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EBV Related Lymphomas: New Approaches to Treatment

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Epstein-Barr virus; Hodgkin lymphoma; Burkitt lymphoma; non-Hodgkin lymphoma; posttransplantation lymphoproliferative disorder; HIV-associated lymphomas; immunodeficiency; adoptive T-cell therapy; EBV-specific cytotoxic T-cells; rituximab; viral latency; EBV-DNA

Introduction

Over 90% of adults worldwide harbor lifelong latent EBV infection in a small fraction of their B-lymphocytes. While persistent latent EBV infection is common, the chance of any individual developing an EBV-related lymphoma is low and is modulated by ethnic, geographic, genetic, immunologic, and infectious cofactors. For example, in areas of holoendemic malaria, primary EBV infection is followed by a period of a few years where there is a propensity to develop EBV-positive Burkitt lymphoma (BL). The specific interplay of viral and malarial infection that leads to lymphoma remains poorly understood. In Europe, primary EBV infection that manifests as infectious mononucleosis is followed within 6 months to 10 years by increased risk of developing EBV-positive Hodgkin lymphoma (HL). Although both are B-lineage lymphomas, HL and BL are distinct, including differences in EBV latent gene expression. Whereas BL tumors typically express only a single viral protein Epstein-Barr nuclear antigen-1 (EBNA-1), HL tumors express EBNA-1 as well as viral latency membrane proteins (LMP)-1 and -2. These differences likely reflect differences in host factors and co-infection.[1]

EBV-related lymphomas are a heterogeneous group of hematologic malignancies but share the feature of harboring *latent* EBV within tumor cells. Certain lymphomas, such as endemic BL or HIV-associated primary central nervous system lymphoma, are EBV-positive in virtually 100% of cases. In HL, around 30% of cases in North America are EBV-positive. Diffuse large B-cell lymphoma (DLBCL) is rarely EBV-related, although cofactors such as chronic inflammation and perhaps immunosenescence of aging are associated with EBV-positivity. Immune dysfunction increases the likelihood of developing an EBV-related lymphoma, with high-risk groups including patients with HIV, congenital immunodeficiencies, post-transplant immunosuppression, or chronic active EBV. PTLD, particularly very early post-transplant, is usually EBV-positive.

There are different patterns of viral gene expression in different lymphomas, with associated ramifications for treatment. Only one or a few proteins may be expressed in a particular lymphoma type. The restricted protein expression allows the viral DNA to persist in cells, evading immune recognition. A corollary of this is that tumors with the least restricted viral

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gene expression patterns don't occur in patients with functional immune systems. EBV-related lymphomas that arise in the setting of immunosuppression may respond favorably to therapies that ameliorate anti-viral immune function, such as reduction of immunosuppression or adoptive immunotherapy. By contrast, EBV-related tumors in immunocompetent individuals typically have more restricted viral gene expression and thus less immunogenicity, rendering the aforementioned therapies less effective. Some viral proteins activate proliferative pathways while others protect infected cells from apoptosis. Thus, it seems likely that viral gene expression modulates the effects of cytotoxic chemotherapy. Furthermore, antiviral therapies such as ganciclovir may be limited insofar as the agent requires phosphorylation for antiviral cytotoxic activity. The viral kinases required for ganciclovir phosphorylation are not typically expressed in tumors. Thus, these antiviral agents are not effective in the treatment of EBV-related lymphomas except perhaps in association with strategies to induce the viral kinases.

Nonetheless, the presence of virus in EBV-related lymphomas may be exploited for diagnostic and therapeutic purposes. Monitoring of EBV-DNA in peripheral blood is emerging as a marker of EBV-related lymphoma that may serve as a prognostic marker that could aid in risk-stratification, or guide response-adapted therapy decisions. Adoptive cellular immunotherapy targeting viral antigens is effective treatment in some settings. The development of strategies for altering viral gene expression patterns to facilitate targeting of virus-infected tumors continues.

TREATMENT

- At present time, the EBV status of a lymphoma guides investigational therapies and the treatment of PTLD, particularly in the setting of allogeneic hematopoietic stem cell transplant (HSCT) where adoptive immunotherapy (either donor lymphocyte infusion or infusion of EBV-specific lymphocytes) has been shown to be effective.

Diet and lifestyle

- There are no specific dietary or lifestyle risk factors for EBV-related lymphomas or lymphoproliferative disorders. However, there are areas of the world where certain EBV-related lymphomas have a much higher prevalence, such as EBV-positive BL in areas of Africa with holoendemic malaria, EBV-positive NK/T-cell lymphomas in parts of Asia, and EBV-positive HL in economically developing countries and among those of Hispanic ethnicity. The age of primary infection is a function of culture and diet but whether age of primary infection influences the overall incidence of EBV-related malignancies is not clear.

EBV-DNA Monitoring

- There is growing interest in quantifying EBV-DNA in the peripheral blood as a surrogate marker for EBV-positive malignancies.[2–6]
- While EBV-DNA is readily detected in the peripheral blood mononuclear cells (PBMCs) of even healthy, EBV-seropositive individuals, reflecting the small population of circulating latently infected B-lymphocytes, cell-free EBV-DNA (in plasma or serum) is not typically present in healthy individuals. Cell-free EBV-DNA can be derived from many sources, including dying EBV-infected tumor cells, dying latently infected benign cells, or virions. Particularly in immunocompromised hosts, cell-free EBV-DNA may be detectable for reasons apart from an underlying EBV-positive malignancy.

- Some institutions monitor EBV-DNA levels in the blood of patients following allogeneic HSCT or solid organ transplantation (SOT).[7] However, the detection of EBV-DNA in blood specimens is not sufficient to diagnose EBV-PTLD.
- In extranodal NK/T-cell lymphoma, EBV-DNA can be detected prior to therapy in the majority of patients. In one study, the treatment response rate was lower for patients with high levels of EBV-DNA in plasma prior to therapy; grade 4 treatment toxicities were also higher for these patients.[8] In extranodal NK/T-cell lymphoma, high pre-treatment plasma EBV-DNA levels correlated with higher International Prognostic Index scores and inferior survival outcomes.[3] Detectable post-treatment plasma EBV-DNA corresponded to inferior survival compared to those with undetectable post-treatment plasma EBV-DNA.[3]
- In HL, there is a strong correlation between EBV-positive tumors and elevated cell-free EBV-DNA.[4, 5] One study showed that high EBVDNA in plasma of HL patients corresponds to higher stage disease and higher International Prognostic scores.[6] Several studies have demonstrated that cell-free EBV-DNA corresponds to active EBV-positive disease, where patients with EBV-positive HL typically have detectable EBV-DNA in their blood at diagnosis or in the setting of relapsed/ refractory disease while patients with EBV-negative HL tumors or EBV-positive tumors in remission will not have EBV-DNA in their blood.[4] In a trial of rituximab-ABVD for patients with advanced Hodgkin lymphoma, those with EBV-positive tumors experienced rapid declines in EBV-DNA viral load after one cycle of therapy, which corresponded to treatment responses in all cases.[9]

Pharmacologic treatment

- In most instances, the approach to EBV-positive lymphomas does not differ from EBV-negative lymphomas of the same histology. The exceptions are in the context of investigational protocols or where an adoptive immunotherapy approach is available.
- When EBV-positive lymphomas arise in the setting of immunosuppression, ameliorating the immune defect can assist in the treatment of these lymphomas. In HIV-associated lymphomas, antiretroviral therapy is generally appropriate although potential drug interactions and the effects of chemotherapy on the ability to maintain HAART therapy in terms of nausea, vomiting and mucositis must be considered with regard to the timing of antiretroviral therapy. However, for EBV-related lymphomas in HIV patients, initiation of antiretroviral therapy alone is inadequate for treatment. This is in contrast to AIDS-associated Kaposi sarcoma where initiation of antiretroviral therapy is often a standard approach in patients who are asymptomatic or minimally symptomatic and are antiretroviral naïve. In EBV-PTLD, select cases may benefit from reduction of immunosuppression as the sole intervention or as part of the treatment plan.

Rituximab

For the treatment of EBV-PTLD, rituximab as a single agent has a reported efficacy of 63% on a systematic review and is considered first-line treatment by many transplant centers.[10] The benefits of rituximab for EBVPTLD are that many patients can be spared the toxicities of combination chemotherapy and that their immunosuppression can be often be continued, leading to lower risk of graft rejection and/or GVHD. Rituximab may be effective in EBV-PTLD not only because it targets the CD20(+) tumor, but also because, through B-cell depletion, it may shift the ratio of EBV-infected B-lymphocytes to EBV-specific cytotoxic T-cells in favor of an antiviral/ anti-tumor immune response. The benefit (or detriment) of

harboring EBV within other lymphomas, as it relates to rituximab efficacy, has not been studied. There is some evidence that LMP-1, via activation of downstream signaling pathways, may alter the sensitivity of EBV-positive lymphomas to rituximab. One recent study showed that rituximab-sensitive EBV-positive lymphoma cells were rendered resistant to rituximab treatment after transfection with LMP-1.[11] Further, LMP-1 transfection induced Akt phosphorylation and Akt inhibition restored rituximab sensitivity of these cells. [11]

Standard dosage: Variable depending on regimen

Contraindications: None, precautions if history of infusion reaction

Main drug interactions: None, although concurrent use with live vaccines may increase risk of infection by the live vaccine.

Main side effects: Infusion reactions, generally mild, can occur. Hypogammaglobulinemia or lateonset neutropenia are recognized to occur in small numbers of patients.

Special points: Extranodal lymphomatous involvement, including the gastrointestinal tract, is often a feature of EBV-associated lymphomas and gastrointestinal perforation has been reported with rituximab.

Cost/cost-effectiveness: Average wholesale price of rituximab is $582.19 per 10 mL (100 mg) vials. At the average dose of 800 mg, the cost of one infusion would be $4,657.52.

Reduction or Modification of Immunosuppression

Reduction of pharmacologic immunosuppression (RI) can be an effective approach to the treatment of EBV-PTLD in some cases, although this strategy must be balanced against the risk of transplant rejection (or GVHD in HSCT). Furthermore, the rapidity with which an EBV-specific immune response will regenerate following RI should be considered. In settings where T-cells have been removed either by T-cell depletion of a stem cell graft as in HSCT or following treatment of the patient with antibodies that specifically target T-cells such as OKT3, antithymocyte globulin, or alemtuzumab, immune reconstitution may be delayed for weeks even after the immunosuppressive agents have been discontinued. In some settings, particularly SOT, where immunosuppression is a result of calcineurin inhibitors and antimetabolites, recovery of anti-viral immune function following RI may occur more rapidly.

As an alternative to RI, modification of the immunosuppression regimen to include agents that have potential anti-tumor and anti-viral properties is an attractive option, allowing treatment of the EBV-PTLD while maintaining the level of immunosuppression necessary to prevent graft rejection and GVHD. Immunosuppressive agents such as mammalian target of rapamycin (mTOR) inhibitors have been explored in PTLD given their antitumor properties in other settings. The mTOR signaling pathway is important in B-cell proliferation and early EBV-PTLD lesions have been shown to have activation of the mTOR pathway.[12, 13] Remissions of EBV-PTLD have been reported in SOT patients upon modification of their immunosuppressant regimen to include an mTOR inhibitor instead of a calcineurin inhibitor. [14, 15] *In vitro*, rapamycin has been shown to inhibit the proliferation of EBV-positive lymphoma cell lines, but not EBV-negative lymphoma cell lines, suggesting possible anti-viral activity as well.[16]

Surgery and Interventional Procedures

- In the setting of PTLD involving a transplanted kidney or other localized disease, surgery to remove lymphoma is sometimes undertaken, particularly if RI has failed.
- Excisional biopsy is valuable in the diagnosis of lymphoma and for performing appropriate virally-related tissue evaluations, including EBV determination by *in situ* hybridization for highly-expressed EBV RNAs (EBER) or immunohistochemistry for LMP-1.

Other treatments

- Adoptive cellular immunotherapy

Adoptive Cellular Immunotherapy

Adoptive T-cell therapy with EBV-specific cytotoxic T-cells (EBV-CTLs) has been used for the treatment or prevention of EBV-PTLD since 1995 and has proven to be safe and effective, even in patients with refractory or relapsed disease.[17, 18] Clinical outcomes of patients with EBV-PTLD treated with EBV-CTLs were recently reviewed, with responses seen in the majority of patients.[19] Among 101 HSCT patients who were deemed highrisk for EBV-PTLD, none developed EBV-PTLD after prophylactic treatment with EBV-CTLs. [17] This same group reported that 11 of 13 patients treated for established EBV-PTLD achieved durable complete remissions. In one series, over two-thirds of HSCT patients with EBV-PTLD had durables remissions following infusion of EBV-CTLs with no cases of GVHD (compared to 17% in patients receiving HLA-matched donor lymphocyte infusion for EBV-PTLD).[20]

EBV-CTLs can be expanded *ex vivo* using lymphoblastoid cell lines (LCLs), a process that requires repetitive antigen stimulation and typically takes 4–12 weeks. However, EBV-PTLD is often aggressive, necessitating urgent treatment. A group in Edinburgh has made efforts to make EBV-CTLs an “off the shelf” product by creating a bank of cryopreserved EBV-CTLs expanded from healthy blood donors.[21] The group has created a bank of EBV-CTLs from donors with various HLA types, making products available for quick matching if a patient develops EBVPTLD. An overall response rate of 48% was observed with these banked allogeneic EBV-CTLs when given to SOT patients with EBV-PTLD. The survival of third-party EBV-CTLs is hindered by allogeneic responses and their efficacy may be limited by ongoing immunosuppressive therapy. Nonetheless, the approach has promise and follow-up investigations are ongoing. Others have reported that overnight stimulation is adequate to generate EBNA-1-specific CTLs from peripheral blood mononuclear cells using EBNA-1-specific interferon gamma secretion as a selection marker.[22] Adoptive therapy with the resultant cellular products led to *in vivo* expansion of EBVCTLs in 80% of patients and clinical responses in 70% of patients.[22] Similar approaches to the generation of cytomegalovirus (CMV)-specific and adenovirus-specific CTLs are ongoing and there has been exploration of multivirus- specific (EBV, CMV, adenovirus) CTLs in HSCT patients receiving T-cell depleted grafts.[23, 24]

Engineering specific CTLs has attracted considerable attention. In the HSCT setting, EBV-CTLs have been shown to persist *in vivo* long-term, demonstrated by detection of gene-marked cells many years after infusion.[17] In the SOT setting where patients are often maintained on immunosuppression for years or for life, EBV-CTLs have been engineered to be resistant to calcineurin inhibitors so as to enhance survival and efficacy.[25, 26] Conversely, concerns that CTLs might induce GVHD have led to the development of cellular products that are engineered with suicide genes, so that in the event that these cells lead to GVHD, the cells may be turned off pharmacologically.[27, 28]

Successes in EBV-PTLD spurred interest in the use of EBV-CTLs for patients with other EBV-related diseases, including EBV-positive HL and EBV-positive undifferentiated nasopharyngeal carcinoma (NPC).[29] One of the barriers to treatment outside the HSCT setting is achieving *in vivo* expansion of infused cells. A variety of approaches have been explored. Among them, short-term lymphodepletion with anti-CD45 monoclonal antibodies to facilitate *in vivo* expansion of EBV-specific CTLs in NPC patients has been followed by adoptive immunotherapy.[30] When the approach was applied, increases in EBV-CTLs were seen, with some patients achieving clinical remission. *In vivo* persistence of EBV-CTLs correlated with tumor regression and improved clinical outcomes.[30] In pre-clinical experiments, IL-15 has been shown to support *in vivo* proliferation of EBVCTLs that were initially expanded *ex vivo*, even in the presence of functional regulatory T-cells.[31] Other investigations involve engineering CTLs to be resistant to tumor-derived immunomodulatory cytokines such as TGF-β that are believed to be important components of the tumor milieu in HL.[32] The viral proteins LMP-1 and LMP-2 appear to drive the transcriptional activation of galectin-1 (Gal1) in HL tumors.[33] It has been suggested that Gal1 expression skews the immune response away from a cytotoxic T-cell response and towards a tolerogenic, regulatory T-cell response, potentially facilitating the tumor immune escape in HL.[34] EBV-CTLs are sensitive to Gal1-mediated apoptosis and Gal1 neutralizing antibodies have been shown to protect EBV-CTLs from Gal1 cytotoxicity.[33] Gal1 neutralizing antibodies may have a future role in the immunotherapeutic approaches to EBV-positive lymphomas.

EBV-specific T-cells are also being investigated as novel therapies for non-EBV associated hematologic malignancies, such as chronic lymphocyte leukemia. In an ongoing clinical trial, anti-CD19 antibodies are joined to T-cells as form a chimeric receptor. In attempts to increase longevity of the chimeric receptor-T-cell, half will have a CD28 protein attached and half will be selectively expanded to recognize EBV. In a similar design, there is a Phase I clinical trial for patients with CD30-posiitve lymphomas where an anti-CD30 antibody is joined to EBV-CTLs.

Standard dosage: Use is investigational

Contraindications: Per clinical trial criteria, but often allogeneic HSCT recipients with GVHD of grade 2 or greater are considered ineligible

Main drug interactions: Some immunosuppressive therapies may hinder CTL expansion *in vivo*

Main side effects: Localized swelling at sites of EBV-PTLD with inflammation and necrosis. Some patients have had GVHD or recurrence of GVHD.

Special points: Limitations include production time, availability of donors outside of HSCT setting, concerns about GVHD, manufacturing cells limited to certain centers

Cost/cost-effectiveness: In 2009, the reported cost of manufacturing and infusing EBV-CTLs at an established facility was reported to be $6,095[17]

Emerging therapies

- Signaling pathway inhibitors
- Lytic inducers coupled with antiherpesvirus agents
- EBV vaccines

Signaling Pathway Inhibitors

The EBV protein LMP-2, which is present in some EBV-related lymphomas, mimics a functional B-cell receptor (BCR) and may activate downstream signaling pathways, including Syk, Akt, and mTOR. The Lyn/Syk pathway is downstream of the BCR and, when activated, functions to promote the survival of B-lymphocytes. In murine NHL tumors, Syk is required for tumor survival *in vitro* and, *in vivo*, tumors that do not express Syk have increased apoptosis.[35] Syk signaling leads to activation of Akt and XIAP, a caspase inhibitor, resulting in inhibition of apoptosis.[36] Inhibition of Syk leads to tumor regression in NHL cell lines and mouse models.[35, 37, 38] Syk inhibitors have clinical anti-tumor activity that has been demonstrated in patients with CLL and NHL.[39–41] In LMP-2A transgenic mice, B-cells can survive and proliferate despite lacking functional BCRs.[42] In LMP-2A/myc double transgenic mice, lymphoid tumors develop, suggesting that LMP-2A functions to allow cells with aberrant myc expression to escape apoptotic signals and survive.[43] Among the Src family kinases, Lyn kinase in particular associates with LMP-2A, leading to activation of B-cell survival signaling pathways that bypass signaling through the BCR.[44] Dasatinib has been shown to inhibit the proliferation of LMP-2A-expressing B-lymphocytes *in vitro*, as well as splenomegaly and lymphomagenesis in LMP-2A/myc double transgenic mice.[45] The mechanism of action of dasatinib appears to be related to the inhibition of LMP-2A-induced Lyn signaling.[45] In EBV-PTLD cell lines, inhibition of Syk led to reduced tumor proliferation and induced apoptosis.[36] As more small molecule inhibitors become available, evaluating these agents in EBV-related lymphomas may prove effective beyond the anti-tumor activity seen in EBV-negative lymphomas, due to the viral association.

The EBV protein LMP-1, a mimic of CD40, is recognized to activate the PI3K/Akt pathway through noncanonical Syk signaling.[46–48] LMP-1 has been shown to increase the localization of PI3K to lipid rafts, with cholesterol depletion resulting in inhibition of PI3K localization and decreased Akt activation.[49] In extranodal NK/T-cell lymphoma, tumor LMP-1 expression, determined by immunohistochemistry on tumor tissue, correlated with Akt phosphorylation, more localized disease, and better overall survival.[50] In EBV-positive NPC, the PI3K/Akt and mTOR pathways are often activated.[51–53] PI3K inhibitors, Akt inhibitors, and dual PI3K/mTOR inhibitors are all in development. Akt inhibitors, such as MK-2206, have been shown to inhibit growth of NPC cell lines.[54] MK-2206 is currently being investigated in Phase II clinical trials for patients with relapsed/refractory HL and NHL, as well as recurrent NPC. LMP-1, by signaling through the JAK/STAT pathway, may also function to increase programmed cell death ligand 1 (PD-L1) expression in EBV-positive tumors, including EBV-PTLD and EBV-positive HL.[55] EBV-transformed LCLs were shown to have high levels of JAK3-associated STAT proteins (pSTAT3 and pSTAT5) and enhanced LMP-1 increased STAT5 activity. JAK3 inhibition led to decreased PD-L1 expression in EBV-transformed LCLs. PD-L1 is a cosignaling molecule that inhibits the function of activated effector T-cells. PD-L1 thus acts to dampen T-cell mediated immune responses, including anti-tumor immunity. PD-1 blockade is a promising targeted therapy in hematologic malignancies and has already proven to be effective in the treatment of other solid tumor malignancies. PD-L1 blockade and reactivation of a dampened immune response may be especially relevant for EBV-related tumors.

Lytic Inducers Coupled With Antiherpesvirus Agents

To persist for the lifetime of the host, EBV maintains tight control over the switch between latent and lytic gene expression, with latency characterized by very restricted gene expression. Lytic proteins, such as viral thymidine kinase, are not expressed during latency.

As a result, antiherpesvirus drugs that rely on phosphorylation by viral thymidine kinase for conversion of the prodrug to its active form, are not effective during latent infection. Thus, there is interest in the pharmacologic lytic activation of EBV to render infected tumor cells susceptible to antiviral agents such as ganciclovir. Furthermore, lytic activation in itself may render the infected cells more susceptible to immune recognition and killing due to a less restricted expression of viral proteins.

Numerous therapies induce lytic viral activation to varying degrees, including chemotherapeutic agents, radiation, steroids, histone deacetylase inhibitors (HDACi), and others. Lytic activation may be part of the DNA damage response or unfolded protein response to endoplasmic reticulum stress. Bortezomib, a proteasome inhibitor, has been shown to activate EBV lytic gene expression in EBV-positive BL cell lines.[56] EBV-positive NPC cell lines treated with arsenic trioxide showed increased lytic gene expression and decreased viability.[57] When ganciclovir was added to arsenic-treated EBV-positive cells, viability was further reduced while EBV-negative cells showed no changes in viability after treatment with arsenic alone, ganciclovir alone, or the combination.[57] LMP-1-positive cells were also shown to have higher levels of promyelocytic leukemia nuclear bodies (PML-NB) protein expression and treatment with arsenic decreased PML-NB protein levels.[57] PML-NB degradation may have an important role in the switch from latent to lytic herpesvirus gene expression and LMP-1 may increase PML-NB expression to maintain latency.[57] EBNA-1 may also mediate the switch between latency and lytic activation through alterations in PML-NB.[58] Viral genes expressed at early stages of the lytic cycle have also been found to participate in PML-NB disruption.[59]

HDACis have been shown to induce lytic gene expression and sensitize EBV-positive lymphoma cell lines to ganciclovir at drug concentrations achievable in patients.[60–62] In EBV-positive NPC cell lines, gemcitabine and valproic acid had a synergistic effect on lytic activation.[62] The addition of ganciclovir increased cytotoxic activity compared to treatment with the lytic inducers alone, which was not observed in EBV-negative cell lines.[62] Notably, the production of viral capsid proteins and new virions were also inhibited.[62] In a patient with a refractory EBV-positive DLBCL, treatment with valproic acid and ganciclovir was associated with a rise in EBVDNA in the plasma that was found to be unencapsidated by DNAse assay, suggesting that the rise in EBV-DNA was tumor-derived.[63] In a clinical trial of 15 patients with refractory EBV-positive lymphomas, 10 responded to treatment with arginine butyrate, an HDACi and lytic inducer, in combination with ganciclovir.[64] However, it remains unclear if the response seen was due to the anti-tumor activity of the HDACi or truly related to lytic induction and ganciclovir sensitization of tumor cells. Epigenetic modifying agents such as 5-azacytidine also induce lytic gene expression in EBV-positive cell lines and, when combined with ganciclovir, lead to caspasemediated apoptosis.[65] Parthenolide, a plant extract, has been shown to induce lytic gene expression in EBV-positive BL cell lines and to have cytotoxic activity that synergizes with ganciclovir.[66]

The ataxia-telangiectasia-mutated (ATM) kinase plays a role in EBV lytic induction, with ATM kinase activity required for agents such as bortezomib, HDACis, and others to effectively induce lytic gene expression.[67] Treatment of EBV-positive cell lines with an ATM inhibitor decreased lytic gene expression, with similar findings in an ATM knockdown model.[67] Drugs which activate ATM appear to result in lytic induction.[67]

EBV Vaccines

A recombinant glycoprotein gp350 vaccine in healthy volunteers appears to reduce the incidence of symptomatic infectious mononucleosis but may not prevent primary infection.

[68, 69] Whether such vaccination would impact on the incidence of EBV-related malignancies is unknown. A peptide epitope-based vaccine aimed at stimulating T-cell mediated immunity against immunodominant latency antigens has been shown to induce EBV-specific T-cell responses in healthy volunteers and may have a role in the prevention of primary infection, or in the prevention or treatment of EBV-related malignancies.[70, 71] In a trial of pediatric patients awaiting SOT, the gp350 vaccine was given pre-transplant with the hopes of improving anti-viral T-cell immunity to prevent EBVPTLD.[72] However, the gp350 vaccine failed to elicit a durable immune response in the majority of trial participants.[72] Phase I EBV vaccine trials have been completed in patients with EBV-positive malignancies in remission using a modified vaccinia Ankara EBNA-1/LMP-1 vaccine.

Pediatric considerations

- Pediatric patients are more likely to be EBV-seronegative, which is a consideration in pediatric transplant patients, where the risk of EBVPTLD is heightened in these circumstances.

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•• Of major importance

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Opinion statement

In the treatment of Epstein-Barr virus (EBV)-related lymphomas, there are few therapies specifically targeted against the latent virus within these tumors; in most cases the treatment approach is not different than the approach to EBV-negative lymphomas. Nonetheless, current and emerging therapies focused on exploiting aspects of EBV biology may offer more targeted strategies for EBV-positive lymphomas in the future. Conceptually, EBV-specific approaches include bolstering the anti-viral/ anti-tumor immune response with vaccines or EBV-specific cytotoxic T-lymphocytes, activating lytic viral genes to render the tumor cells susceptible to antiviral therapies, and inhibiting the downstream pro-survival or anti-apoptotic pathways that may be activated by latent EBV proteins.

EBV-specific cytotoxic T-cell infusions have proven effective in EBV-related posttransplantation lymphoproliferative disorder (EBV-PTLD) and expanding such adoptive immunotherapies to other EBV-related malignancies is an area of active research. However, other EBV-related lymphomas typically have more restricted, less immunogenic arrays of viral antigens to therapeutically target with adoptive immunotherapy compared to EBV-PTLD. Furthermore, the malignant EBV-positive tumor cells of Hodgkin lymphoma are scattered amid a dense infiltrate of regulatory T-cells, macrophages, and other cells that may dampen the anti-tumor efficacy of adoptive immunotherapy. Strategies to overcome these obstacles are areas of ongoing pre-clinical and clinical investigations.

Some emerging approaches to EBV-related lymphomas include the coupling of agents that induce lytic viral replication with anti-herpesvirus agents, or the use of small molecule inhibitors that block signaling pathways that are constitutively activated by EBV. EBV vaccines seem most promising in the treatment or prevention of EBV-related malignancies, rather than the prevention of primary EBV infection. EBV vaccine trials in patients with residual or low-bulk EBV-related malignancies or for the prevention of EBV-PTLD in EBV-seronegative patients awaiting solid organ transplantation are ongoing.