Epstein-Barr Virus as a Paradigm in Nasopharyngeal Cancer: From Lab to Clinic

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OVERVIEW

Nasopharyngeal carcinoma (NPC) of the undifferentiated subtype remains endemic in southern China, with a peak incidence in this region approaching 30 cases per 100,000 population per year. Despite advances in chemotherapy and radiation delivery techniques in localized disease, distant metastasis is still common and NPC remains the seventh leading cause of cancer death in the region. There is great need for early diagnosis, developing novel therapies, and identifying patients with localized disease at higher risk of future recurrence or metastasis to appropriately tailor their treatment and improve outcomes. Knowledge of the integral involvement of Epstein-Barr virus (EBV) in the pathogenesis of undifferentiated NPC has been of seminal importance in developing strategies to optimize disease management. The close association with EBV is being evaluated in multiple settings including screening of at-risk populations, disease prognostication, development of targeted therapies, optimizing adjuvant treatment, and early recurrence detection. These translational studies are likely to have an enormous effect on management of undifferentiated NPC and significantly improve the landscape of the disease in years to come.

Epstein-Barr virus (EBV), a linear double-stranded DNA virus, infects over 90% of the world’s population before adolescence. During acute infection B lymphocytes are targeted and lytic phase replication occurs, resulting in the release of more infectious virions. A small number of B cells retain the infection in latent phase with minimal antigen expression. This latent episomal virus has oncogenic potential and many cancers have been associated with EBV latency. Undifferentiated nasopharyngeal cancer (NPC) is the most consistently associated cancer, with latent EBV occurring in more than 95% of cases irrespective of geographic or ethnic origin of the disease. The earliest association between EBV infection and NPC was demonstrated in 1966 by Old et al. Increased titers of immunoglobulin (Ig) G against viral capsid antigen (VCA) and diffuse-early antigen (D-EA) as well as aberrant immune response with IgA antibodies were later identified in patients with NPC. Latent EBV is present in high-grade dysplasia and NPC cells, but not in normal epithelium or low-grade dysplasia. Monoclonality of the viral genome in NPC has been shown by Southern blot hybridization, suggesting that EBV latency in these cells occurred before clonal expansion. EBV DNA is also consistently shed from tumor tissue into the plasma and is a valuable biomarker. Knowledge of this integral role of EBV in NPC carcinogenesis is being translated into clinical practice with the goal of improving disease outcomes.

EPSTEIN-BARR VIRUS IN DIAGNOSIS AND MANAGEMENT OF NASOPHARYNGEAL CANCER

Outcome in NPC is highly correlated with disease stage. In early localized disease, radiotherapy alone results in 80% to 90% 5-year survival. However, more than 60% of patients present with locally advanced disease; in this group 5-year survival rates drop to approximately 60% to 70%. Early diagnosis may improve outcomes, and serologic and EBV DNA-based screening modalities have been studied for screening at-risk populations. High titers of IgA VCA, IgA D-EA, and EBV DNAse with increasing titers during follow-up correlate with subsequent diagnosis of NPC. However, a false-positive rate of 2% to 18% has been noted with serologic testing alone. Plasma EBV DNA is a very sensitive marker for NPC. EBV DNA is shed consistently from NPC tumor cells and can be routinely detected in the plasma of patients with NPC with more than 95% sensitivity. A recent large population-based study of 1,318 volunteers in Hong Kong evaluated plasma EBV DNA as a screening modality for NPC. Sixty-nine people (5.2%) had a baseline positive test among whom three early-stage NPC cases were identified by nasal endoscopy and magnetic resonance imaging (MRI). Only one of these patients had positive IgA VCA serology. Of the 69 people with an initial positive test, only 17 had persistent positive plasma EBV DNA at 18 months, and no new patients with NPC were identified among these people.
ple in 2 years’ follow-up. In an expanded phase II project, the same group is studying about 20,000 healthy men between ages 40 and 60 screened by plasma EBV DNA (NCT02063399). Another serum-based polymerase chain reaction (PCR) test evaluating changes in the epigenome in seven candidate genes in NPC has also demonstrated some promise in identifying NPC cases. Cases identified through such screening of asymptomatic patients may uncover earlier-stage disease amenable to curative therapy. Optimal cost-effective methods, a suitable target population for screening, and the survival benefit of such screening must be evaluated.

NPC therapy is tailored to disease stage: stage I disease is typically managed with radiotherapy alone; stage II may be treated with radiation alone or concurrent chemotherapy; and stage III, IVA, and IVB is typically managed with concurrent chemoradiation. Phase III trials have shown an overall survival benefit for concurrent chemoradiation over radiotherapy alone in locally advanced disease. However, the role of adjuvant therapy for these patients remains debated. The U.S. Intergroup 0099 study evaluated concurrent high-dose cisplatin and radiation followed by adjuvant cisplatin and 5-fluorouracil (FU) for three cycles, which showed a survival benefit compared to radiotherapy alone. However, the relative contribution of concurrent compared with adjuvant chemotherapy remains unclear, and only approximately 50% of patients were able to complete the planned adjuvant treatment. In a recent randomized study from China, no significant benefit in failure-free or overall survival was seen for chemoradiation followed by adjuvant cisplatin/5-FU compared with chemoradiation alone. Distant metastasis remained the most common cause of treatment failure. Identifying a subgroup of patients at high risk for distant metastasis and an adjuvant chemotherapy regimen with better tolerability is a high research priority. Plasma EBV DNA is an excellent biomarker to identify patients at high risk of distant metastasis. During radiotherapy, plasma EBV DNA declines with a half-life of 3.8 days between the third and seventh weeks of treatment. Persistent positive plasma EBV DNA after completion of radiotherapy or concurrent chemoradiation is a strong predictor of residual disease and independently correlates with poorer disease-free and overall survival. The Hong Kong NPC group is conducting a randomized phase III study in patients with locally advanced disease and detectable plasma EBV DNA after 6 to 8 weeks of completing radiation or concurrent chemoradiation, comparing adjuvant chemotherapy with cisplatin/gemcitabine to observation alone (NCT00370890). Harmonization of the plasma EBV DNA PCR assay across four centers in the United States, China, Hong Kong, and Taiwan has facilitated an international collaborative study. In the forthcoming NRG oncology trial led by the RTOG (NRG-HN001), patients with persistent positive plasma EBV DNA post-chemoradiation will be randomly assigned to receive cisplatin/5FU or gemcitabine/paclitaxel in a randomized phase II fashion to evaluate 2-year progression-free survival as a primary endpoint. Patients with post-chemoradiation negative plasma EBV DNA will be randomly assigned in a phase III fashion to adjuvant cisplatin/5FU or observation alone to evaluate 2-year overall survival in a noninferiority design (Fig. 1). Results of these trials will be crucial in identifying an appropriate population for adjuvant treatment of locally advanced disease and the optimal chemotherapy regimen for these patients.

**KEY POINTS**

- Type II Epstein-Barr virus (EBV) latency is universally associated with the undifferentiated subtype (World Health Organization [WHO] type III) of nasopharyngeal carcinoma.
- Aberrant serologic responses to EBV are seen in patients with NPC as well as those at high risk for future disease development.
- EBV DNA detected in cell-free plasma in patients with NPC is an excellent marker of residual disease post-therapy and an independent predictor of disease-free and overall survival.
- Detectable plasma EBV DNA is being studied as a biomarker for screening and diagnosis in at-risk populations and determining adjuvant therapy options in locally advanced disease.
- EBV-based targeted therapies, including cytotoxic T lymphocytes, EBV-directed vaccines, and epigenetic treatments, are being investigated as stand-alone or combined modalities.

**EPSTEIN-BARR VIRUS-DIRECTED TARGETED THERAPIES FOR NASOPHARYNGEAL CANCER**

The use of targeted therapies against EBV as stand-alone or adjunctive treatments is being explored. Undifferentiated NPC is characterized by type II EBV latency where latent genes EBER, EBNA1, LMP1, LMP2A, LMP2B, and BARTs are expressed. EBNA1 protein is essential for stabilization of the viral episome and proliferation of the virus using the host DNA machinery. Latent membrane protein 1 (LMP1) is the major oncogene of EBV. It bears homology to the TNF receptor family (TNFR) and activates multiple downstream signaling pathways including MAPK, JNK, PI3K/AKT, and NF-kB, resulting in inhibition of apoptosis, promotion of cellular proliferation, and possibly facilitation of metastasis. LMP2A and LMP2B are more consistently expressed in NPC cells, however, their role in NPC oncogenesis is less clear. LMP2A is able to activate Akt and PI3K pathways, resulting in cell division. Both LMP2A and LMP2B are involved in modulating cell adhesion and motility, potentially contributing to metastatic spread. BART miRNAs may facilitate immune evasion and inhibit apoptosis of tumor cells.

The host immune response against these EBV latency antigens is suboptimal. A higher number of circulating regulatory T cells (Tregs) has been demonstrated in patients with NPC. These Tregs have been shown to suppress the CD8+ T-cell response to these already weakly immunogenic anti-
Although tumor-infiltrating lymphocytes appear to lack EBV-specific cytotoxicity, anti-EBV responses can still be generated from circulating CD8+ lymphocytes. The ability to stimulate anti-EBV responses in circulating CD8+ T cells has been used as a basis for adoptive immunotherapy and vaccine development.

### Adoptive T-Cell Therapy

Autologous cytotoxic T lymphocytes (CTL) expanded and developed against LMP2 antigens have shown some durable clinical responses with minimal toxicity. A phase II study of gemcitabine/carboplatin-based chemotherapy followed by LMP2-specific CTL infusions revealed promising results. The NATELLA study is a phase I trial looking at LMP1- and LMP2-specific CTLs in relapsed or refractory disease. An adenovirus vector–based polysaccharide vaccine combining the antigenic epitopes of EBNA1, LMP1, and LMP2 has shown high potency in rapid expansion of autologous T cells and further clinical studies are awaited. Autologous cytokine-induced natural killer (NK) cells have also shown efficacy in treatment of metastatic disease in combination with chemotherapy.

### Epstein-Barr Virus-Directed Vaccines

Vaccines have been developed against the latent EBV antigens. EBNA1, LMP1, and LMP2 antigens are not dominant targets of T-cell response, however, certain epitopes can be presented to the CD4+ or CD8+ T lymphocytes. EBNA1 predominantly induces a CD4+ T cell response; LMP2, a weak CD8+ response. Both antigens have been combined in the modified vaccinia virus vaccine MVA-EL which expresses the CD4 epitope-rich C-terminal domain of EBNA1 fused to full-length LMP2 with CD8 epitopes. The combination facilitates CD4+ mediated CD8+ immune responses, which are more robust. The vaccine was well tolerated in a phase I study in EBV-positive patients with NPC in remission after primary therapy. T-cell responses to one or both vaccine antigens were increased in 15 of 18 patients. The range of these responses suggested a direct relationship with vaccine dose, with all six patients at the highest dose level having strong EBNA1/LMP2 responses. This vaccine may be better targeted for patients with persistent positive EBV DNA in plasma following first-line therapy. An ongoing phase II trial is addressing this question (NCT01094405).

In a phase II study of an autologous dendritic cell vaccine developed by adenoviral transduction of LMP2 and truncated LMP1 antigenic domains, in vitro expansion of immunoreactive T cells was seen, but similar findings were not seen in vivo and efficacy was limited. In another trial of LMP2A-directed dendritic cell vaccination in localized NPC post-radiotherapy, patients who developed a delayed-type hypersensitivity response to the vaccine were able to mount a T-cell immune response and demonstrate a significant decline in post-radiotherapy plasma EBV DNA levels (p = 0.0310).40

### Genetic and Epigenetic Therapies

Gene therapy targeted to EBV-positive NPC cells has been explored using transgenes whose transcription will be regulated by the origin of replication of EBV (ori-P). Both adenoviral and nonviral vectors have been developed in this fashion and different genes, including pro-apoptotic p53 or...
**CONCLUSION**

We are in an exciting era in the history of NPC. Basic scientific knowledge of EBV and NPC carcinogenesis is being rapidly translated into clinical practice. The development of EBV-based screening tools, EBV-directed therapies, and biomarker-driven adjuvant treatment are on the horizon. Results of later-phase clinical trials in these areas are eagerly awaited. This knowledge will no doubt be practice-changing and will guide further research in other oncogenic virus–related malignancies, including human papillomavirus–related head and neck cancers.

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**Disclosures of Potential Conflicts of Interest**

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**References**


