# A phase 1/2 trial of arginine butyrate and ganciclovir in patients with Epstein-Barr virus–associated lymphoid malignancies

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**Malignancies associated with latent Epstein-Barr virus (EBV) are resistant to nucleoside-type antiviral agents because the viral enzyme target of these antiviral drugs, thymidine kinase (TK), is not expressed. Short-chain fatty acids, such as butyrate, induce EBV-TK expression in latently infected B cells. As butyrate has been shown to sensitize EBV$^+$ lymphoma cells in vitro to apoptosis induced by ganciclovir, arginine butyrate in combination with ganciclovir was administered in 15 patients with refractory EBV$^+$ lymphoid malignancies to evaluate the drug combination for toxicity, pharmacokinetics, and clinical responses. Ganciclovir was administered twice daily at standard doses, and arginine butyrate was administered by continuous infusion in an intrapatient dose escalation, from 500 mg/(kg/day) escalating to 2000 mg/(kg/day), as tolerated, for a 21-day cycle. The MTD for arginine butyrate in combination with ganciclovir was established as 1000 mg/(kg/day). Ten of 15 patients showed significant antitumor responses, with 4 CRs and 6 PRs within one treatment cycle. Complications from rapid tumor lysis occurred in 3 patients. Reversible somnolence or stupor occurred in 3 patients at arginine butyrate doses of greater than 1000 mg/(kg/day). The combination of arginine butyrate and ganciclovir was reasonably well-tolerated and appears to have significant biologic activity in vivo in EBV$^+$ lymphoid malignancies which are refractory to other regimens. (Blood. 2007;109: 2571-2578)**



## Introduction

Epstein-Barr virus (EBV) is a common and worldwide pathogen.[1] Whereas childhood infection is generally asymptomatic, approximately 50% of individuals with delayed exposure develop a self-limited lymphoproliferative syndrome, infectious mononucleosis.[2,3] Although EBV is associated with a number of human malignancies (reviewed in Hsu and Glaser[4]), EBV likely plays a causal role in 2 endemic tumors: African Burkitt lymphoma[5,6] and nasopharyngeal carcinoma.[7,8]

The development of large patient populations with T-cell dysfunction, caused by iatrogenic immunosuppression required for organ and marrow transplantation and by AIDS, led to the discovery of additional EBV-related illnesses: hairy leukoplakia,[9] B-cell lymphoproliferative disease,[10] and lymphomas in patients who received a transplant[11] and patients with AIDS.[12] Sporadic T-cell and B-cell lymphomas,[13-15] 50% of Hodgkin lymphomas,[16-20] AIDS-related sarcomas,[21,22] gastric carcinomas,[23,24] and certain breast carcinomas[25] have been found to contain EBV.[26-29] Whether or not the EBV genome is causally associated with these malignancies or lymphoproliferative diseases, the viral genome represents a potential tumor-specific target for therapeutic modalities in many malignancies.

Like herpesviruses, such as herpes simplex virus (HSV) and varicella-zoster virus, EBV encodes a thymidine kinase (TK) enzyme.[30] In a rate-limiting step, the TK from these viruses can convert nucleoside analogs to their monophosphate form.[30,31] Cellular enzymes then complete their conversion to biologically active triphosphates. A viral DNA polymerase preferentially incorporates the toxic metabolites into viral DNA, leading to premature termination of the nascent DNA, with resulting apoptosis of infected cells. Acyclovir (ACV) and ganciclovir (GCV) are purine nucleoside analogs with a linear side chain replacing the cyclic sugar of guanosine. In some studies, HSV TK preferentially phosphorylates ACV, whereas EBV-TK preferentially phosphorylates GCV,[32,33] although this substrate specificity is controversial.[34]

Studies also suggest that the EBV protein kinase BGLF4, another gene product induced early on stimulation of the lytic cycle, may be a major mediator of ganciclovir phosphorylation in EBV-infected cells.[35] Because GCV triphosphate accumulates to higher levels and persists for longer periods in EBV-infected cells than ACV, GCV produces more interference with cellular DNA synthesis than does ACV.

The susceptibility of EBV to antiviral drugs that inhibit replication of other herpesviruses has been difficult to assess. When ACV and later GCV were used to treat AIDS-related herpesvirus infections, regression of hairy leukoplakia was unexpectedly observed, establishing the efficacy of these agents in vivo for treatment of lytic EBV disease.[32,36,37] Latent EBV disease, however, was unaffected by these antiviral agents. Latently infected B cells do not express the EBV-TK transcript or protein. However, exposure of these cells in vitro to arginine butyrate (or the sodium

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salt) results in modest induction of some lytic-phase genes and gene products, including TK.[38-42] We previously found that arginine butyrate induces EBV-TK activity in EBV-immortalized B cells and in patient-derived tumor cells.[38-41] Furthermore, induction of EBV-TK activity with arginine butyrate in EBV-immortalized B cells and patient-derived B-cell lymphoma tumor cells rendered these latently infected, previously GCV-resistant cells susceptible to ganciclovir.

There are isolated case reports of administration of butyrates to patients for various malignancies.[43,44] Arginine butyrate has been administered as a single agent over extended periods of time without major side effects, to induce a gene for fetal hemoglobin in sickle-cell anemia and β-thalassemia.[45-47] We therefore hypothesized that treatment of patients with EBV-associated malignancies with the combination of arginine butyrate (to induce the EBV-TK gene) and GCV (to then eliminate EBV-TK–expressing tumor cells) might be an effective, tumor-targeted therapeutic approach.

A phase 1/2 trial was therefore undertaken to evaluate the safety and tolerability of arginine butyrate when administered in combination with ganciclovir in EBV-associated lymphoid malignancies and lymphoproliferative disease and to determine whether any antitumor activity occurred, albeit in a group of refractory patients, and in the context of a phase 1/2 trial.

## Patients, materials, and methods

### Study population

Eligibility for this combination therapy included the following criteria: (1) a microscopically documented neoplasm, which was $EBV^+$ as determined by immunohistochemistry ($EBNA\text{-}2^+$ and/or $LMP\text{-}1^+$); (2) presence of evaluable disease; (3) patients with lymphoma must have been refractory to at least one combination chemotherapy regimen, which could include high-dose chemotherapy and marrow or stem-cell rescue or stem-cell transplantation, or immunotherapy, and were deemed incurable by standard therapy; (4) patients must have recovered from prior chemotherapy or radiotherapy and at least 3 weeks must have elapsed since the last course of chemotherapy; (5) adequate bone marrow function (absolute neutrophil count [ANC] $> 1.0 \times 10^9$/L, platelet count $> 50 \times 10^9$/L); (6) adequate hepatic function (total bilirubin level $< 25.65$ μmol/L [$< 1.5$ mg/dL], serum aminotransferases $< 2$ times the upper limit of normal); (7) adequate renal function (serum creatinine 265.2 μmol/L [$< 3.0$ mg/dL]), and calculated creatinine clearance 0.5 mL/second ($> 30$ mL/minute). There were no limitations on functional status for eligibility. Male or female patients at least 3 years of age were eligible. The study was conducted under an IND from the US Food and Drug Administration.

These investigations were performed with approval by the Institutional Review Boards of the Boston University Medical Center and at each treating hospital. Informed consent was obtained from each subject or each subject's guardian in accordance with the Declaration of Helsinki.

### Patient evaluations

Patient evaluations before beginning the protocol included the following: complete history and physical examination; performance status assessment; tumor evaluation (of selected assessable sites); complete blood count with leukocyte differential; serum electrolytes; blood urea nitrogen; creatinine; creatinine clearance; calcium; magnesium; phosphorous; total bilirubin; liver transaminases; alkaline phosphatase; total protein; albumin; uric acid; prothrombin time (PT); partial thromboplastin time (PTT); urinalysis; electrocardiogram, chest X-ray (or chest computed tomography if part of tumor evaluation); tumor measurement with computed tomography (CT) or magnetic resonance imaging (MRI) scan of lesions if appropriate, pathologic confirmation of cancer diagnosis; and appropriate tumor markers. These studies were repeated at the treating physicians' discretion and prior to any subsequent courses of treatment.

Study evaluations during each cycle of therapy included daily physical examination and assessment of performance status, complete blood counts with differential, serum electrolytes, blood urea nitrogen, creatinine, magnesium, phosphorous, calcium, and liver function tests every 3 days.

### Treatment plan and drug administration

Pretreatment studies included confirmation of tissue diagnosis, obtaining informed consent, documentation of immunosuppression (if any), placement of central venous access if not already in place, and performance of a pregnancy test within 2 days of beginning therapy, unless the patient was postmenopausal or not fertile for medical reasons.

On days −1 or day 0, ganciclovir was begun at standard doses (5 mg/kg intravenously over 1 hour twice per day), unless it had already been initiated, and continued throughout the cycle. On day 0, infusion of arginine butyrate was begun at a starting dose of 500 mg/(kg/day) by continuous infusion. In the absence of intolerable toxicity, dose escalation was conducted according to the following scheme: level 1, 500 mg/(kg/day) intravenously [20.8 mg/(kg/h)] for 2 days; level 2, 1000 mg/(kg/day) intravenously [41.6 mg/(kg/h)] for 2 days; level 3, 1500 mg/(kg/day) intravenously [62.5 mg/(kg/h)] for 2 days; level 4, 2000 mg/(kg/day) intravenously [83.2 mg/(kg/h)] until day 21. If toxicities required interruption of the arginine butyrate, the agent was reinstituted at the last dose tolerated. Ganciclovir was not interrupted. Renal, hepatic, and hematologic monitoring was performed after 3 weeks. Repeat staging of the tumor was carried out 1 to 4 weeks after institution of treatment. A second cycle of treatment was allowed, starting on day 29, if there was evidence of response. Patients could receive a total of 3 cycles of therapy as long as tumor response was evident, unless they met one of the criteria for removal from the study. For any additional cycles, arginine butyrate and ganciclovir were administered at the highest dose tolerated in the previous cycle.

Toxicities were scored according to the National Cancer Institute common toxicity criteria (CTC), version 2.0. Toxicity of grade 3 or 4 was considered dose limiting.

### Scoring of tumor responses

Evaluation of tumor measurement or disease response was performed after the first cycle of arginine butyrate plus ganciclovir; a few patients were evaluated earlier, particularly when a patient was removed from the study for management of a complication (eg, pneumonia, sepsis) or disease progression was suspected. A complete response (CR) was defined as disappearance of detectable malignant disease on imaging or physical examination where appropriate, (eg, for skin lesions or tonsilar masses). A partial response (PR) was defined as 50% decrease in tumor size (sum of the product of the largest perpendicular diameters) of measurable lesions chosen for analysis prior to beginning the treatment. For tumors which could only be measured in one dimension, a greater than 50% decrease in the largest dimension qualified as a PR. In 3 patients who died of other morbidities, responses were confirmed pathologically at autopsy.

### Pharmacokinetic sampling and analytical assay

Plasma was obtained before treatment and 4 hours into infusion on days 2, 4, 6, 8, 14, and 21 of treatment for measurement of butyrate and arginine levels. Plasma samples were also obtained between 4 and 6 hours and 12 and 18 hours after each dose escalation and after discontinuation. L-arginine levels were analyzed by a Beckman amino acid analyzer (Beckman Instruments, Palo Alto, CA) as previously described.[48] Butyrate levels were assayed by liquid chromatography and mass spectrometry (LCMS).[49]

## Results

Fifteen patients with $EBV^+$ lymphoid malignancies were treated with arginine butyrate and ganciclovir to evaluate toxicity and tolerance of the patients. Although either immunohistochemistry (IHC) or in situ hybridization (ISH) criteria were permitted for

diagnosis, all enrolled patients were diagnosed by tumor-cell positivity for LMP-1 and/or EBNA by IHC. Characteristics are provided in Table 1. EBV$^+$ lymphoid malignancies included monoclonal lymphoproliferative disease (PTLD), B-cell non-Hodgkin lymphomas (NHL) (including one HIV-associated anaplastic diffuse large B-cell lymphoma), T-cell NHL (including one subcutaneous panniculitis-like T-cell lymphoma), natural killer (NK)/T-cell lymphomas, and Hodgkin disease. Most of the patients had been previously treated with chemotherapy and radiation before entry into this study, although entry criteria precluded therapy within 3 weeks of beginning this protocol.

### Adverse events and determination of MTD

Dosing parameters for the arginine butyrate component were selected on the basis of previous tolerability studies for arginine butyrate as a single agent, wherein infusions delivering 500 to 2000 mg/(kg/day) were associated only with tolerable nausea, headache, and anorexia.[48] All recorded adverse events are listed in Table 2. The most common adverse events that were likely attributable to the treatment were nausea and vomiting, which were controllable with conventional antiemetic therapies, and headache. Elevation of BUN was observed in 4 patients, but without changes in creatinine levels, and was an expected event, secondary to metabolic conversion of L-arginine from the arginine butyrate to urea. Hypokalemia occurred in 3 patients and was severe in 1 patient, requiring interruption of therapy. Severe dyspnea occurred in one patient and was attributed to bacterial pneumonia. The most common severe adverse events were related to the central nervous system and included stupor or somnolence (4 patients), confusion (6 patients), acoustic hallucination (1 patient), lethargy or fatigue (2 patients), and visual changes (1 patient). Because many of the patients were gravely ill at the time the treatment combination was initiated, it is not clear that somnolence or lethargy were related entirely to the experimental protocol drugs. However, the severe lethargy and stupor experienced by 2 patients at the highest dose of arginine butyrate [2000 mg/(kg/day)] was likely to be attributable to the combination protocol, as it was reversible and did not recur when the dose of arginine butyrate was reduced to 1000 mg/(kg/day).

In 13 patients, dose escalation proceeded to the maximum dose allowed by the protocol, 2000 mg arginine butyrate/(kg/day). In 2 of the patients, the dose escalation was discontinued at 1500 mg arginine butyrate/(kg/day), because of concerns about tumor progression in one case, and because of grade 3 toxicities in one case. At the dose levels of 500 and 1000 mg arginine butyrate/(kg/day), no dose-limiting toxicities were observed. The dose-limiting toxicities occurred at the 1500 to 2000 mg arginine butyrate/(kg/day) dose levels. At the 1500 mg/(kg/day) dose level, marked lethargy and confusion was observed in 2 patients, with acoustic hallucinations in one and severe hypokalemia in another patient. At the 2000 mg/(kg/day) dose, severe stupor and somnolence developed in 2 patients, one of whom had experienced somnolence at 1500 mg/(kg/day). Although the other patients tolerated 1500 mg arginine butyrate/(kg/day) without serious adverse events, the dose to be used in the follow-on phase 2 trial of intravenous arginine butyrate in combination with ganciclovir will be 1000 mg/(kg/day).

Several severe adverse events occurred and were evaluated by the treating physicians as secondary to rapid tumor lysis. One patient developed a fatal hemorrhage after regression of a lymphoma which was invading the carotid artery. A second patient with a lymphoma of the small bowel developed a shock lung/ARDS syndrome associated with regression of the small bowel lymphoma on CT scan, which was suspected to be related to pathologically documented small bowel necrosis. A third patient experienced hepatic and pancreatic necrosis 3 days after completing one cycle of the protocol with complete disappearance of a nasal NK/T-cell lymphoma and was considered by the treating physician to be due to release of Fas ligand from the lymphoma which underwent complete necrosis.[50]

**Table 1. Patient characteristics**

| | Values |
|---|---|
| **Age, y** | |
| Mean | 38.1 |
| Median | 39 |
| Range | 3-65 |
| **Sex, no.** | |
| Male | 5 |
| Female | 10 |
| **No. of courses** | |
| No more than 1 | 12 |
| 1-2 | 2 |
| 2-3 | 1 |
| **Diagnosis, no.** | |
| PTLD | 6 |
| B-cell NHL | 2 |
| T-cell NHL | 2 |
| NK/T-cell NHL | 4 |
| Hodgkin's disease | 1 |
| **Prior transplantations, no.** | |
| Lung | 2 |
| Bone marrow | 3 |
| Renal | 1 |
| **No. of patients with prior therapy for this diagnosis** | |
| Radiation | 6 |
| Chemotherapy | 14 |
| **No. of prior chemotherapy regimens for this diagnosis** | |
| Median | 3 |
| Range | 0-6 |

PTLD indicates post-transplantation lymphoproliferative disease; NHL, non-Hodgkin lymphoma.

### Antitumor responses

Of the 15 patients treated, all were evaluated for response, although several received only 7 to 10 days of therapy rather than 21 days (Table 3). Eleven received at least one full cycle of therapy (21 days), and 1 patient received 3 cycles. Four patients received fewer than 21 days (1 cycle) of treatment, because of concomitant complications (2 patients), grade 3 toxicity (1 patient), and possible disease progression (1 patient).

Four patients demonstrated complete responses (2 PTLD, 1 extranodal NK/T-cell lymphoma, 1 peripheral T-cell lymphoma). Six patients demonstrated partial responses (3 PTLD, 1 diffuse large-cell B-cell lymphoma, 1 extranodal NK/T-cell lymphoma, and 1 subcutaneous panniculitis-like T-cell lymphoma). Three patients demonstrating complete responses died shortly after completing the therapy as a result of comorbid conditions and complications of tumor lysis and had postmortem examinations. Two of these 3 patients had complete disappearance of the tumor by pathologic examination, and a third patient had significant necrosis of the residual lymphoma.

Of the 5 nonresponding patients, 2 did not complete a full treatment cycle. However, 2 patients who had complete responses received only 15 or 16 days of therapy, demonstrating that responses could occur within this time frame, and one is a long-term survivor, more than 5 years after treatment without

**Table 2. Adverse events by organ system**

| Adverse event | Mild, no. | Moderate, no. | Severe, no. | Life-threatening, no./no. fatal | Total, no. |
|---|---|---|---|---|---|
| **Hematologic** | | | | | |
| Anemia | 0 | 1 | 0 | 0 | 1 |
| Thrombocytopenia | 1 | 3 | 0 | 0 | 4 |
| **GI** | | | | | |
| Nausea/vomiting | 4 | 5 | 0 | 0 | 9 |
| Diarrhea | 0 | 2 | 0 | 0 | 2 |
| Constipation | 0 | 1 | 0 | 0 | 1 |
| Hepatomegaly | 0 | 1 | 0 | 0 | 1 |
| Hepatitis and pancreatitis | 0 | 0 | 0 | 0/1 | 1 |
| Other GI Symptoms | 2 | 3 | 0 | 0 | 5 |
| **CNS** | | | | | |
| Stupor/somnolence | 0 | 2 | 2 | 0 | 4 |
| Confusion/disorientation | 1 | 5 | 1 | 0 | 7 |
| Hypoesthesia | 1 | 0 | 0 | 0 | 1 |
| Hallucination | 0 | 0 | 1 | 0 | 1 |
| Lethargy/fatigue | 1 | 3 | 2 | 0 | 6 |
| Visual changes | 0 | 1 | 0 | 0 | 1 |
| Restlessness | 2 | 1 | 1 | 0 | 4 |
| Headache | 2 | 1 | 0 | 0 | 3 |
| Insomnia | 1 | 0 | 0 | 0 | 1 |
| Rejection of transplanted lung | 0 | 0 | 0 | 0/1 | 1 |
| **Metabolic** | | | | | |
| Hypokalemia | 1 | 1 | 1 | 0 | 3 |
| Elevated BUN | 1 | 2 | 1 | 0 | 4 |
| **Cardiorespiratory** | | | | | |
| Orthostasis | 1 | 0 | 0 | 0 | 1 |
| Increased dyspnea | 0 | 0 | 1 | 0 | 1 |
| ARDS, pulmonary hemorrhage | 0 | 0 | 0 | 1 | 1 |
| **Infectious** | | | | | |
| Pneumonia | 0 | 1 | 0 | 1 | 2 |
| Fungal (noninvasive, GI tract) | 0 | 1 | 0 | 0 | 1 |
| Fever/suspected sepsis | 0 | 0 | 1 | 0 | 1 |
| Staph sepsis, port infection | 0 | 2 | 0 | 0 | 2 |
| **Other** | | | | | |
| Back pain | 0 | 1 | 0 | 0 | 1 |
| Sinus pain | 1 | 0 | 0 | 0 | 1 |
| Rash | 1 | 0 | 0 | 0 | 1 |
| Pharyngitis | 0 | 2 | 0 | 0 | 2 |
| Mucositis | 1 | 1 | 0 | 0 | 2 |
| Deep vein thrombosis | 0 | 0 | 1 | 0 | 1 |
| Complications of tumor lysis (hemorrhage or bowel perforation) | 1 | 0 | 0 | 0/2 | 2 |
| Total | 22 | 40 | 12 | 2/4 | 80 |

GI indicates gastrointestinal; CNS, central nervous system; BUN, blood urea nitrogen; ARDS, adult respiratory distress syndrome.

disease recurrence. The single patient with Hodgkin disease demonstrated no response to the protocol. Review of pathology before therapy was instituted showed that only a single lymph node was positive for EBV antigens, whereas the patient's large central mediastinal masses were negative for EBV.

### Pharmacokinetic studies

Butyrate was not detectable during or after infusion of doses equal to or less than the MTD of 1500 mg/(mL/day), consistent with previous pharmacokinetic studies of arginine butyrate.[45,47]

## Discussion

The lymphomas and lymphoproliferative diseases associated with EBV are in some cases causally related to the disease, whereas, in other cases, the nature of the association is unclear. For example, clonal EBV is found in association with certain T-cell lymphomas. EBV-associated T-cell lymphomas are highly site-restricted and are morphologically indistinguishable from EBV$^-$ T-cell lymphomas.[51] The finding that EBV is found in almost all tumor cells in most cases of primary extranodal, and especially nasal, T-cell lymphomas[52-54] and not in primary nodal T-cell lymphoma, where the proportions of EBV-infected neoplastic cells varies greatly,[55] strongly suggests an etiologic role for EBV in the former. Of extranodal NK/T-cell tumors, EBV is closely linked to nasal/pharyngeal NK/T-cell lymphoma, but shows geographic and racial variations in other subtypes.[56] All types of NK or NK/T lymphomas/leukemias have an extremely poor prognosis with a median survival of less than a year.[57]

For more than 20 years, a role for EBV in the pathogenesis of Hodgkin disease has been postulated, based on epidemiologic evidence linking Hodgkin patients with EBV seropositivity and elevated EBV titers.[58] The association between EBV and Hodgkin disease remained speculative until 1987, when molecular genetic analysis showed that some Hodgkin tissues contained monoclonal EBV DNA[59] and that the virus was localized to Reed-Sternberg

**Table 3. Courses of individual patients**

| Patient | EBV-related disease, other | Maximum dose/MTD, mg/kg | No. cycles | Outcome, 1 cycle | Prior therapies/chemotherapy regimens | Adverse events |
|---|---|---|---|---|---|---|
| 1 | PTLD; s/p lung transplant | 500/500 | < 1, 15 d | CR | CHOP × 6 | Confusion; diarrhea; emesis-coffee ground; rejection of transplanted lung* |
| 2 | DLBCL (CNS); s/p BMT × 2 for AML, GVHD | 1800/1800 | < 1, 16 d | CR | ACV, IL-2, IgG, dexamethasone; XRT (brain) (2 regimens) | Confusion; mucositis; headache; N/V; abdominal pain |
| 3 | PTLD; s/p BMT/PBSC transplant for AML, GVHD | 2000/2000 | < 1, 19 d | PR | IDA/ARA-C; mitoxantrone | Confusion; N/V; tumor lysis leading to bowel perforation* |
| 4 | Anaplastic DLBCL; s/p HIV | 2000/2000 | 1 | PR | Vinblastine, anthracyclines, AraC; cisplatinum, Steroids | Confusion; N/V; anorexia |
| 5 | DLBCL (paranasal) | 2000/2000 | 1 | NR | XRT, CHOP × 6; MTX, doxorubicin, vincristine (3 regimens) | Confusion; restlessness; somnolence; N/V, abdominal pain; visual changes; orthostasis |
| 6 | PTLD, s/p renal transplant | 1000/1000 | 1 | NR | BMT, CHOP × 2 | Headache; N/V; abd. pain thrombocytopenia |
| 7 | Extranodal T-cell lymphoma | 2000/1500 | 1 | CR | CHOP × 3, XRT | Lethargy/stupor/confusion; hypotonia/hypoesthesia; fungal infection/mucositis; tumor lysis leading to hemorrhage* |
| 8 | Extranodal NK/T-cell lymphoma | 1500/1000 | 1 | PR | XRT, APO × 2, cytoxan, MTX × 2; cisplatinum/AraC/VP-16; (4 regimens) | Acoustic hallucinations; somnolence; hypokalemia; sepsis, DVT |
| 9 | Extranodal NK/T-cell lymphoma | 2000/2000 | 1 | CR | ACVBP, DHAP, ICE, MTX, dexamethasone (4 regimens) | Confusion; fatigue; elevated BUN; tumor lysis leading to pancreatitis/hepatitis* |
| 10 | PTLD; s/p lung transplant | 1000/800 | 1 | NR | None | Elevated BUN; encephalopathy |
| 11 | PTLD; s/p BMT and SCT, GVHD | 1500/1500 | < 1, 8 d | PR | VCR/prednisone, L-Asp; Daunorubicin, IT MTX, AraC, cytoxan, 6-MP/MTX (2 regimens) | Diarrhea; hepatomegaly |
| 12 | Hodgkin: 1 nodule $EBV^+$, mediastinal mass $EBV^-$ | 2000/2000 | 1 | NR | MOPP, ABVD, DHAP, CCNU; VP-16, cytoxan XRT, BMT (6 regimens) | Nausea; pneumonia; port infection |
| 13 | Subcutaneous panniculitis-like T-cell lymphoma; pulmonary metastases | 938/938 | 3 | PR | Hyper-CVAD × 3; ICE, VP-I6, anti-CD3 Ab; HuM291; denileukin diftitox × 3 (5 regimens) | Nausea, anorexia, weight loss; anemia; thrombocytopenia; lethargy; insomnia; hypokalemia |
| 14 | Extranodal NK/T-cell lymphoma | 1250/1250 | < 1, 19 d | NR | CHOP × 4; ESHAP, ICE × 3-(3 regimens), XRT | Sinus, throat, back pain; thrombocytopenia; hypokalemia; lethargy |
| 15 | DLBCL | 1000/1000 | < 1, 5 d | PR | CHOP × 6 | Lethargy; increased dyspnea; polymicrobial pneumonia/ARDS |

DLBCL indicates diffuse large B cell lymphoma; s/p, status post; BMT, bone marrow transplant; GVHD, graft-versus-host disease; PBSC, peripheral blood stem cell; SCT, stem cell transplant; EBV, Epstein Barr virus; MTD, maximum tolerated dose; NR, no response; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; IL-2, interleukin 2; IgG, immunoglobulin G; XRT, radiotherapy; AraC, cytarabine; MTX, methotrexate; APO, vincristine, adriamycin, and prednisone; VP-16, etoposide; ACVBP doxorubicin, cyclophosphamide, vindesine, bleomycin, and prednisone; DHAP, dexamethasone, high-dose cytarabine, and cisplatin; ICE, ifosfamide, carboplatin, and etoposide; VCR, vincristine; IT, intrathecal; 6-MP, 6-mercaptopurine; MOPP, mechlorethamine, vincristine, prednisone, and procarbazine; ABVD, doxorubicin, bleomycin, vinblastine, and dacarbazine; CCNU, lomustine; CVAD, cyclophosphamide, vincristine, adriamycin, and dexamethasone; HuM291, visilizumab; ICE, ifosfamide-carboplatin-etoposide; ESHAP, etoposide, methylprednisolone, cytarabine, cisplatin; and N/V, nausea/vomiting.

*Fatal adverse event.

(RS) cells.[16] Subsequent immunohistochemical and serologic data support an association between EBV and Hodgkin disease and confirmed the localization of the virus to cytologically malignant-appearing RS cells and variants.[60,61] In most reported series, EBV is associated with approximately half of mixed cellularity Hodgkin disease and a lower percentage of the nodular sclerosing subtype.

In contrast to the unclear relationship and variable association of EBV with the non-Hodgkin lymphomas and Hodgkin disease, the virus is almost certainly causally related to the post-transplantation lymphoproliferative disorders. The incidence of PTLD after transplantation varies between less than 1% and more than 20%, depending on the number of risk factors.[62-64] Despite a number of therapeutic approaches, including reduction of immunosuppression, antiviral drugs, adoptive immunotherapy, and administration of anti-CD20 monoclonal antibodies, the mortality of PTLD remains high.[62,63]

All of the patients with PTLD in our study had the early-onset form of PTLD (occurring during the first year after transplantation), which often shows a rapid disease progression, with a median survival of 0.6 months.[63] The disease in each of these patients would have been classified as polymorphic, being monoclonal and exhibiting architectural effacement of involved lymphoid tissue and destructive extranodal masses.[65] Effective therapeutic options for PTLD of this type have been limited, with unpredictable responses to reduction in immunosuppression (where possible), and high mortality with chemotherapy.[66] Adoptive immunotherapy, including infusion of donor leukocytes or EBV-specific donor-type T-cell lines, has been used and may be effective in some, but may be ineffective in advanced cases of PTLD, or in disease with CNS involvement.[67,68] The administration of monoclonal antibodies against B-cell epitopes such as CD20 has produced responses in some cases of PTLD but not in others.[69] Monoclonal antibodies directed against this B-cell epitope are also used in the treatment of B-cell NHL. Fourteen of the patients treated in this study had failed to respond to, or had become refractory to, aggressive combination chemotherapy regimens or immunomodulatory therapies, such as reduction in the level of immunosuppression where relevant, or rituximab.[70,71]

Many herpesvirus family–infected cells, including cells infected by herpes simplex and cytomegalovirus, can be killed by nucleoside analog antiviral drugs such as ganciclovir and acyclovir. Unlike other members of the herpesvirus family, however, EBV is resistant to the antiviral agents such as ganciclovir, presumably because of low levels of viral thymidine kinase expression during lytic phase and lack of expression during latency. Although a number of nucleoside analogs show activity against replicating EBV,[72,73] antiviral treatment of latent EBV has been unsuccessful in vitro[74] and as prophylaxis,[75] and there are only rare reports of potential activity in the treatment of EBV neoplasms in vivo.[76,77] We have demonstrated in in vitro studies that exposure of EBV-transformed B cells or tumor cells to arginine butyrate induces EBV-TK and renders them sensitive to ganciclovir. It should be noted that this potential therapeutic approach does not depend on the associated EBV genome being the cause of the tumor. Rather, just the presence of the EBV genome in latent form would be predicted to make a tumor susceptible to this combination approach.

Butyrate, a naturally occurring short-chain fatty acid, itself has demonstrated some antitumor activity in vitro and in animal models. Butyrate and its derivatives exert a number of antiproliferative effects on transformed cell lines in vitro, including decreased DNA replication leading to arrest of cell division in the $G_1$ phase, modification of cellular morphology, and alteration of gene expression consistent with differentiation.[78-89] The mechanism(s) of action proposed for these effects on differentiation are varied and are not fully understood, but are likely attributable in large part to its actions as a histone deacetylase (HDAC) inhibitor. Similarly, the $G_1$-phase cell-cycle arrest induced by butyrate and related short-chain fatty acids is dependent on HDAC-inhibitory activity and the resulting inhibition of cyclin D1 expression[90] and induction of cyclin-dependent kinase p27.[91] Sodium butyrate has been used clinically in patients with acute myelogenous leukemias,[43,44] and we have extensive experience with arginine butyrate, a salt of butyrate, in clinical studies for the treatment of β-hemoglobinopathies[45,47] and of refractory solid neoplasms.[92]

However, although other histone deacetylase inhibitors have demonstrated some antitumor activity in cultured lymphoma cells and in preclinical models of lymphoma,[93,94] butyrate and derivatives, including phenylbutyrate, phenylacetate, and tributyrin, have shown minimal or no activity as single agents against a variety of hematopoietic and solid malignancies. For example, a phase 1 trial in 12 patients with advanced refractory neoplasms, using arginine butyrate at intravenous doses comparable to those used in this study, produced no sustained partial or complete responses.[92] It is therefore not likely that the clinical responses observed in this study are due to the arginine butyrate component of the combination therapy alone.[95-97] Similarly, it is unlikely that ganciclovir, as a single agent, contributed independently to the responses observed, as the majority of the patients (12 of 15) had already received ganciclovir at therapeutic antiviral doses in an attempt to control their disease prior to the initiation of the combination protocol. In EBV-infected B-cell lines and viable tumor tissue obtained from patients, arginine butyrate induced TK gene expression and rendered the tumor cells susceptible to ganciclovir in vitro, whereas neither arginine butyrate nor ganciclovir as single agents had a significant antitumor effect.[39]

The induction of the TK gene and gene product by arginine butyrate is a result of the HDAC-inhibitory activity of the compound. Diverse, structurally distinct HDAC-inhibitory compounds can also induce TK expression in cells latently infected with EBV.[41] Histone hyperacetylation leads to induction of the EBV immediate-early transcriptional activators ZTA and RTA, which initiate a program of gene induction, including the viral TK gene.[41] There are at least 4 different types of latent gene expression patterns in tumor cells and lymphoblastoid cells infected with EBV. The differing patterns of latent gene expression for PTLD and for NHL are well established. Although we did not verify the latency patterns in this study, our finding that both PTLD and NHL tumors, which display different patterns of latent gene expression, responded in vivo, as did $EBV^+$ lymphoblastoid cell lines in vitro, suggests that the pattern of gene expression (with regard to these 3 types of latency patterns) does not affect the ability to respond to the combination of arginine butyrate and ganciclovir.

The dose-limiting toxicity observed in these studies was somnolence or stupor, which occurred in 3 of the 14 patients who reached dose levels of arginine butyrate of 1500 mg/(kg/day) or higher and appeared dose related. This adverse event was reversible and did not recur at lower doses. The mechanism underlying this toxicity is not clear. Confusion or stupor is not a recognized common side effect of ganciclovir, although somnolence and confusion were rarely observed during a study of ganciclovir for CMV prophylaxis in a population that received a transplant.[98] Similarly, in 25 patient-years of experience with arginine butyrate as a single agent in patients with hemoglobinopathies, most of which was delivered at infusion rates twice as rapid as administered in this study, somnolence and stupor have not been observed.[45] Infusions with sodium phenylbutyrate, however, have been reported to produce somnolence and confusion as a dose-limiting toxicity.[47,95] Elevations of BUN have been observed in patients receiving high doses of arginine butyrate, or arginine alone, from conversion of L-arginine to urea (not secondary to renal compromise).[48] These elevations in BUN did not cause changes in mental status in subjects treated with arginine butyrate as a single agent, and BUN levels were no higher in the AB + GCV combination reported here than they were in prior studies of AB alone. It is possible, however, that prior radiation therapy and/or transplantation and ganciclovir predisposed the patients receiving both agents to CNS effects, as observed with ganciclovir administered for CMV following transplantation. In contrast, the more common adverse effects of ganciclovir on hematopoiesis did not generally occur in the present study.

Limiting the dose of arginine butyrate to 1000 mg/(kg/day) is not likely to compromise the antineoplastic actions of this combination. In the majority of the responding patients, responses were noted within the

first week of treatment, whereas the dose of arginine butyrate was still being escalated from 500 to 1000 mg/(kg/day) and before the 1500 mg/(kg/day) dose was reached. Indeed, unexpectedly rapid tumor lysis contributed to some of the fatal outcomes in this pilot study, with regression of tumor leading to uncontrollable hemorrhage in one patient, rejection of a transplanted lung, and suspected bowel perforation in another patient. Release of Fas ligand from a NK/T-cell lymphoma, which contains high levels of this apoptosis-inducing protein, likely contributed to acute hepatic and pancreatic necrosis in one patient following a course of treatment and complete tumor regression.[50] These fatal outcomes and other comorbidities prevented assessment of the durability of the observed antitumor activity in most cases. One patient who was treated for end-stage PTLD following marrow transplantation for acute myelogenous leukemia, and had extensive PTLD involvement of the CNS, has had no recurrence of PTLD for longer than 5 years following a single treatment cycle.

Recent in vitro studies now suggest that exposure of $EBV^+$ tumor cells for as little as 6 hours may be sufficient to sensitize them to nucleoside analog antiviral agents.[99] Shorter, more convenient infusion regimens of this combination merit evaluation for efficacy.

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## Authorship

Contribution: S.P.P. and D.V.F. designed and performed the research, collected and analyzed data, and wrote the paper; O.H., T.S., F.S., R.O., F.B., J.F., A.L., S.J.M., M.D., A.M.G., C.K., and S.H. contributed and collected data; M.A. collected data.

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