Experimental Treatment of Epstein-Barr Virus-associated Primary Central Nervous System Lymphoma


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ABSTRACT

Primary central nervous system lymphoma (PCNSL) that arises in immune-deficient patients is an aggressive B-cell neoplasm that is universally associated with the EBV. Patients with EBV+ PCNSL face a particularly poor prognosis with median survival times of 2–12 months despite aggressive management with radiation therapy. We have developed a preclinical model of EBV+ PCNSL to explore strategies that specifically target EBV-infected B lymphoblasts in vivo. Stereotactic implantation of EBV-transformed human lymphoblastoid B-cell lines into the caudate nucleus of the nude rat resulted in lethal CNS tumor burden manifested by the onset of focal neurological symptoms within 21 days. Histological evaluation at autopsy revealed a multifocal, perivascular human EBV+ lymphoblastic B-cell infiltrate that displayed a latency type III EBV gene expression profile similar to PCNSL that develops in some immune-deficient patients. Radiation (1600 Gy) of lymphoblastoid B-cell lines resulted in up-regulation of the EBV thymidine kinase (EBV-TK) transcript and sensitization of these cells to drug-induced apoptosis using nucleoside analogs. Enhanced expression of EBV-TK mRNA in EBV+ PCNSL tumors by radiation therapy occurred in a dose-dependent fashion. In vivo trials using the nude rat PCNSL model demonstrated significantly improved mean survival time (MST) with single fraction whole-brain radiotherapy (WBRT) and antiviral therapy consisting of zidovudine (AZT) and ganciclovir (GCV; MST 41.3 ± 3.3 days; P = 0.05), compared with either antiviral therapy (MST 32.1 ± 1.1 days) or WBRT alone (MST 22.8 ± 0.8 days). We found constitutive and abundant EBV-TK mRNA expression in a stereotactic core biopsy specimen from a solid organ transplant patient with EBV+ PCNSL. Withdrawal of immunosuppression did not result in disease regression. This patient achieved a complete response after therapy with high-dose AZT and GCV in the absence of WBRT, and remains in remission on oral maintenance AZT/GCV therapy 3 years after diagnosis. These results suggest that antiviral therapies can be effectively explored in vivo using a preclinical animal model of human EBV+ PCNSL with subsequent translation to patients with EBV+ PCNSL.

INTRODUCTION

Individuals with acquired, iatrogenic, or congenital immunodeficiency are at increased risk for PCNSL, which commonly presents as a monoclonal, B-cell neoplasm classified as either diffuse large-cell or immunoblastic non-Hodgkin’s lymphoma associated with the EBV (1, 2). The reported incidence of PCNSL in 1999 was 2–11% of patients with AIDS, 1–7% of post-transplant patients with iatrogenic immune suppression, and 4% of those with congenital immune deficiencies (3). Patients with PCNSL face high mortality and a median survival time of 2–12 months after diagnosis (4–6). For PCNSL tumors associated with AIDS or iatrogenic immune suppression, standard treatment is chemotherapy and WBRT, which at best provides patients with a minimal improvement in survival (7, 8). WBRT-induced neurotoxicity, including symptoms of cognitive impairment and ataxia, occurs in a substantial fraction of PCNSL patients and is progressively debilitating (9).

PCNSL in immune-deficient patients is unique among intracranial neoplasms because of the strong association with EBV. These tumors demonstrate specific patterns of latent viral gene expression that likely influence tumor development and pathogenesis (10). The presence of viral gene expression in tumors may represent a unique opportunity to develop targeted therapeutic strategies. In the past 10 years, antiviral therapy with nucleoside analogs, mainly GCV, have targeted EBV-associated diseases with a pattern of EBV lytic gene expression (11). Nucleoside analogs require phosphorylation by the lytic viral gene product TK before being incorporated into viral and cellular DNA. During lytic infection, the EBV open reading frame BXLF1 encodes the viral thymidine kinase (TK) enzyme, that radiation can up-regulate EBV-TK expression that, in turn, can sensitize EBV+ tumor cells to the cytotoxic effects of antiviral therapy. Our results suggest that radiation-induced EBV-TK expression followed by antiviral therapy in EBV+ PCNSL may be an effective therapeutic strategy to reduce morbidity and mortality in EBV+ PCNSL. Finally, we document the regression of EBV+ PC-
NSL found to constitutively express EBV-TK in a patient treated with antiviral therapy.

MATERIALS AND METHODS

Cell Lines. CB17 SCID mice were engrafted with human peripheral blood leukocytes (Hu-PBL-SCID) from a healthy EBV-seropositive donor (19). Eight weeks later, a human EBV+ B-cell lymphoblastic tumor that developed spontaneously in the hu-PBL-SCID mouse was harvested, dispersed into a single cell suspension, passed over a Ficoll-Hypaque density gradient, and then implanted s.c. into the flanks of five SCID mice (104 cells per animal). After two successive passages in vivo, EBV+ B-cell tumors were harvested, dispersed into single cell suspensions, and cultured in vitro for 2 weeks. Cells were subcloned in 96-well plates by limiting dilution assay and allowed to weekly injections of antiasialo-GM1 and resulted in faster progression of CNS rat natural killer cells. Immune suppression of animals was maintained with characteristic of EBV IN) were maintained in pathogen-free, isolated barrier cages. Before implantation, LCLs, nude rats were chosen randomly to receive either mock treatment or placebo (buffered saline).

Establishment of a Preclinical Animal Model for Human PCNSL. Four to 6-week-old male athymic, homozygous NIH nude rats (Harlan, Indianapolis, IN) were maintained in pathogen-free, isolated barrier cages. Before implantation, all of the animals received an immunosuppressive conditioning regimen consisting of whole body irradiation (137Cs 300 cGy) and antiasialo-GM1 (50 mg/kg i.p.; Wako Chemical, Osaka, Japan) antisera for depletion of rat natural killer cells. Immune suppression of animals was maintained with weekly injections of antiasialo-GM1 and resulted in faster progression of CNS symptoms in rats treated with combination chemotherapy. The EBV+ LCL 4A1A was implanted into the CNS of nude rats after methods described previously (20). Animals were anesthetized with a mixture of ketamine (90 mg/kg) and xylazine (15 mg/kg). The head was shaved, sterilized with a betadine scrub, and immobilized in a stereotactic frame (Model 900; David Kopf Instruments, Tujunga, CA). A 2-mm midline incision on the scalp was made from the mid-frontal to the mid-parietal cranial plate. The frontal bone, lateral to the right of the midline, was exposed. The location of implantation was the right caudate nucleus and is described with respect to the bregma: 2.0 mm lateral to the bregma, 1.4 mm anterior to the bregma, and 5.8 mm deep (21). A burr hole was created with a sterile drill and was filled with a plastic screw (#60 screw; Plastics One, Ruanoke, VA). Plastic screws were manufactured with a needle-sized hole to perpendicularly align a 27-gauge Hamilton syringe for injection. Between 0.5 × 106 and 5.5 × 106 EBV+ LCLs (4A1A) were resuspended in 25 μl of RPMI 1640 and 25 μl 1% agarose (gel temperature of 37°C), and injected with a Hamilton syringe over a 5-min interval through the plastic screw. Unless specified, all of the experiments where rats received antitumor therapy were performed using an inoculum of 5 × 106 of 4A1A cells. The screw hole was filled with bone wax and the incision closed with a single sterile clip. Postoperatively, the animals were monitored three times daily for 1 week and then daily thereafter. Animals were euthanized with a lethal dose of anesthetic on the manifestation of irreversible neurological symptoms (seizure activity or hemiparesis) or a ≥10% loss of body mass over a 5-day period. All of the animal research was reviewed and approved by the University Laboratory Animal Resources at The Ohio State University.

Antiviral Pharmacokinetics and Treatment. Dosage and delivery schedule of AZT and GCV were determined in a series of pharmacokinetic studies. The MTD of AZT and GCV was determined in a dose escalation trial by i.p. administration of four doses of combined AZT and GCV: AZT 30 mg/kg and GCV 20 mg/kg, AZT 60 mg/kg and GCV 40 mg/kg, AZT 80 mg/kg and GCV 60 mg/kg, and AZT 120 mg/kg and GCV 80 mg/kg. Drugs were delivered daily over a 4-week period. Every 7 days, animals were weighed and peripheral blood collected for cell counts and differential, measurement of liver enzymes and serum chemistries (performed by Antech Diagnostics, Chicago, IL). After determining the MTD (80 mg/kg AZT and 60 mg/kg GCV), pharmacokinetic studies were performed to assess drug distribution in the plasma and CSF. Serum and CSF samples were taken at 1, 4, 6, 12, and 24 h after a single dose of AZT and GCV at the MTD. Plasma and CSF levels of AZT and GCV were measured using a modified reverse-phase high-performance liquid chromatography assay as described previously (22). For in vivo treatment, rats were implanted as described with 4A1A and after 10 days, given daily low-dose antiviral therapy (AZT 60 mg/kg and GCV 40 mg/kg), high-dose (MTD: AZT 80 mg/kg and GCV 60 mg/kg) antiviral therapy, or placebo (buffered saline).

WBRT. On day 10 after stereotactic intracranial implantation of EBV+ LCLs, nude rats were chosen randomly to receive either mock treatment or WBRT. Rats were anesthetized with ketamine/xylazine and placed on the linear accelerator treatment table. Six rats were arranged in a circle with heads aligned toward the central axis. One cm of Superlab (tissue equivalent bolus) was placed over the rat skulls, with a central opening to allow for adequate ventilation. A cerrobend custom block (<5% transmission) was used to protect the rat torso. Rats were implanted as described with 4A1A cells and treated with a single dose (0 simulated but not radiated, 400, 800, 1200, or 1600 cGy; n = 3/group) of WBRT at day 10 (after implantation) and monitored for symptoms of tumor burden. Treatment was delivered using a single anterior field with 6 MV (megavolt) photons, and the dose was prescribed to D-max (depth of maximum dose) (1.6-cm depth). The linear accelerator was calibrated daily, and the dose rate was 300 MU/min (approximately 300 cGy/min). For rats treated with a combination of WBRT and antiviral therapy (Table 1; n = 10/group), WBRT dose was 1600 cGy. For in vitro work, cell lines were exposed to a γ-irradiation from a 137Cs source. Cells were exposed to 300, 500, 800, or 1600 cGy under sterile conditions. The instrument was calibrated to deliver 103 cGy/min.

Histology. After euthanization, the peripheral circulatory bed was perfused with 125 ml of PBS followed by 125 ml of 10% buffered formalin. The intact contents of the cranial cavity were either snap frozen (−70°C) or placed in 10% buffered formalin, and subsequently embedded in paraffin blocks for sectioning and histology. Paraffin-embedded tissues were sectioned (6 μm) and stained with H&E. IHC staining was performed on 6-μm frozen sections as described previously (19). Antibodies used for IHC included antihuman CD20, CD45, EBV LMP-1 or isotype control mouse IgG1 (Dako, Carpinteria, CA). A staff pathologist at The Ohio State University evaluated all of the specimens.

In Situ RT-PCR to Detect EBV-TK. EBV+ LCL (4A1A) or CNS sections from nude rats were fixed in 10% formalin and analyzed in a single-blinded fashion by in situ RT-PCR for EBV-TK transcript expression as described previously (17).

Informed Consent. The patient treated in this report provided written informed consent before therapy that was approved by the Institutional Review Board of The Ohio State University Hospitals.

Statistical Analyses. To test for a significant trend in survival time by radiation dose, a Jonckheere-Terpstra test was performed, which is a nonparametric test for trend. An exact P for this test was computed. The survival distributions for the placebo versus low-dose and high-dose antiviral treatments were compared using an exact Wilcoxon’s rank-sum test. The survival distributions for the radiation versus antiviral treatment (alone and in combination with radiation) were compared using a log-rank test. The latter two analyses involved multiple comparisons and, hence, a Bonferroni adjustment was made (23, 24). The Proportional Hazard Assumption necessary for the log-rank test to have optimal power was examined (23, 24). All of the statistical analyses were performed using SAS software (version 8.02; Cary, NC).
The survival data were expressed as the mean ± SE. Sigma Plot 7.0 graphing software was used to display data.

RESULTS

Dose-dependent Survival in Nude Rats Implanted with CNS EBV+ B-Cell Tumors. After immunosuppressive conditioning of nude rats, the human EBV+ LCL 4A1A was stereotactically implanted into the right caudate nucleus with a range of cell doses (0.5, 1, and 5 × 10^6 cells/rat), and rats were observed for the development of irreversible neurological symptoms before euthanization. Nude rat survival times were dependent on cell dose (Fig. 1A). The 5 × 10^6 cell dose resulted in 100% mortality within 21 days (19–23 days) after implantation and was used in all of the subsequent in vivo experiments.

Postmortem examination of the CNS consistently revealed a large, space-occupying lymphoid lesion with focal areas of cell dropout/necrosis and meningeal infiltration (Fig. 1, B and C). In addition to the large primary lesion, there were satellite lesions consistent with perivascular infiltration (Fig. 1, D and E). The majority of tumor cells were comprised of collections of immunoblastic cells with occasional plasmacytoid differentiation and cellular pleomorphism or atypia (Fig. 1, D and E). There was a high mitotic rate, high nuclear:cytoplasmic ratio, and prominent nucleoli. Histological features were consistent with a medium to high-grade malignant lymphoid process. The tumors could be identified in vivo by the human CD20 antigen (Fig. 2A). EBV+ B-cell tumors expressed LMP-1 (Fig. 2B) consistent with viral gene expression found in EBV+ PCNSL (10, 25).

Radiation and Antiviral Treatment of Rat PCNSL. It has been demonstrated recently that AZT and GCV are cytotoxic to EBV+ LCLs in vitro (16). We evaluated treatment of rat PCNSL with either antiviral therapy (AZT and GCV) or WBRT. Dose escalation studies with nucleoside analogues demonstrated an MTD for AZT (80 mg/kg) and GCV (60 mg/kg; data not shown). Pharmacokinetics performed for single AZT and GCV infusions showed that a daily dose at the MTD provided measurable and sustained concentrations of both drugs in plasma and CSF (Fig. 3A). Rats treated with a low-dose combination of antiviral therapy did not have a statistically significant change in survival, compared with placebo, whereas treatment with combination high dose antiviral therapy (MTD) demonstrated a significant improvement (Fig. 3B; P < 0.0079). Treatment of nude rat PCNSL with low but escalating single doses of WBRT also resulted in a significant prolongation of survival (Fig. 3C; P < 0.0002).

Radiation Induces EBV Lytic Cycle and EBV-TK Transcript in Vitro and in Vivo. We sought to determine whether radiation treatment of EBV+ LCLs could induce expression of EBV-TK mRNA in vitro. In a dose escalation of γ-radiation (0–1600 cGy) of the EBV+ LCL 4A1A, we demonstrated that EBV-TK transcript was induced in a direct dose-dependent fashion (Fig. 4A, shown at 48 h after treatment). EBV-TK transcript was expressed as early as 12 h after treatment and persisted for as long as 72 h (Fig. 4B).

We next assessed whether WBRT could induce EBV lytic gene expression in PCNSL lesions from the nude rat. Rats were treated with a single dose of WBRT (1600 cGy) 10 days after tumor implantation and assessed for EBV-TK gene expression in the CNS (17). Tumors from mock-irradiated nude rat brains did not display EBV-TK transcript (Fig. 5A), whereas tumors from nude rats given a single fraction of WBRT expressed increasing amounts of EBV-TK transcripts over time, with a peak of 40% of cells expressing EBV-TK at 72 h (Fig. 5B).

Therapeutic WBRT Enhances the Efficacy of AZT and GCV, and Promotes Survival in Vivo. TK has been shown to increase the cytotoxic activity of nucleoside analogs like GCV and AZT (13, 26).
We evaluated the potential for combining a single fraction of WBRT (1600 cGy) and high-dose combination antiviral therapy (at the MTD) for the treatment of PCNSL in the nude rat. Animals were implanted with tumor as described and treated at day 10 after implantation of tumor with a single dose of WBRT, daily AZT and GCV, combined treatment (WBRT, AZT, and GCV), or mock simulation plus daily placebo (n = 10/group). A summary of the results is shown in Table 1. The most significant improvement in survival time resulted when a single dose of WBRT was combined with high-dose antiviral therapy compared with either modality alone (P < 0.05). Identical results were obtained in a second trial when glucocorticoid treatment with dexamethasone was added to each treatment arm (data not shown).

Successful Treatment of a Patient with Post-Transplant EBV+ PCNSL. A 45-year-old man received a living-related kidney allograft in 1977 and suffered from an episode of acute rejection treated successfully in 1997. In 1999, the patient developed a seizure, and MRI revealed four enhancing lesions in the brain, the largest being a 5 × 6 × 5 cm lesion in the frontal lobe (Fig. 6A). Stereotactic core biopsies of one lesion demonstrated features of monomorphic, large cell, immunoblastic non-Hodgkin’s B-cell lymphoma. Core biopsies demonstrated the presence of EBV and EBV-TK expression by in situ RT-PCR (Fig. 6B). Staging by computed tomography showed no evidence of systemic disease. Initial therapy included discontinuation of all of the immunosuppressive medications except prednisone, which was maintained (60 mg daily) for control of CNS swelling. Phenytoin was also added for seizure prophylaxis. Prednisone was additionally reduced to 30 mg once daily, and after 1 month there was no radiological improvement in the tumor or evidence of renal allograft rejection. Given the constitutive expression of EBV-TK, the patient was admitted for therapy with high dose i.v. AZT (1.5 g Q12H) and GCV (2.5 mg/kg once daily) without WBRT. After 2 weeks of this antiviral therapy, a repeat MRI revealed a decrease in the size of two lesions, including the largest lesion in the frontal lobe, with no clinical evidence of allograft rejection. The patient was discharged on maintenance AZT (300 mg p.o. bid) and GCV (500 mg p.o. bid) therapy, and prednisone was gradually tapered to 5 mg every other day. Follow-up MRI 1 month later showed significant regression of two lesions. Outpatient follow-up (MRI) was repeated at least every 3 months over the ensuing 3 years, documenting an absence of tumor regrowth and unchanged white matter lesions likely because of hemorrhagic foci and infarct-related scarring (Fig. 6C). The patient has been maintained on oral GCV and AZT, and remains disease-free 36 months out from diagnosis with EBV+ PCNSL. The patient eventually developed chronic rejection of the renal allograft 18 months after diagnosis of PCNSL and underwent a second successful renal transplant in 2001.

DISCUSSION

The population at risk for immune deficiency-related EBV+ PCNSL includes the growing number of solid-organ transplant patients treated with iatrogenic immune suppression and HIV-positive patients with the AIDS (3, 5). EBV+ PCNSL has an incidence of 2–6% in AIDS patients and 1–7% in transplant recipients (3). In 2000, there
w ere ~20,000 new organ transplants performed in the United States\textsuperscript{5} and 36 million people in the world living with HIV infection (27), underscoring the fact that thousands of people will be diagnosed with EBV\textsuperscript{+} PCNSL each year. Diagnosis of EBV\textsuperscript{+} PCNSL is universally associated with a poor prognosis. The standard therapeutic options that exist often use high-dose WBRT, provide modest improvement (12 months) in DFS, and are often associated with devastating morbidity (2, 4–6, 28, 29). The development of more effective treatment strategies for PCNSL has been hindered for at least two reasons. First, the number of patients presenting with a performance status that is suitable for enrollment on a clinical trial is small, which has made large, randomized trials virtually impractical. Second, meaningful evaluation of novel therapies can be hampered by the late presentation of the patients and their profound immune-deficient status. The present report describes a preclinical animal model of human EBV\textsuperscript{+} PCNSL to evaluate novel therapies \textit{in vivo}. In addition, we present one case where a relatively unconventional antiviral therapy of post-transplant EBV\textsuperscript{+} PCNSL in a patient with iatrogenic immune deficiency has been associated with a significantly prolonged DFS.

We demonstrated that implantation of human EBV\textsuperscript{+} LCL into the nude rat CNS leads to the development of irreversible neurological symptoms after 19–22 days. The focal neurological symptoms were because of an expanding tumor mass in the CNS that led to neurological deficits in a majority of animals. Over the course of developing this nude rat model, we identified three characteristics important to its validity as a preclinical tumor model of EBV\textsuperscript{+} PCNSL (30). First, the EBV\textsuperscript{+} tumors have genotypic and phenotypic characteristics of most EBV\textsuperscript{+} PCNSL. The implanted cell lines were derived from spontaneously developing human EBV\textsuperscript{+} B-cell tumors that display an activated, mature B-cell phenotype. Second, the tumor is localized in the CNS, is multifocal and perivascular in nature, and reproducibly

leads to development of CNS symptoms and fatality in all animals, similar to the human counterpart. Third, the animals are congenitally immune deficient and iatrogenically immune suppressed, thereby minimizing the interaction of the rat immune system with experimental therapies targeting the EBV/H11001 PCNSL. Consequently, we have developed an EBV/H11001 PCNSL nude rat model that has the potential to closely parallel such disease in humans.

To explore our ability to evaluate treatments for EBV/H11001 PCNSL in this model, we tested two strategies: antiviral therapy and WBRT. Nucleoside analogs have been developed as antiviral drugs to target viral DNA synthesis. The mechanism of action for nucleoside analogs can occur via phosphorylation by herpes-encoded TKs that have higher affinity for the analogs than cellular kinases (12, 13). Subsequent modifications by cellular enzymes enable the nucleoside analogs to enter viral DNA synthesis and interrupt viral DNA polymerase. During lytic gene activation, the EBV open reading frames BXLF1 and BGLF4 encode proteins with kinase activity that possess the capacity to phosphorylate AZT (31) and GCV (32), respectively. Together, these reports suggest that a broader range of antiviral drugs including AZT may be applicable for testing in diseases associated with herpesvirus infections such as EBV. In fact, other investigators have demonstrated that both AZT and GCV are cytotoxic to EBV/LCL in vitro (16), and we have confirmed these findings (data not shown). In this report, we have evaluated combination antiviral treatment with AZT and GCV in the rat model of EBV/H11001 PCNSL. High doses of combined AZT and GCV demonstrated a significant improvement in survival compared with placebo or either drug alone.

For the past 30 years, WBRT has been a major component of EBV/H11001 PCNSL care. Immune-deficient patients with EBV/H11001 PCNSL typically receive high-dose WBRT (5000 cGy) and whereas they may initially respond, they rarely experience prolonged DFS. In addition, WBRT is often accompanied by delayed neurotoxicity manifested by cognitive impairment, weakness, fatigue, incontinence, and behavioral disturbances (1, 5, 6, 9, 28). Despite this comorbidity, WBRT has been generally accepted as the best palliative measure to offer patients with EBV/H11001 PCNSL. We evaluated WBRT in our nude rat model of EBV/H11001 PCNSL. Rats given a single dose of WBRT (1600 cGy) displayed initial improvement in survival; however all of the animals eventually developed irreversible neurological deficits secondary to progressive PCNSL. On the basis of our in vitro and in vivo animal data demonstrating the induction of EBV-TK expression with a single dose of WBRT, we explored the possibility that a single dose of WBRT could demonstrate synergy with a combination of antiviral therapy for effective treatment of EBV/H11001 PCNSL. We hypothesized that induced expression of EBV-TK and possibly other lytic gene products (BGLF4; Ref. 31) would support the phosphorylation of AZT and GCV, and enhance the cytotoxic activity against EBV/H11001 PCNSL in vivo. Indeed, we have shown a significant improvement in survival of tumor-bearing rats using this combination, and a poten-
tially therapeutic application of WBRT (1600 cGy) in combination with high dose AZT and GCV for treatment of EBV+ PCNSL.

Additionally, we have described a case report where we document, for the first time, the constitutive expression of EBV-TK in a patient brain biopsy of EBV+ PCNSL. This patient with PCNSL after organ transplantation did not receive WBRT, proved highly responsive to a course of high-dose antiviral (AZT and GCV) therapy, and has been disease-free for 3 years. Raaz et al. (33) described five AIDS-related PCNSL cases where four of five patients responded to treatment with interleukin 2, AZT, and GCV. Because viral kinetics can phosphorylate AZT and GCV, our demonstration of constitutive EBV-TK expression within the patient tumor provided rationale for using this antiviral strategy in the absence of WBRT. Demonstration of EBV-TK expression in EBV+ PCNSL biopsies may prove to be useful in selecting a strategy with AZT and GCV. Alternatively, EBV+ PCNSL and systemic lymphomas may still be rendered sensitive to antiviral therapy through induction of EBV-TK expression (15, 34). In addition to ionizing radiation, other agents, such as arginine butyrate, have been successfully used to induce EBV lytic gene expression in EBV+ tumors in vitro (16, 35). This case report suggests that there should be additional consideration for the use of high-dose antiviral therapy with AZT and GCV in EBV+ PCNSL.

In the past 20 years, there has been little improvement in therapy for EBV+ PCNSL, which is virtually always diagnosed in immunocompromised patients. In this report, we have defined a preclinical animal model for EBV+ PCNSL that can be used to evaluate experimental therapies. We used in situ RT-PCR to document the induction of EBV-TK expression with a single dose of WBRT. Our data demonstrating a significantly improved survival with the combination of WBRT (1600 cGy), and high-dose combination antiviral therapy demonstrates the utility of the rat model for evaluating novel and rational therapies in an in vivo setting. Efficacy of WBRT with combination AZT and GCV may represent a potential improvement in patient quality of life as well, by reducing comorbidity of WBRT (5).

The clinical case presented here supports application of this therapeutic approach with antiviral treatment of EBV+ PCNSL before palliative procedures such as high-dose fractionated WBRT are considered. Thus, this model may prove to be a useful tool for investigators studying PCNSL, thereby expediting the development and evaluation of new therapeutic approaches in this disease.

REFERENCES

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