8th edition (treatment decisions per 7th edition); stage II 20.4%, III 47.7%, IVA 31.8% (in analysed cohort)	Radical IMRT; concurrent cisplatin-based chemoradiation ± induction or adjuvant chemotherapy (distribution reported)	Baseline immediately before treatment, then weekly during treatment until undetectable in two consecutive measurements	Mid-RT (weeks 4–5/mid-IMRT)
Huang *et al* [12]	China (Fuzhou, Fujian); Retrospective cohort (observational)	2,203 (from 2,613 M0 NPC screened)	Newly diagnosed, histologically confirmed non-metastatic NPC (M0), stage I–IVA (TNM-8)	IC followed by radical IMRT (69.3–72.0 Gy), with concurrent cisplatin in 69.8%; IMRT alone in 30.2%; adjuvant chemotherapy in 9.2%	T0 baseline (≤28 days pre-IC); T1 end-IC/around RT start (24 days pre-RT to 1 week after RT start); T2 end-RT (≤21 days post-RT)	Dynamic (Post-IC/Pre-RT (end-IC) + End-RT/Early post-RT)
Lan *et al* [2]	China (Sun Yat-sen University Cancer Center, Guangzhou); Retrospective cohort	775	Stage II–IVA (AJCC 8th); non-metastatic, curative-intent CCRT	IMRT + concurrent cisplatin 100 mg/m² q3w; ± concurrent cetuximab/nimotuzumab; radiation dose schema described	Pre-DNA: within 7 days before CCRT start. Mid-DNA: within 7 days before the 2nd cycle cisplatin during RT	Mid-RT (weeks 4–5/mid-IMRT)

Continued

S3.2. Study design, population, treatment and sampling. *Continued*

Study	Country/design	*N*	Population/stage	Treatment	Sampling timepoints	Timing category
Xu *et al* [27]	China (seven academic hospitals across regions); Multicentre, single-arm phase two clinical trial (PLATINUM; NCT03984357)	152	Untreated LANPC; nonkeratinising histology; stage III–IVA	Three cycles induction chemo-immunotherapy: gemcitabine + cisplatin + nivolumab → IMRT + three cycles nivolumab (no concurrent cisplatin) → adjuvant nivolumab (six cycles) [Non-standard curative regimen: induction chemo-immunotherapy + IMRT without concurrent cisplatin]	Serial blood samples 'pre-treatment" and during treatment; reported EBV DNA status after induction, after radiotherapy and after adjuvant phases	Post-IC / Pre-RT (end-IC)
Yang *et al* [29]	China; Observational cohort (NR if prospective/ retrospective not explicitly stated)	1,881	Non-metastatic NPC; stage III–IVa (AJCC 8th); adults (≥18)	IMRT with or without concurrent chemotherapy; excluded induction and adjuvant chemotherapy; mid-IMRT sampling occurred before cumulative cisplatin 200 mg/m²	Three timepoints: pre-treatment, middle of IMRT, post-treatment / end of IMRT	Mid-RT (weeks 4–5/mid-IMRT)
Zheng *et al* [31]	China (Xiamen); Retrospective observational cohort	172	Non-metastatic NPC; stages III–IVA (8th TNM); ECOG 0–1	Three cycles IC → IMRT + two cycles platinum-based CCRT	Blood collected before IC and after completion of IC (prior to radiotherapy)	Post-IC/Pre-RT (end-IC)

S3.2. Study design, population, treatment and sampling. *Continued*

Study	Country/design	*N*	Population/stage	Treatment	Sampling timepoints	Timing category
Le *et al* [33]	USA (Stanford) + Hong Kong (Queen Mary Hospital); Prospective observational cohort (assay comparison + prognostic biomarker)	58	Newly diagnosed NPC, no distant metastasis; Stage I–II: [8], Stage III–IV: 47 (majority LRA)	Definitive RT (median 70 Gy); RT alone 12; concurrent CT 46 (cisplatin-based); neoadjuvant PF 14; adjuvant CT 21	Blood: pre-treatment, halfway into RT, completion of RT, then every 3–6 months after completion	Mid-RT (weeks 4–5/mid-IMRT) + End-RT/Early post-RT
Zhang *et al* [34]	China (Sun Yat-sen University Cancer Center, Guangzhou); Retrospective comparative cohort; propensity score matching (1:1)	256 (Routine 150; New 106); PSM: 192 (96 pairs)	Histologically confirmed stage III–IVA NPC (AJCC/UICC 8th ed.); M0; age 18–70; baseline cfEBV-DNA >0	Induction TPF q3w (1–4 cycles) → IMRT + concurrent cisplatin q3w ×2. Routine: standard three IC cycles. New: IC cycles stage + post-IC cfEBV-DNA–guided (stop IC if cfEBV-DNA becomes undetectable)	≤2 weeks pre-IC; after each IC cycle; within/during 1 week of CCRT completion	Post-IC/Pre-RT (end-IC)
He *et al* [13]	China (Guangzhou; Sun Yat-sen University Cancer Center); Retrospective cohort	949	Newly diagnosed NPC; restaged AJCC 8th: I (9.6%), II (25.9%), III (47.4%), IVAB (17.1)	IMRT ± chemotherapy (stage-adapted): IMRT alone (I); IMRT + concurrent chemo (II); CCRT ± neoadjuvant/adjuvant chemo (III–IVAB). Neoadjuvant/adjuvant: cisplatin+5FU and/or taxane (2–3 cycles). Concurrent cisplatin weekly/triweekly. Some received adjuvant cetuximab/nimotuzumab.	Baseline (pre-treatment), mid-treatment (after ~4 weeks RT), end of treatment, 3 months post-treatment	Dynamic (≥2 timepoints including on-treatment)

S3.2. Study design, population, treatment and sampling. *Continued*

Study	Country/design	*N*	Population/stage	Treatment	Sampling timepoints	Timing category
Lertbutsayanukul *et al* [11]	Thailand; Secondary analysis of a prospective randomised trial cohort (prognostic biomarker analysis)	105	Non-metastatic NPC; stages II–IV (II 13.3%, III 51.4%, IVa–b 35.2%); included only patients with detectable pre-treatment plasma EBV-DNA and complete serial samples	IMRT 70 Gy/33–35 fx + concurrent weekly cisplatin 40 mg/m² (up to seven cycles) for >T1 or N+; adjuvant cisplatin 80 mg/m² + 5-FU 1,000 mg/m²/day continuous infusion (96 hours) q4w ×3	Pre-IMRT (baseline), week 5 of RT (mid-treatment) and 3 months post-completion	Mid-RT (week 4–5/mid-IMRT) + End-RT / Early post-RT
Lv *et al* [14]	China (Sun Yat-sen University Cancer Center, Guangzhou); Prospective observational cohort (EP-SEASON; per-protocol ctDNA assessments)	1,000	Newly diagnosed non-metastatic, EBV+ non-keratinising NPC; Stages II–IVA (majority stage III–IVA)	Sequential chemo-radiotherapy: no-NAC CCRT ± AC (24.8%) versus NAC→CCRT ± AC (75.2%); NAC regimens GP/TPF/TP/PF	11 timepoints: baseline (T0); post-NAC cycles (T1–T3); weekly during RT (T4–T9); within 1 week post-RTend (T10); 3 months post-RT (T11)	Dynamic (≥2 timepoints including on-treatment)
Lu *et al* [8]	China (single centre); Retrospective cohort	173	Locoregionally advanced NPC; non-metastatic; stage III–IVA (NR for exact staging system edition)	IC followed by CCRT; targeted therapy/ICIs used in a subset (NR for exact criteria)	Baseline and pre-each-chemotherapy-cycle (21–28-day intervals) during both IC and CCRT	Mid-RT (weeks 4–5/mid-IMRT)
Liu *et al* [35]	China (Guangxi); Retrospective cohort; model development + internal split validation (7:3)	581	LA-NPC stage III–Ivan (AJCC 8th), ECOG ≤2	Induction chemo (2–3 cycles; TP/PF/GP/TPF) + CCRT with IMRT + concurrent cisplatin	Before treatment; after completion of IC (post-IC); follow-up testing includes EBV DNA (routine)	Post-IC / Pre-RT (end-IC)

S3.2. Study design, population, treatment and sampling. *Continued*

Study	Country/design	*N*	Population/stage	Treatment	Sampling timepoints	Timing category
Prayongrat *et al* [15]	Thailand (Bangkok); Retrospective cohort (medical records)	204	NPC non-metastatic, stages I–IVB (AJCC 7th), curative IMRT; recurrences excluded	IMRT; definitive 70–74 Gy (PTV-HR) + 50–56 Gy (PTV-LR), 33–35 fractions; with/without CT; concurrent CT (most commonly cisplatin) + adjuvant platinum/5-FU CT in 93.1%	Pre-IMRT; mid-IMRT (S5); post-IMRT (3 months); then q6 months until relapse	Mid-RT (weeks 4–5/mid-IMRT) + End-RT/Early post-RT
Liu *et al* [3]	NR; Prospective observational cohort	185	Advanced-stage nonmetastatic NPC (stage III–IVb)	NACT → CCRT (curative intent)	Before & after NACT; also reported after CCRT	Post-IC / Pre-RT (end-IC)
Li *et al* [28]	Endemic area (NR); Retrospective cohort	2,942	Non-metastatic NPC stage I–IVa (AJCC 8th)	Radical IMRT-based curative treatment	Pre-treatment; pre-RT; post-RT EBV-DNA	Baseline + Post-IC/Pre-RT + End-RT/Early post-RT
Lin *et al* [36]	Taiwan; Observational cohort (serial plasma sampling)	99	Advanced NPC, stage III–IV (AJCC 1997)	10 weekly chemo → RT; dose 70 Gy (T1–3)/74 Gy (T4)	Pre-treatment (D-1); D35 & D64 during chemo; 1 week post-RT; then q6mo follow-up	Dynamic (≥2 timepoints including on-treatment)
Zhang *et al* [16]	China; Retrospective cohort	273	Non-metastatic NPC; staged UICC/AJCC 7th	Radical IMRT; stage I RT only, stage II–IVB CCRT ± neoadj/adjuvant	Pre-treatment; end of treatment (±1 week); 3 months post-treatment (±1 week)	End-RT/Early post-RT
Chen *et al* [37]	Taiwan; Prospective cohort (consecutive enrolment)	60	Newly diagnosed non-metastatic NPC (AJCC 7th; Stage I 5%, II 15%, III 40%, IVA 40%)	Definitive RT (≥64.8 Gy) + concurrent cisplatin-based chemo (cisplatin 50 mg/m² D1 + tegafur 800 mg/day + leucovorin 60 mg/day D1–14 q2w during RT); no induction chemo	Pre-treatment + during CCRT while receiving 35–40 Gy of RT	Mid-RT (week 4–5/mid-IMRT)

S3.3. Assay, EMR construct, outcomes and adjusted evidence.

Study	EBV-DNA assay	EMR definition/construct	Outcomes	Effect estimates	Covariates	Follow-up
Alfieri *et al* [30]	Plasma EBV-DNA by CE-IVD qPCR (single-copy gene assays); units IU/mL or copies/mL; undetectable versus detectable (including unquantifiable)	EBV-DNA classified as negative versus positive (quantifiable or unquantifiable); no validated quantitative cut-off; kinetics assessed descriptively (Detectable versus Undetectable; Kinetics (longitudinal descriptive))	Recurrence-free survival (RFS)	No Cox HRs reported; associations assessed via RFS comparisons, ROC curves, predictive values	Multivariable logistic regression including EBV-DNA at T1, T2, T4	60 months (range 9–134)
Huang *et al* [32]	Plasma EBV DNA measured 'as described previously'; unit copies/mL; baseline cut-off 2000 copies/mL; LOD/LOQ Not reported	'Elevation' defined as ≥5% increase versus prior value (to account for fluctuation/variation) between adjacent chemo cycles or during RT (this is a kinetic change definition, not clearance/undetectable) (Increase/decrease (% change))	1-year DFS; EBV DNA elevation rate	OR for EBV DNA elevation; HR for DFS (incl. multivariable)	Multivariable logistic regression for EBV DNA elevation; multivariable Cox for DFS (specific covariate list Not provided in extracted pages)	17.7 months (median)
Chan *et al* [9]	Plasma; real-time quantitative PCR targeting BamHI-W; ABI Prism 7000; results in EBV DNA copies/mL; 'undetectable' = 0 copies/mL; duplicate runs (discrepancy <2%); LOD/LOQ not explicitly reported in the extracted text	Kinetics modelled assuming exponential decay: log-transformed weekly ratios versus baseline; half-life = ln2/k. Cut-off for 'slow' versus 'fast' clearance derived by ROC to predict progression; half-life >15 days used as main discriminator (Kinetics (half-life/clearance slope))	Pre-specified: PFS, DMFS, OS	Yes (Cox). Multivariable: half-life >15 days prognostic for DMFS (HR 4.91) and OS (HR 5.24); for PFS, half-life >15 days and GTV_P+N prognostic	Age, sex, T, N, overall stage, GTV_P, GTV_N, GTV_P+N, half-life	30.3 months (range 6.0–74.2)
Huang *et al* [12]	Plasma EBV DNA by real-time qPCR targeting BamHI-W; copies/mL; quantifiable range 20–4,950,000 copies/mL	Binary D/U at each timepoint (T0/T1/T2) → trajectory types: I (U-U-U), II (D-U-U), III (D-D-U), IV (D-D-D), V 'resurgence' (D-U-D / U-D-U / U-D-D / U-U-D) (Trajectory (T0/T1/T2 patterns))	PFS, OS, LRFS, DMFS	Adjusted Cox (reference = Type IV): Type I HR 0.35 (PFS), 0.44 (OS); Type II HR 0.29 (PFS), 0.31 (OS); Type III HR 0.40 (PFS), 0.34 (OS); Type V HR 0.51 (PFS), 0.38 (OS). Also reported for LRFS/DMFS.	Age, sex, tumour category, node category, histological subtype, adjuvant chemotherapy	53.5 months (IQR 43.1–66.9)

Continued

S3.3. Assay, EMR construct, outcomes and adjusted evidence. *Continued*

Study	EBV-DNA assay	EMR definition/construct	Outcomes	Effect estimates	Covariates	Follow-up
Lan *et al* [2]	Real-time quantitative PCR for plasma EBV DNA; technical details referenced as previously described (not fully detailed in-text)	Pre-DNA high versus low using 4,000 copies/mL cut-off; Mid-DNA detectable (>0) versus undetectable (=0) (Quantitative threshold (cut-off); Detectable versus Undetectable)	Primary: PFS. Secondary: DMFS, LRRFS, OS; definitions provided	Multivariable Cox: Pre-DNA ≥4,000 versus <4,000 → PFS HR 2.242; DMFS HR 2.990; OS HR 4.118. Mid-DNA >0 versus =0 → PFS HR 1.652; LRRFS HR 1.965 (others as reported)	Cox model considered age, gender, T stage, N stage, cumulative cisplatin dose (CCD), pre-DNA, mid-DNA and combined pre+mid categories	44 months
Xu *et al* [27]	Plasma cfDNA (2 mL plasma) extraction; RT-qPCR targeting BamHI-W; results in copies/mL	'Early clearance' operationalised as undetectable EBV DNA after induction-phase treatment (versus detectable), evaluated for association with FFS (Detectable versus Undetectable)	Primary: Failure-free survival (FFS). Secondary: OS, locoregional recurrence-free survival, DMFS, safety/QoL	Early EBV-DNA clearance after induction associated with improved FFS: HR 0.40 (95% CI 0.17–0.97), $p = 0.032$	NR	43 months (median; IQR 41–45)
Yang *et al* [29]	qPCR assay targeting BamHI-W; plasma; results in copies/mL; EBV-DNA = 0 considered negative	Primary 'on-treatment' definition: plasma EBV-DNA >0 copies/mL in the middle of IMRT; risk-grouping reported as EBV-DNA =0/EBV-DNA decrease/EBV-DNA increase (operational definition of decrease/increase NR) (Increase/decrease (% change); Detectable versus Undetectable)	OS; PFS; LRFS; DMFS	Multivariable (PFS): HR 0.633 for key treatment factor (context NR in extracted text segment); "increase in plasma EBV-DNA level" HR 3.075 (95% CI 1.991–4.75; $p < 0.01$)	Cox model included sex, age, tumour stage, radiotherapy technique, chemotherapy dose (as reported)	68 months (IQR 53–81)
Zheng *et al* [31]	Plasma EBV-DNA extracted and quantified by ddPCR; results reported as IU/mL; post-IC EBV-DNA classified as detectable if >0 IU/mL	Post-IC EBV-DNA detectable (>0 IU/mL) versus undetectable (0 IU/mL) (Detectable versus Undetectable)	LRFS; DMFS; DFS; OS	Multivariable Cox (Detectable versus undetectable post-IC EBV-DNA): LRFS HR 4.274 (1.131–16.148), $p = 0.032$; DMFS HR 3.949 (1.394–11.191), $p = 0.010$; DFS HR 3.504 (1.499–8.192), $p = 0.004$; OS HR 3.455 (0.889–13.423), $p = 0.073$	Multivariable model variables reported: clinical stage, pre-treatment EBV-DNA (<430 versus ≥430 IU/mL), EBV-DNA after IC (detectable versus undetectable)	30 months (range 9–55)

S3.3. Assay, EMR construct, outcomes and adjusted evidence. *Continued*

Study	EBV-DNA assay	EMR definition/construct	Outcomes	Effect estimates	Covariates	Follow-up
Le *et al* [33]	Real-time qPCR Pol-1 (EBV Pol-1 single-copy) on ABI7700; comparison assays: BamHI-W and LMP2 (subset pre-treatment; LMP2 on LightCycler)	EBV-DNA detectable versus undetectable at the end of RT and post-treatment (≤3 months) (clearance = response); timepoint mid-RT explored (Detectable versus Undetectable)	Freedom-from-relapse (FFR), OS	Post-tx EBV-DNA detectable versus undetectable: 2-year FFR 28% versus 91% (p < 0.0001); 2-year OS 63% versus 94% (p < 0.005). Multivariate Cox (FFR): HR 21.7 (95%CI 4.5–105; p < 0.0001). Overall stage HR 5.3 (95%CI 1.3–21.8; p = 0.02)	WHO histology, N stage, overall stage, post-tx EBV-DNA	25 months (range 11–65)
Zhang *et al* [34]	Plasma cfEBV-DNA qPCR; unit copies/mL; LOD/LOQ NR; uses 4,000 copies/mL as a cut-off for 'pre-EBV-DNA'	Complete disappearance to 0 copies/mL after a given IC cycle (used as 'clearance' and to adapt IC cycle number) (Detectable versus Undetectable; Trajectory)	DFS (primary), OS; LRFS, DMFS	After PSM: HR (New versus Routine): LRFS 0.66 (95% CI 0.25–1.73; p = 0.39); DMFS 0.92 (0.41–2.04; p = 0.84); PFS 0.59 (0.19–1.81; p = 0.35). OS multivariable: Pre-EBV-DNA >4,000 HR 2.635 (0.981–7.080; p = 0.055); GAP >68 days HR 2.874 (1.233–6.697; p = 0.014). LRFS multivariable: Pre-EBV-DNA >4,000 HR 3.360 (0.957–11.791; p = 0.059).	PSM covariates: clinical stage, N stage, T stage, BMI, sex, age. Other Cox covariates NR (beyond those explicitly shown)	Entire cohort: median 54 months (range 1–125); Matched: median 49 months (range 1–118)
He *et al* [13]	Plasma EBV-DNA real-time qPCR targeting BamHI-W; TaqMan (Roche); unit copies/mL; undetectable defined as $<10^3$ copies/mL	Timepoint-specific ROC cut-offs for 'low versus high': pre-EBV 2,500; mid-EBV 871; end-EBV 721; 3-month EBV 211 copies/mL. Dynamic grouping also assessed (e.g., Group 2 = detectable end-EBV + detectable 3-month EBV). (Quantitative threshold (cut-off); Trajectory)	OS (primary), DMFS, PFS	Group 2 (detectable end-EBV + detectable 3-month EBV) versus Group 1: OS HR 4.71 (95% CI 2.159–10.27); DMFS HR 2.396 (1.073–5.351); PFS HR 2.71 (1.085–6.766). In detectable end-EBV subgroup: adjuvant therapy associated with improved OS HR 2.419 (1.297–4.51) and DMFS HR 2.45 (1.243–4.828) (comparison direction NR as reported).	NR	55.43 months (range 2.67–88.65)
Lertbutsayanukul *et al* [11]	Plasma EBV-DNA by RT-qPCR (QIAsymphony extraction; QIAGEN artus EBV kit on Rotor-Gene Q; target BamHI-W); LOD 316 copies/mL	Mid-EBV: undetectable (<316 copies/mL) versus detectable at week 5; Post-EBV: undetectable versus detectable at 3 months (Detectable versus Undetectable)	OS, PFS, DMFS (also LPFS, RPFS reported)	Multivariable: post-EBV detectable associated with worse outcomes, OS HR 6.881 (95% CI 1.209–39.155); PFS HR 3.303 (95% CI 1.025–10.655); DMFS HR 14.400 (95% CI 2.821–73.510). Mid-EBV HRs: NR (reported via survival curves/p-values)	Multivariable Cox considered factors including age, sex, stage, pre-/mid-/post-EBV, WHO subtype, IMRT technique (model inclusion rule reported as p < 0.25 in univariable)	45.3 months

S3.3. Assay, EMR construct, outcomes and adjusted evidence. *Continued*

Study	EBV-DNA assay	EMR definition/construct	Outcomes	Effect estimates	Covariates	Follow-up
Lv *et al* [14]	Plasma cfEBV DNA by qPCR targeting BamHI-W (copies/mL); β-globin control	Early response defined by undetectable cfEBV DNA (0 copies/mL) at early on-treatment points (e.g., post-NAC1 T1 = 0; also refined to include post-RT2w T5 = 0) (Detectable versus Undetectable; Trajectory)	DFS, OS (also DMFS, LRFS referenced as survival endpoints)	HRs for cfEBV DNA positivity across timepoints reported (Tables/figures referenced); numeric HR/CI: NR in PDF text extract	Multivariable Cox included baseline cfEBV DNA load, TNM stage, treatment options (others NR)	34.0 months (IQR 25.0–43.0)
Lu *et al* [8]	qPCR targeting EBV BamHI-W region; plasma; threshold >400 copies/mL; DNA extracted from 500 µL plasma	Early EBV-DNA status conversion (ESC): undetectable cEBV-DNA before the 3rd chemotherapy cycle; Late EBV-DNA status conversion (LSC): conversion from the 3rd cycle to the end of CCRT; No EBV-DNA status conversion (NSC): persistently positive throughout treatment (Trajectory (EBV-DNA status conversion patterns))	OS; PFS (NR for DFS/DMFS/EFS)	NR (HR not reported for clearance groups); Kaplan–Meier/log-rank reported (OS $p = 0.026$; PFS $p = 0.016$)	Multivariable Cox included factors such as smoking, tumour response and targeted therapy exposure (full model: NR)	31 months (6–91)
Liu *et al* [35]	Real-time quantitative PCR (RT-qPCR) on plasma (target NR)	EBV DNA after IC ≤5,660 versus >5,660 copies/mL (risk strata); IC response CR/PR versus SD/PD (Quantitative threshold (cut-off))	OS (primary), DMFS, LRFS, PFS	Multivariable OS (training): EBV DNA after IC >5660 HR 2.188 (95% CI 1.290–3.710); SD/PD versus CR/PR HR 1.820 (95% CI 1.187–2.790)	Age, LDH, EBV DNA after IC, IC response (clinical stage added to nomogram)	53 months
Prayongrat *et al* [15]	QIAmp; RTQ-PCR (BamHI-W), LightCycler 2.0; undetectable threshold <600 copies/mL	Disappearance/clearance to 'undetectable' (<600 copies/mL) at mid-IMRT and/or post-IMRT; dynamic categories (Upre–Umid/post, Dpre–Umid/post, etc.) (Detectable versus Undetectable; Trajectory)	DMFS, PFS, OS	EBV-DNA detectable post-IMRT versus undetectable (MVA): DMFS HR 14.331 (5.124–40.078); PFS HR 12.167 (5.133–28.840); OS HR 16.956 (6.511–44.157)	Model; Cox: age, T stage, EBV-DNA (pre-, mid-, post-IMRT); variables with $p \leq 0.25$ in univariable analysis	35.1 months

S3.3. Assay, EMR construct, outcomes and adjusted evidence. *Continued*

Study	EBV-DNA assay	EMR definition/construct	Outcomes	Effect estimates	Covariates	Follow-up
Liu *et al* [3]	Real-time quantitative PCR	Post-NACT EBV-DNA undetectable versus detectable (cut-off 0 copies/mL) (Detectable versus Undetectable)	PFS, DMFS, LRFS	MV: Post-NACT EBV-DNA→ PFS HR 2.31 (95%CI 1.18–4.54); DMFS HR 2.99 (1.25–7.15)	MV model reported incl. tumour response to NACT, T stage, N stage, age, gender (EBV-DNA included in primary MV for PFS/DMFS)	NR
Li *et al* [28]	qPCR targeting BamHI-W (60-bp), copies/mL	EBV-DNA negative = 0 copies/mL at all three timepoints; positive otherwise (Detectable versus Undetectable)	OS; LRFFS	EBER-/EBV-DNA- versus EBER+/EBV-DNA+: 5 years OS 90.8% versus 94.0% (p = 0.002); LRFFS 88.2% versus 94.3% (p < 0.001); adjusted HR LR recurrence 4.368 (2.617–7.291)	NR	NR
Lin *et al* [36]	RT-qPCR targeting BamHI-W; QIAamp extraction; ABI Prism 7,700	Detectable versus undetectable EBV-DNA after completion of treatment (1 week post-RT); baseline cutpoint reported (≥1,500 copies/mL) (Detectable versus Undetectable)	Relapse-free survival; Overall survival	Detectable EBV-DNA after treatment: HR 22.9 (3.0–173.5) relapse; HR 34.5 (7.4–162.1) OS	Tumour response; T stage; N stage; overall stage; pre-treatment EBV-DNA	NR
Zhang *et al* [16]	QIAamp extraction; RT-qPCR targeting BamHI-W region	Post-treatment change pattern: Group 1–4 by detectability at end-DNA and 3-month-DNA (Trajectory (post-treatment detectability patterns))	DFS; DMFS; OS (3-year rates reported)	Cox (versus Group 1): Group 3 HR DFS 4.69 (1.13–19.47), DMFS 4.42 (1.06–18.48), OS 2.91 (0.64–13.20); Group 4 HR DFS 4.20 (0.96–18.33), DMFS 4.20 (0.96–18.33), OS 2.85 (0.62–13.09)	NR	NR
Chen *et al* [37]	Plasma EBV DNA extraction/quantification per previously described method; copies/mL (qPCR details NR)	Interim EBV-DNA dichotomised: detectable versus undetectable (undetectable became dominant; thus binary) (Detectable versus Undetectable)	OS; RFS; response at 3 months	Prognostic score (age ≥55 + detectable interim EBV-DNA + TLG reduction ratio ≤0.6): score 2/3 versus 0 HR 18.973 (p = 0.001); score 1 versus 0 HR 4.756 (p = 0.074); 3 years OS 55.6% (score 2/3) versus 77.8% (score 1) versus 96.2% (score 0). (HR/CI for interim EBV-DNA alone: NR)	Multivariable risk factors used in score: age ≥55, detectable interim EBV-DNA, TLG reduction ratio ≤0.6	4.32 years (range 5–77 months)

Note: Details reproduce the report-level extraction used in the review. NR indicates not reported. Assay thresholds and reporting units are study-specific and should not be interpreted as analytically equivalent. The Prayongrat *et al* [15] and Lertbutsayanukul *et al* [11] reports provide complementary analyses from one underlying Thai cohort and are counted once at the study level. Potential partial overlap among large institutional series could not be quantified; nominal participant counts should therefore not be summed as a unique-patient total