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PROTOCOL

A PHASE I TRIAL OF BUTYRATE and GANCICLOVIR IN EBV-ASSOCIATED MALIGNANCIES

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1.0 Objectives

1.1 To evaluate the safety of administration of arginine butyrate, and to define any drug-related toxicity and the reversibility of such toxicity.

1.2 To study the clinical pharmacology of arginine butyrate in combination with Ganciclovir, in the setting of EBV-INDUCED MALIGNANCIES and LYMPHOPROLIFERATIVE DISEASE, including plasma half-life and the major routes of elimination.

1.3 To assess the biologic effects of arginine butyrate to induce sensitivity to ganciclovir in tumor samples (Samples will be obtained only in selected patients with accessible lesions who give specific consent).

1.4 To observe any antitumor activity (within the context of a Phase I study).

2.0 Background and Rationale:

We describe a virus-directed strategy for treating Epstein-Barr Virus (EBV)-associated tumors: Nasopharyngeal Carcinoma, Hodgkin's Disease, African Burkitt's lymphoma, certain Non-Hodgkin's lymphomas, and B cell lymphoproliferative disorders (EBV-LPD). This strategy is based on induction of the EBV thymidine kinase gene in latently infected tumor cells employing the experimental drug Arginine Butyrate. After induction of the viral thymidine kinase gene by Arginine Butyrate, addition of the FDA-approved nucleoside antiviral, ganciclovir, then leads to specific killing of virus-infected cells which express the viral thymidine kinase and spares normal cells.

A. Epstein-Barr Virus:

EBV is a 172 kb *herpesvirus*.[1] Virus tropism is determined by complement receptor type 2[2,3] which mediates attachment of the envelope protein gp350/220[4] to B and some T lymphocytes, follicular dendritic cells and epithelial cells. *In vivo* EBV undergoes lytic replication after initial infection of oropharyngeal epithelia. The genome in linear form is duplicated, packaged into the viral capsid and extruded from the cell by budding or lysis. ~100 viral proteins are synthesized during this lytic stage of the virus life cycle.[5,6] In contrast, normal B-cells incubated with EBV *in vitro* are efficiently immortalized and develop into continuously growing lymphoblastoid cell lines (LCLs). The cellular events regulating these distinct outcomes are not understood. In immortalized cells, the genome circularizes, amplifies and replicates coordinate with, and dependent upon, cell division. Because no viral particles are produced, infection is considered to be latent.[5,6] EBV persists in B cells for life. Outgrowth of latently infected B-cells is prevented by T-Cell immune surveillance. In immortalizing latent infection, only 11 gene products are detected, including 6 nuclear antigens (EBNA-1, -2, -LP, -3A, -3B, -3C), 3 membrane proteins (LMP-1, LMP-2A/2B) and two small, non-poly(A) RNAs (EBER-1/-2).[5-7] In EBV(+) tumors such as Burkitt's lymphoma, neoplastic genetic events have often superseded the requirement for viral immortalizing functions, and gene expression may be limited to EBNA-1.[8]

EBV is a common and worldwide pathogen. Childhood infection is asymptomatic. ~50% of individuals with delayed exposure, however, develop a self-limited lymphoproliferative syndrome, infectious mononucleosis.[1] EBV is also detected in 2 endemic tumors: African Burkitt's lymphoma (BL)[9] and nasopharyngeal carcinoma (NPC).[10] Recently, some T-cell and B-cell lymphomas,[11] as well as ~50% of Hodgkin's lymphomas[12] have been found to contain EBV.

The development of a large patient population with T-cell dysfunction in the 1980's, caused by profound iatrogenic immunosuppression required to facilitate solid organ and bone marrow transplantation and by the AIDS epidemic, led to the discovery of 2 additional EBV-related illnesses: hairy leukoplakia,[13] and B-lymphoproliferative disease (EBV-LPD).[14]

B. Burkitt's Lymphoma. Incidence in U.S.A.: 60 EBV(+) patients per year

Burkitt's lymphoma occurs several years after the primary infection with EBV, in immunocompetent patients. Burkitt's lymphoma is a monoclonal lymphoma, as opposed to infectious mononucleosis, which is a polyclonal disease.[15] The disease is distinctly different in Africa (called "endemic"; 97% EBV-positive and common [100:10^6 children]) versus America (called "sporadic"; 20% EBV-positive and rare [1-2:10^6 children, 300 cases per year]). African Burkitt's lymphoma is characterized by rapid growth of the tumor at nonlymphoid sites such as the jaw or the retroperitoneum. The tumor is of B cell origin and is closely related to the small noncleaved cells of normal lymphoid follicles.[16] The biopsy specimens from African Burkitt's lymphoma invariably contain the EBV genome and are positive for EBNA.[17] This contrasts with the non-African Burkitt's lymphoma, in which only 15% to 20% of the tumors contain the EBV genome. EBV has a worldwide distribution and infects most (more than 90%) individuals before adulthood. The clustering of Burkitt's lymphoma in the equatorial belt of East Africa remains unexplained. It has been hypothesized that alterations of the immune system, possibly due to hyperstimulation by endemic malaria, may play an important role in the outcome of an EBV infection to individuals in this region.[18,19] Individuals from this region show impairment in virus-specific cytotoxic T-cell activity. Normally, it is the T-cell response to EBV infection that limits B-cell proliferation, and this T-cell response is directly stimulated by EBV.[20] It has been postulated that the failure of the T-cell immune response to control this proliferation could lead to excessive B-cell proliferation and, as such, provide a suitable background for further mutation, oncogenic transformation, and lymphomagenesis. A scenario has been suggested for the involvement of EBV in the etiology of African Burkitt's lymphoma.[21] The first step involves the EBV-induced immortalization of B lymphocytes in a primary infection. The second step involves the stimulated proliferation of EBV(+) B cells. This step is facilitated in the geographic areas where Burkitt's lymphoma is endemic (presumably because of the presence of malaria), through B-cell triggering and the suppression of T-cells involved in the control of the proliferation of EBV-infected cells. This pool of cells becomes increased in size as a target cell population for random chromosomal rearrangements. The third and final step is the reciprocal translocation involving a chromosomal locus with an immunoglobulin gene and the c-*myc* gene on chromosome 8. This leads to the deregulation of the c-*myc* gene, to the development of the malignant clone, and to the appearance of a tumor mass.[22] Alternative scenarios have been proposed in which the order of the steps are rearranged such that the B-cell activation by malaria precedes the chromosomal translocation and is followed by EBV infection.[23] Regardless, the components of these two scenarios each account for the geographic distribution of Burkitt's lymphoma, the critical involvement of EBV in lymphomagenesis, and the eventual selection and clonal outgrowth of a population of cells with the critical translocation involving the deregulation of the c-*myc* gene on chromosome 8.

Treatment of Burkitt's lymphoma is most commonly chemotherapy, with radiotherapy playing a minor role. Prolonged survival rates of 50% can be achieved.

C. Nasopharyngeal Carcinoma Incidence in U.S.A.: 1,200 patients per year

Nasopharyngeal carcinoma (NPC) has also been linked to EBV.[10] The incidence of nasopharyngeal carcinoma (NPC) in the United States is approximately 0.6 per 100,000 (1,200 cases per year), with a male to female ratio of 2.4:1.[24] The disease can occur at almost any age, but mean age at diagnosis in North America is 51 years. In areas where nasopharyngeal carcinoma is endemic, especially southeastern China, the Philippines, North Africa and the Mediterranean basin, a bimodal age distribution curve (peaks at 15-25 years and 40-60 years) has been described. Although worldwide the annual incidence rates are low, there are areas in China (especially the southern province) in which there is a high rate of about 10 cases per 100,000 persons per year. Almost 20% of malignant tumors diagnosed in the Chinese population of Asia are nasopharyngeal carcinoma, and the incidence is remarkably high in the city of Canton, where the disease sometimes is called "Cantonese cancer."[25-28] Because the incidence among individuals of Chinese decent remains high, irrespective of where they live, a genetic susceptibility has been proposed. For the Cantonese in Singapore, the annual rates of 29 per 100,000 are higher than for other racial groups living in the same locale. In a British Columbia study, the rate of nasopharyngeal carcinoma in native-born Chinese was 118 times that of whites; for North American-born Chinese, it was seven times that of whites.[27] Risk of contracting the disease is greater for American-born Caucasians who relocate to Southeast Asia than for those residing in the United States.[26] A correlation of certain HLA haplotypes has been noted among the Chinese, but these associations do not hold true for evaluation of NPC in Tunisia. A disease susceptibility gene has been indicated at the human leukocyte antigen HLA-2 histocompatibility locus.[25] Although genetics are believed responsible for nasopharyngeal carcinoma incidence in the Chinese population, environmental factors, including fumes, chemicals, smoke, and ingestion of salt-cured fish also are considered very important.

EBV genomes are found in nearly all biopsies of undifferentiated NPC specimens from all over the world.[20,29] The genome exists in the epithelial cells of the tumors.[30] The EBV genome is transcriptionally active within these tumors, and the regions transcribed in the NPC biopsies are the same as those expressed in latently infected lymphocytes.[31] These molecular observations are consistent with an active role for EBV in the neoplastic processes involved in NPC. No characteristic chromosomal translocations have been found in NPCs. Attempts to identify mutated or activated cellular oncogenes in NPC also have not been successful. Viral proteins associated with EBV-induced *in vitro* cellular production are seen in tumor cells,[27,28,32] and elevated levels of IgA and IgG antibodies to viral capsid and early antigens are found in 90% of cases. Treatment is predominantly *via* supervoltage irradiation, as most such tumors are not resectable and are not responsive to chemotherapy. 5 year survivals in excess of 50% have been reported with modern techniques.

D. Hodgkin's Disease: Incidence in U.S.A.: 3,500 EBV(+) patients per year

For more than 20 years, a role for EBV in the pathogenesis of Hodgkin's disease (HD) was postulated based on epidemiologic evidence linking Hodgkin's patients with EBV seropositivity and elevated EBV titers.[33-36] A number of studies have found an increase (2-5 fold) in the incidence of HD after infectious mononucleosis. However, some Hodgkin's patients were seronegative for EBV. Therefore, the association between EBV and Hodgkin's disease remained speculative until 1987, when molecular genetic analysis showed that some Hodgkin's tissues contained monoclonal EBV DNA[37] and that the virus was localized to Reed-Sternberg (RS) cells (the malignant cells in HD).[12] Subsequent immunohistochemical [38] and serologic data [39] support an association between EBV and Hodgkin's disease and confirm the localization of the virus to cytologically malignant-appearing RS cells and variants.[40-44] EBV also infects variable numbers of small B and T lymphocytes in the reactive inflammatory cell infiltrate that composes the bulk of Hodgkin's tissues.[45,46]

In most series of cases reported to date, EBV is associated with approximately half of mixed cellularity Hodgkin's disease cases and a somewhat lower percentage of the nodular sclerosing subtype. In contrast, lymphocyte-predominant Hodgkin's disease is rarely EBV-associated.[45] Although EBV DNA has been identified in the lymphocyte depletion variant of Hodgkin's disease, there is controversy over the morphologic criteria for diagnosis of this variant and its distinction from anaplastic large-cell lymphoma, in which EBV is also implicated as a pathogen.

Clonal and non-clonal EBV genomes are present in Hodgkin's disease. Expression of the viral oncogene LMP (latent membrane protein) is seen in RS cells. Expression of the oncogene LMP (latent membrane protein) is seen in RS cells. In HD, the region of the (viral) BNLF1 oncogene coding for the amino terminal and transmembrane domains (associated with oncogenic function) of LMP appears to be homogeneous, whereas the region coding for the intracytoplasmic (carboxy terminal) domain of LMP is heterogeneous. Cytological similarities between SR cells and immunoblasts of known EBV-induced infectious mononucleosis and EBV-induced AIDS-related lymphomas are consistent with the hypothesis that the EBV-BNLF1 oncogene is an inducer of morphological features of RS cells. Whether chromosomal integration of EBV DNA is an important factor in activation of such a transforming activity remains to be elucidated. Therefore the RS cells appear to be derived from lymphocytes beyond the pre-B-cell or common thymocyte stage, which may or may not subsequently become infected by EBV.

The high prevalence of EBV in Hodgkin's disease implies an etiologic role for the virus in Hodgkin's tumorigenesis. This pathogenetic theory is supported by the monoclonality of EBV DNA in these tumors.[37,46-55] In one series, monoclonal EBV DNA was detected in all 17 cases having EBNA1-positive RS cells.[56] Because tumor-associated viral DNA is monoclonal, it is likely that virus infection preceded clonal expansion. This reinforces the hypothesis that the virus is not an innocent bystander, but rather plays a role in the pathogenesis of the Hodgkin's disease and the other tumor types in which it is found.[57] The observation of EBNA1 expression in the RS cells of clonally-infected cases indicates that the clonal virus is localized to these cells and suggests that Hodgkin's disease results from the transformation of an EBV-competent cell. Other studies, however, suggest that this virus is modulating rather than an etiologic agent in a considerable proportion of HD cases.

Previous investigations into the biology of EBV infection have shown that only one viral particle successfully infects a given cell.[58] Once the viral DNA is established inside the cell, it circularizes and reproduces itself to yield multiple identical copies of viral DNA.[59] In this way, tumors derived from infected cells can have multiple copies of EBV per cell, while maintaining clonal viral DNA structure. The average amount of clonal EBV DNA in Hodgkin's disease tissues varied from 0.5 to 5 copies per cell. Because RS cells comprised only a small fraction (<1%) of all HD tissue cells, the content of EBV DNA in each RS cell is estimated to be at least 100 times higher than the measured average copy number per cell, or at least 50 copies of viral DNA per RS cell. This is comparable with, or greater than, the viral burden in infected non-Hodgkin's lymphomas.[47] The high copy number of EBV in RS cells may relate to the pathobiology of this complex lymphomatous disorder. In agreement with the studies cited above, EBV DNA is abundant and monoclonal in infected RS cells [56] and the presence of EBV in RS cells was strongly and independently linked to mixed cellularity histology and Hispanic ethnicity.

This close and common association of EBV with certain subsets of Hodgkin's disease provides impetus for the novel approach we propose to the therapy of this tumor.

E. T-Cell Non-Hodgkin's Lymphoma. Incidence in U.S.A.: 3,100 T-cell, EBV(+) NHL patients per year

Clonally-integrated EBV is found in association with T-cell lymphomas as well as B-cell lymphomas. Currently, three populations of tissue-restricted T lymphocytes have been recognized; i.e.,

mucosa-associated, cutaneous and nodal T lymphocytes. Therefore, T-cell lymphomas arising from different sites but with similar morphology may show differences in lymphomagenesis, and thus in expression of oncogenes, adhesion molecules, presence of certain DNA/RNA viral sequences, and in clinical presentation and behavior. Primary cutaneous CD30(+) large-cell T-cell lymphomas often remain localized to the skin for a long time, express a unique cutaneous lymphocyte antigen (CLA), known as the skin-homing receptor, have been postulated to be associated with the presence of human T-cell leukemia/lymphoma virus type I (HTLV 1), and have a good clinical course;[60-62] while, in contrast, morphologically similar T-cell lymphomas of nodal origin often behave more aggressively, are CLA-negative, and have been associated with the presence of EBV.[60,63,64] There was no relation between primary cutaneous T-cell lymphoma and EBV. In agreement with this finding, EBV is not present in lymphomatoid papulosis, a premalignant cutaneous lymphoproliferative disorder.[65] EBV-associated T-cell lymphomas are highly site-restricted and are morphologically indistinguishable from EBV(-) T-cell lymphomas.[66] Nasal T-cell lymphomas are EBV-associated.[67-69] The frequency of EBV(+) primary pulmonary T-cell lymphoma is similar to the frequency of EBV(+) primary nodal T-cell lymphoma.[70,71] EBV-associated primary gastrointestinal T-cell lymphomas seem to be rare, but recently a considerable number (36%) of enteropathy-associated T-cell lymphoma cases (EATCL) were reported to be EBV(+),[72] although other studies did not find such an association.[66] Angiocentric immunoproliferative lesions (AILs) of nose (also known as lethal midline granuloma), lung (also known as lymphomatoid granulomatosis), and skin are frequently associated with EBV.[73] Although all AILs in the nose were EBV-positive, the relation between angiocentricity and EBV seems to be more complex. Primary cutaneous and gastrointestinal AILs are EBV(-), whereas primary pulmonary AILs can be EBV(+) or EBV(-). There is no clear relation between angiocentricity and EBV, and only primary site seems to be important in the relation between peripheral T-cell lymphoma and EBV. Thus, there are site-restricted differences in the occurrence of EBV-infected peripheral T-cell lymphomas.

T cells can be infected by EBV, through a yet unidentified mechanism, but probably via CR2 or CR2-like receptors.[74] The close contact between T cells and the upper respiratory tract epithelium, known for its reservoir function for EBV, probably make T cells in this region more vulnerable for EBV infection. The finding that EBV can be found in almost all tumor cells in most cases of primary extranodal, and especially nasal, T-cell lymphoma,[67-69] in contrast to primary nodal T-cell lymphoma, where the number of EBV-infected neoplastic cells varies greatly between the cases [70,75] argues for an etiologic role for EBV in these cases. Moreover, these cases often express LMP-1,[69] known for its transforming and oncogenic properties *in vitro*,[76-79] and are reported to be monoclonal for EBV.[67,80] Thus, there are site-restricted etiologic differences between morphologically identical T-cell lymphomas, of which EBV might be one of many factors.

F. EBV-Induced Lymphoproliferative Disease or Lymphoma in <u>Immunodeficiency</u>

Incidence in U.S.A.: 10,000 B-cell, EBV(+) lymphoma patients per year

EBV is very commonly associated with lymphomas in patients with acquired or congenital immunodeficiencies. These lymphomas can be distinguished from the classical Burkitt's lymphomas in that the tumors may be polyclonal. The tumors also do not demonstrate the characteristic chromosomal abnormalities of Burkitt's lymphoma described earlier. The pathogenesis of these lymphomas involves a deficiency in the effector mechanisms needed to control EBV-transformed cells. The prototypic model for this disease has been the X-linked lymphoproliferative (XLP) syndrome.[81] Patients with XLP who develop acute infectious mononucleosis exhibit the usual atypical lymphocytosis and polyclonal elevation of serum immunoglobulins and increases in specific antibody to VCA and to EA. During these infections, patients with XLP fail to mount and sustain an anti-EBNA response after acute EBV infection. The unique vulnerability of males with XLP to EBV infection is most likely due to an inherited immune regulatory defect that results in the failure to govern the cytotoxic T cells and NK cells required to cope with EBV.

Patients with iatrogenic immunodeficiencies, such as organ transplant recipients, are also at an increased risk for lymphomas, and these lymphomas often contain EBV DNA and EBNA. Also, patients with AIDS are at a higher risk for developing polyclonal lymphomas associated with EBV.

1. EBV-Induced Lymphomas Associated with Immunosuppression:

EBV-Associated Lymphoproliferative Disease (EBV-LPD) is characterized by actively proliferating EBV(+) B-cells, frequently without overt malignant change. These immunoblastic lymphoma-like lesions have been identified in a variety of transplant patients, in patients with congenital immunodeficiency, and in patients infected with HIV.[82] These so called "post-transplant lymphoproliferative disorders" (PTLD) are observed after all transplants, including kidney, bone marrow, heart, liver and lung transplantation. This increased incidence of EBV-LPD in this setting is likely due to the aggressive immunosuppression required after these transplants. Phenotypically, the immunoblastomas resemble large cell lymphomas (LCLs) *in vitro*. They express EBNA1-EBNA6 and LMP. Obviously, these cells proliferate because the surveillance of the host has broken down, rather than owing to any cellular escape.[83] The tumors share several common features, including rapid progression, clinical aggressiveness and a tendency to grow at extranodal sites.[82,84] These EBV(+) B lymphomas generally have an ominous prognosis, especially if monoclonal. In some cases, rapid immune reconstitution has led to spontaneous regression of the tumor. In most cases, however, this has not been possible or is ineffective. EBV-LPD has been particularly tragic in the transplant setting, causing the death of recently 'cured' patients who would be unlikely to develop such disease as their natural immune reconstitution occurred over time. Empirically, nonclonal PTLD may regress with decreased immunosuppression (if that is clinically feasible), whereas regression is less predictable in polymorphic, monoclonal tumors. In contrast, monoclonal monomorphic tumors are frequently more aggressive and tend to progress despite immune modulation.[85,86] Treatment with nucleoside antivirals alone, surgery, cytotoxic chemotherapy, a-interferon with immune globulin, or B-cell directed complement fixing monoclonal antibodies, has rarely been successful. Recently, autologous T-cell transfusions were applied with some success in LPD after allo-BMT,[87] but are unlikely to become a routine therapy and would be inappropriate in tumors of host origin.

The EBV association with PTLD is based on several clinical observations. Almost all patients with PTLD have serologic evidence of an EBV infection and the disease involves a proliferative disorder of B cells. The B cell lymphoproliferation can be either monoclonal or polyclonal. EBV-LPD is characterized by latent viral replication. The genome persists as a circular episome within the infected B-cells, and lytic replication is restricted.[84] Additional evidence that EBV is associated with post-transplant lymphoproliferative disorders is the finding that patients without any prior exposure to EBV are at highest risk for developing the lymphoproliferative disorder. Patients who are serologically EBV(-) are at the highest risk when receiving an EBV(+) transplant.[88-90] Supporting this observation is the finding that EBV-associated PTLD is more frequent in children receiving transplants than in adults. The incidence of EBV-associated PTLD is three-fold greater in some series of pediatric transplantation.[91] An intriguing report suggested that the EBV is carried with the graft. In this case, a common donor gave rise to PTLD in two separate organ recipients. Both recipients demonstrated the same chromosomal translocation in their tumors.[88-90] We have recently reported a similar finding (Mentzer and Faller, 1994).

Lung transplant recipients are a special sub-group of patients at risk for developing PTLD. Most lung transplant recipients require relatively high immunosuppression for prevention of acute and chronic graft rejection. The development of PTLD and these patients has been estimated at 5% to 10%. The clinical presentation has ranged from systemic adenopathy to masses and infiltrates in the transplanted lung. A review of the literature again suggests two distinct clinical syndromes in lung transplant

recipients with PTLD. One scenario involves the polyclonal lymphoproliferative disorder commonly associated with mononucleosis and other benign lymphoproliferative diseases. This syndrome appears to respond to a decrease in the immunosuppressive regimen. The second clinical presentation involves a more aggressive form of lymphoproliferative disorder; one which histologically resembles immunoblastic non-Hodgkins lymphoma (NHL). This EBV-associated lymphoma is often monoclonal and can display an aggressive clinical course. The tumor is typically unresponsive to conventional chemotherapy and has been uniformly fatal. Although withdrawal of immunosuppression has been attempted in many cases, the recipients' dependency on graft function for adequate oxygenation complicates any reduction in the immunosuppression regimen.

2. AIDS-Related, EBV-Associated Lymphomas

As a result of effective antiretroviral therapy, prophylactic measures, and rigorous treatment of opportunistic infections, HIV-infected individuals now live longer. More than 40% of people with AIDS will develop a neoplastic disease, resulting in severe morbidity and, often, death. Intermediate or high-grade B-cell lymphoma was added as an AIDS-related malignancy in 1985. Prolonged survival in HIV-infected patients is associated with increased incidence of lymphoma. Between 1940 and 1980, the US incidence of lymphoma doubled. The AIDS epidemic, however, has superimposed on this underlying trend an additional risk of lymphoma between 60 and 100 times that expected in the HIV-negative population. Lymphoma is likely to be a late manifestation of HIV infection, as documented in France, where 33% of lymphomas occurred after an AIDS-defining illness. In the US, only about 3% of cases of AIDS are diagnosed simultaneously with lymphoma. Lymphoma occurs among all population groups at risk for HIV in all age groups and in diverse geographic regions. In the HIV-negative population, women have a lower incidence of lymphoma than men, and the incidence increases with age in homosexual/bisexual men and in hemophiliacs. The same incidence patterns are seen in the HIV-positive population, except among intravenous drug users, who may succumb relatively early to various infections. 80-90% of AIDS patients who are diagnosed with lymphomas are either intermediate or high-grade B-cell tumors, such as immunoblastic or large-cell types, or small noncleaved lymphoma, which may be subclassified as either Burkitt's or 'Burkitt-like.' Among HIV-negative lymphoma patients, however, high-grade lymphomas represent only 10% to 15% of all lymphomas.

The etiology and pathogenesis of lymphomas in patients infected with HIV is multifactorial and at present are not fully understood. Immunosuppression is thought to be a primary factor correlated with an increased incidence of lymphoma in several settings, including certain congenital immune deficiency diseases, autoimmune disorders, and the chronic use of immunosuppressive drugs, as occurs in patients who have undergone organ transplantation. The lymphomas which develop in these settings are similar to the AIDS lymphomas in terms of the pathologic type, the high frequency of extranodal disease at presentation, and the relatively poor prognosis. It is clear that EBV plays a major role in AIDS lymphomagenesis. This may perhaps be the result of damaged immunosurveillance of EBV-infected cells in AIDS patients.

There appears to be some biologic significance to the site of disease. Patients with lymphoma primary to the central nervous system have an extremely poor prognosis and progressive HIV infection, with about 75% having a history of AIDS prior to development of the lymphoma as well as low CD4 cell counts ($<50\ mm^3$). Pathologically, these primary central nervous system lymphomas are almost always immunoblastic or large-cell lymphomas, and are uniformly associated with EBV. In contrast, improved prognosis has been associated with the large-cell type of systemic lymphoma, exclusive of the central nervous system. Clinicopathologic correlations are imprecise at best, because patients who develop lymphoma as the first AIDS-defining diagnosis may be distinct from those who develop lymphoma after preceeding opportunistic illness.

RATIONALE FOR COMBINATION THERAPY

Like herpes simplex virus (HSV) and varicella-zoster virus (VZV), EBV encodes a thymidine kinase (TK) enzyme localized to the BAM HI X fragment of the genome.[92] In a rate-limiting step, the TK converts nucleoside analogs to their monophosphate form.[92,93] 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. ACV is a purine nucleoside analog with a linear side chain replacing the cyclic sugar of guanosine. GCV differs from ACV in the addition of a hydroxymethyl group to the side chain. However, ACV and GCV differ in functional assays. Whereas HSV TK preferentially phosphorylates ACV, EBV-TK preferentially phosphorylates GCV.[94,95] Furthermore, because GCV triphosphate accumulates to higher levels and persists for longer periods in infected cells than ACV, GCV produces more interference with cellular DNA synthesis than occurs with ACV. In a recent and exciting study, selective toxicity of GCV for cells expressing HSV-TK was utilized to promote tumor killing in the CNS. Rapidly dividing murine glioma cells were infected *in vivo* with an amphotropic retrovirus containing HSV-TK. Animals were then treated with GCV, which killed TK+ tumor cells, sparing adjacent normal cells that replicated too slowly for efficient infection and viral TK expression ([96], see also [97,98]).

EBV's susceptibility to antiviral drugs that inhibit replication of other herpesviruses has been difficult to assess, as no adequate system for studying lytic replication *in vitro* exists (i.e., no plaque assay exists, and efficient *in vitro* infection of epithelial cells followed by lytic replication does not occur). When ACV (acyclovir) and later GCV (ganciclovir) were used to treat AIDS-related herpesvirus infections, regression of hairy leukoplakia was inadvertently observed, establishing the efficacy of these agents *in vivo* for treatment of lytic EBV disease.[94,99,100] Latent EBV disease, however, was unaffected.

Latently-infected B-cells do not express TK. However, exposure of these cells to the compound Arginine Butyrate or the orally-bioavailable analogue Isobutyramide results in modest induction of lytic replication. Our preliminary studies suggest that even when virus production is minimal, many EBV genes active during the lytic cycle are induced by such treatment. Activation of EBV-TK has been previously reported using sodium butyrate.[101] We have demonstrated that Arginine Butyrate and Isobutyramide induce EBV-TK activity in EBV-immortalized B-cells and patient-derived tumor cells (see below). Recent isolated case reports of administration of butyrates to patients for malignancies exist.[102,103]. We have administered Arginine Butyrate and Isobutyramide to adults and children over extended periods of time without major side effects (Perrine, et al, 1993b; 1994c[104,105]). These drugs were recently approved for human studies to induce fetal Hb in children with sickle cell anemia and b-thalassemia (Perrine, et al, 1993b; 1994c[104,105]). Our preliminary *in vitro* studies demonstrate that induction of EBV-TK activity in EBV-immortalized B-cells and patient-derived tumor cells using these drugs is possible, and that these previously-resistant cells are rendered susceptible to ganciclovir therapy (see below). We hypothesize that sequential treatment of patients with EBV-associated tumors with Arginine Butyrate (to induce the EBV-TK) and GCV (to eliminate EBV-TK expressing tumor cells) might be an effective, nontoxic therapy.

It should also be noted that this potential therapy does not depend on the associated EBV genome being the cause of the tumor. Just the presence of the EBV genome in latent form would be predicted to make the tumor susceptible to this combination protocol.

2.1 Introduction: Butyrates

Butyrate is one many naturally-occurring short-chain fatty acids that are generated in the small and large bowel by metabolism of carbohydrates. Butyrate is a four-carbon fatty acid with weakly acidic

properties, and is rapidly absorbed and metabolized. Butyrates have shown significant anti-tumor effects. Sodium butyrate (NaB) has been used clinically in patients with acute myelogenous leukemias[102,103] and we now have extensive experience with Arginine Butyrate, a salt of Butyrate, in clinical studies for the treatment of b-hemoglobinopathies,[105] and more recently with refractory solid neoplasms.[106-109] Butyrate, and derivatives of the parent compound, including Arginine Butyrate, have demonstrated several effects upon transformed cell lines *in vitro* which include 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 of a given cell type examined.[110-116] For example, human tumor cell lines as diverse as colon, breast, melanoma, hepatoma, squamous cell carcinoma of the cervix, endometrial, adenocarcinoma, teratocarcinoma cell lines, leukemic cells (HL-60) and normal human keratinocytes can all be induced to differentiate in the presence of butyrate concentrations ranging from 2-5 mM.[117-135]

Mechanisms of Action of Butyrates: Multiple mechanisms of action for Butyrate have been postulated and include:

1) Differentiation of Tumor Cells

Butyrates have been shown to induce differentiation of tumor cell lines. The mechanism(s) of action proposed for these effects upon differentiation are varied, and are not fully understood. Butyrate is known to inhibit histone (nuclear) deacetylase, which results in hyperacetylation of histones H3 and H4.[136,137] When histones are acetylated, they have a reduced affinity for chromatin, thus allowing for chromosomal unfolding, and possibly enhancement of expression of certain genes. It is postulated that Butyrate acts to prevent histones from binding to key regulatory regions on chromatin designated as nuclear scaffolding-attached regions,[110] and nuclear matrix-attached regions, with a net result that promoter regions of certain genes are exposed for expression.

Butyrate-associated induction of genes have been characterized for various cell types, and the genes are consistently in the class of "differentiation markers" of a cell. For example, in colon cancer cell lines, morphologic changes observed in the presence of Butyrate correlate with increased expression of alkaline phosphatase, plasminogen activator and CEA, all markers of differentiation.[123,125,127,130,132,133,138,139] Hepatoma cell lines increase expression of alpha fetoprotein.[111,113] Breast cancer cell lines express milk-related glycoproteins, epithelial membrane antigens and increased lipid deposition.[121,122,135,140] NaB can also induce expression of cellular proteins associated with converting basal keratinacytes into committed epithelial cells.[126,128]

2) Alteration of Expression of certain Transcription Factors that Regulate Cellular Gene Expression and Regulation of the Cell Cycle.

In the breast cancer cell line MCF-7, Butyrate induces a block in cellular proliferation which is associated with decreased expression of estrogen and prolactin hormone receptor mRNA expression, thus blocking the potential growth stimulation by estrogen and prolactin.[121,122,135] These effects are associated with increased expression of the EGF receptor. Butyrate also has been shown to induce downregulation of c-*myc* and p53 mRNA, and upregulate expression of the c-*fos* transcription factor. In mouse fibroblasts, butyrate will block the cell cycle in the G_1 phase.[112] When these cells are stimulated to proliferate with serum, TPA or insulin, the immediate-early response transcription factors c-*myc* and c-*jun* are upregulated. However, the late G_1 phase "downstream gene" marker *cdc*-2 mRNA is not expressed, and the cells are prevented from entering S phase. Butyrate is an effective cell cycle blocker, associated with a putative restriction point, related to termination of expression of a labile

protein.[141-143] It is generally thought to block cell cycle progression in G_1 [119,140] but might also inhibit some cell types at a point in G_2.[144] Decreased p53 levels in correlation with butyrate treatment[142,145,146] and the inhibition of polyomavirus DNA replication in p53 and Rb-knockout primary mouse fibroblasts in response to butyrate appear to rule out a direct involvement of these gene products individually in the mechanism of butyrate and imply that a putative control point perhaps lies at a later step in the cell cycle. It therefore appears that the butyrate effect is likely to involve a mechanism fundamentally conserved among cell types, but it does not appear to be exerted directly via the Rb or p53 gene product.

3) Regulation of Viral Growth:

The *Herpesvirus* family members are capable of bypassing the butyrate-mediated block, which is probably due to the role of viral early genes in DNA synthesis, such as the viral DNA polymerase, DNA-binding protein, and helicase genes.[147] Butyrate treatment has been reported to result in the induction of the major CMV major immediate-early protein (IE1) by activating the IE1 promoter via cellular factors[148] in a human epithelial thyroid papilloma carcinoma cell line, and in cultured endothelial cells[149] under conditions that are conducive to terminal differentiation.[150] Similarly, EBV early antigen is induced by butyrate in the P3HR-1 cell line[151-155] as well as Raji [101,151,152,156] and NC37[101,153] cell lines. These results suggest that Butyrate exerts some of its effects on viral growth at the level of gene transcription. This conclusion is also supported by the observation that butyrate activates the long terminal repeat-directed expression of human immunodeficiency virus[157] and induces the Moloney murine sarcoma virus *via* a putative butyrate response enhancer-promoter element.[158,159] Therefore, butyrate appears associated with a general induction of early viral proteins. Butyrate has been reported to exert additional cytostatic effects such as G_2/M blockage and antiviral activity against RNA viruses.[114,144,160-162]

Many *Herpes*-family virus-infected cells, including cells infected by HSV and CMV, can be killed by nucleoside analog antiviral drugs like ganciclovir and acyclovir. Unlike other members of the *Herpesvirus* family, EBV is resistant to the antiviral agent ganciclovir, because of low levels of viral thymidine kinase. We have demonstrated in the *in vitro* studies shown above that exposure of EBV-transformed B-cells or tumor cells to Arginine Butyrate induces EBV-TK and renders them sensitive to ganciclovir.

4) Preclinical and Clinical Toxicity and Pharmacology:

The published acute oral LD_{50} of sodium butyrate in rats is 8.79 g/kg. Sodium butyrate was given at 1 g/kg IV tid in rhesus monkeys, with the only side effects recorded as decreased appetite and transient anemia that resolved within 3 days of infusion. There were no adverse effects upon liver or renal function tests (FDA Investigator's Brochure). Continuous infusions in dogs at 2 g/kg/day reached steady-state butyrate plasma levels of 0.28 mM which persisted less than 5 minutes after infusion. Adult baboons treated with NaB at 1 g/kg/day (continuous Iinfusion) demonstrated an increase in fetal hemoglobin synthesis without adverse effects. Acute LD_{50} levels of a single IV dose of Arginine Butyrate was determined by SRI International (Menlo Park, CA) in rats to be 513 mg/kg. One post-partum rhesus monkey was treated with 2 gm/kg/day of Arginine Butyrate for 3 courses of up to 3 weeks in length. The only side-effect noted was anorexia and occasional vomiting, which resolved upon discontinuation of the drug.

Human experience with butyrates have been performed in children with acute myelogenous leukemia in Israel, and in children with neuroblastoma.[102,103,163] Doses of sodium butyrate ranging from 0.5-1.5 g/kg/day for 14 days, and 10 g/kg/day IV infusions were given without toxic side effects. IV

infusion of similar doses of sodium and Arginine Butyrate in adults resulted in no toxic side effects, and elevated plasma butyrate levels returned to normal within 15 minutes of discontinuation. Eleven pediatric patients and twelve adults with b-hemoglobinopathies were treated with IV Arginine Butyrate at a starting dose of 500 mg/kg/day for 2-7 weeks (six of these patients have been reported in Ref. [105]). If no side effects were noted, the rate was increased by 250 mg/kg/day to a final rate of 1500 mg/kg/day in 4 patients, and 2000 mg/kg/day in 2 patients. Side effects were a transient, slight rise in serum aminotransferase in a patient with sickle cell disease. This same patient, and one other also, had a brief increase in blood urea nitrogen, but not creatinine, at the end of a continuous infusion over 2 weeks. The rise in BUN returned to normal within 12 hours of discontinuation of drug, and is thought to be secondary to effect of arginine upon ureagenesis. Transient anorexia developed in one patient. Peak serum levels of butyrate ranged between 0.04-0.05 mM, and butyrate was not detectable 15 minutes after infusion was discontinued. Plasma arginine levels were also rapidly cleared, with a $t_{1/2}$ of 15 minutes, and baseline arginine levels were reached within 12 hours after discontinuation of infusion. In all six patients, Arginine Butyrate was able to induce fetal hemoglobin synthesis by 6-45% above pretreatment values. Butyric acid is already approved by the FDA for oral use in foods and flavoring.

In patients with refractory malignancies, to date, three patients with refractory neoplasms (breast, melanoma, adenocarcinoma of unknown primary) have been enrolled and completed 1-2 cycles of Arginine Butyrate at a dose of 500 mg/kg/day over 6-8 hours for 10 days.[106,107,109] There were no objective toxicities noted for the hepatic, renal, cardiac, pulmonary or hematopoietic systems. 2 of 3 patients experienced grade 1 nausea/vomiting which was controlled with oral anti-emetics. No objective tumor responses were noted. 3 patients (breast cancer, bowel cancer, Hodgkins disease) have completed 2 cycles of Arginine Butyrate at a dose of 750 mg/kg/day for 10 days. 1 of the 3 patients had grade 1 nausea/vomiting which was controlled with oral anti-emetics. 1 patient had a transient elevation of serum BUN, without elevation of serum Cr, and proteinuria measured by dipstick. These abnormalities resolved within 5 days of discontinuation of the drug. Otherwise, there were no other objective measures of organ toxicity. 2 patients had stable disease after two cycles, with no progression. The tumors progressed after treatment was stopped. 1 patient had a partial response (>80%) after 1st cycle, with resolution of lymphadenopathy and leukocytosis, and complete resolution of back pain, with all narcotics stopped. He further resolution of disease during the 2nd cycle. Disease progression was noted 1 month after 2nd cycle was completed. At 1000 mg/kg/day, 3 patients have been enrolled on study. It is too early for tumor response evaluation at this level.

3.0 Drug Formulation and Availability:

Arginine butyrate: Arginine butyrate injection (butyric acid) 50 mg/ml (for investigational study use only). Butyric acid is a short chain fatty acid present in foods such as dairy products. Arginine is an amino acid which is commonly used in hyperalimentation solutions in hospitals throughout the country. Butyric acid is obtained from Aldrich, catalog number W-212. L-Arginine is anhydrous. Arginine is combined with butyric acid, pH 5.0-5.5, in sterile, non-pyrogenic water. The drug will be manufactured under GMP conditions at the University of Iowa College of Pharmacy under FDA approval. Stability testing has shown the drug to be stable for two years. Since arginine butyrate solution is hypertonic (50 mg/ml), it must be infused through a long or deep IV access to avoid peripheral venous irritation. Arginine butyrate will be infused via a portable cassette infusion pump. European pharmacokinetic studies of arginine butyrate given as a “slow” and 90 minute bolus of 500 mgs of a 5% solution (2.5 g IV) in two normal volunteers, and found a 15 minute elimination period.

Ganciclovir: Ganciclovir sodium is a sterile powder which is reconstituted for intravenous administration only. Each vial of ganciclovir contains the equivalent of 500 mg gancyclovir as the sodium salt (46 mg sodium). Reconstitution with 10 ml of sterile water for injection. USP, yields a solution with pH 11 and a gancyclovir concentration of approximately 50 mg/ml. Further dilution in an appropriate intravenous solution must be performed before infusion. The chemical name of gancyclovir sodium is 9-(1,3-dihydroxy-2-propoxymethyl)guanine, monosodium salt, with a molecular formula of C9H12N5NaO4 and a molecular weight of 277.21.

Ganciclovir is a synthetic nucleoside analogue of 2'-deoxyguanosine that inhibits replication of herpes viruses both in vitro and in vivo. Sensitive human viruses include cytomegalovirus, herpes simplex virus, Epstein-Barr virus, and varicella zoster virus. Available evidence suggests that there is preferential phosphorylation of gancyclovir in virus infected cells compared to uninfected cells (10-fold difference). The catabolism of gancyclovir is slow in vitro, with up to 60-70% of the gancyclovir-triphosphate in the cells at 18 hours. The antiviral activity is thought secondary to inhibition of DNA synthesis by two known mechanisms: (1) Competitive inhibition of viral DNA polymerases, and (2) direct incorporation into viral DNA, resulting in eventual termination of viral DNA elongation. Cellular DNA polymerase alpha is inhibited also, but at a much higher concentration than for viral DNA polymerase.

Ganciclovir inhibits mammalian cell proliferation in vitro at 10-60 ug/ml, with bone marrow colony forming cells being the most sensitive (ID50 > 10 ug/ml) of all cell types tested.

The pharmacokinetics of ganciclovir have been evaluated in immunocompromised adults with serious CMV infections. 22 adults with normal renal function received 5 mg/kg doses infused IV over 1 hour. The plasma level of gancyclovir at the end of the first hour infusion (Cmax) was 8.3 +/- 4.0 ug/ml, and the plasma level 11 hours after the start of infusion (Cmin) was 0.56 +/- 0.66 ug/ml. The plasma half-life was 2.9 +/- 1.3 hours, and systemic clearance was 3.64 +/- 1.86 mL/kg/min. Multiple dose kinetics were measured in eight patients with normal renal function who received gancyclovir 5 mg/kg twice daily for 12-14 days. After the first dose, and after multiple dosing, plasma levels at the end of infusion were 7.1 ug/ml and 9.5 ug/ml, respectively. At 7 hours post-infusion, plasma levels after the first dose were 0.85 ug/ml and 1.2 ug/ml after multiple dosing.

Renal excretion of the unchanged drug by glomerular filtration is the major route of elimination of ganciclovir. In patients with normal renal function, more than 90% of the administered gancyclovir was recovered as unmetabolized drug in the urine. For patients with creatinine clearance of 50-79 ml/min/1.73 m2, the systemic clearance was 128 +/- 63 ml/min/1.73 m2, and the plasma half-life was 4.6 hours. There was clear correlation with systemic clearance of ganciclovir and creatinine clearance.

The most frequently reported side effects of ganciclovir administration is suppression of bone marrow function (granulocytopenia/neutropenia/thrombocytopenia). This has required interruption or withdraw of therapy in 1-32% of treated patients. Up to 18% of patients treated with gancylovir have had transient renal impairment with serum Cr rising to >2.5 mg/dl. Other reported toxicities include headache in 17% of patients, confusion in 6% of patients. Approximately 2% of patients experienced anemia, fever, rash, abnormal liver function tests. Recommended dosage is 5 mg/kg twice per day for 14-21 days, and then maintenance treatment of 5mg/kg/day.

Due to the frequency of granulocytopenia and thrombocytopenia, it is recommended that neutrophil and platelet counts be performed every two days during bid dosing of gancyclovir, and then weekly once on daily therapy.

4.0 Patient Selection:

4.1 All patients must have a microscopically documented neoplasm, (which if B-cell lymphoid, must be monoclonal or oligoclonal, but not polyclonal), and EBV(+) as determined by immunohistochemistry [EBNA-2(+) and/or LMP-1(+)] and/or *in situ* hybridization [EBER + (EcoRI J] or [internal repeat + BamHI W(+)] or [EBNA-1 + (BamHI K(+)]. Serology for EBV must be obtained from patient (and preferably from donor in the transplant setting), but need not be EBV(+).

4.2 Patients with evaluable tumor are preferred.

4.3 Patients with EBV-LPD who have not had prior cytotoxic chemotherapy or radiotherapy for this disease are preferred.

4.4 With respect to other EBV-associated malignancies, all patients must have been refractory to at least one combination chemotherapy regimen, which may include high dose chemotherapy and stem cell rescue or Bone Marrow Transplant, if appropriate, and are incurable by standard therapy. Patients should have recovered from prior chemotherapy or radiotherapy. At least 3 weeks should have elapsed since the last course of chemotherapy (6 weeks for nitrosoureas or mitomycin C).

4.5 Granulocyte count $\geq$ 1000, platelet $\geq$ 50,000/mm^3.

4.6 Patients must have a serum bilirubin $\leq$ 1.5mg/dl X ULN, serum aminotransferases less than 2 times normal, and serum creatinine of less than 3.0 mg/dl. The calculated creatinine clearance must be greater than 30 ml/min.

4.7 Patients may not have had an acute myocardial infarction, or onset of atrial fibrillation within 6 months of study.

4.8 Patients must have the ability to give informed consent.

4.9 There are no limitations on functional status for eligibility.

4.10 Pregnancy must be ruled out in any female patient of childbearing age prior to treatment. A negative blood pregnancy test must be documented within 48 hours of starting treatment. Patients should also be counseled in the use of birth control since the effect of arginine butyrate on the unborn fetus is unknown.

4.11 Male or female patients greater than or equal to one (1) year of age are eligible.

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Amendment #4, 12/8/98
Amendment #5, 8/25/00

5.0 Treatment Plan:

5.1 Pretreatment Studies:

Tissue diagnosis
Submit the following sample tissues to Reference Pathology
5mm tissue snap frozen
5mm tissue in culture medium (10% FCS) on ice
Pathology slides for review
Submit copies of serial X-Rays, MRI or CT scan of primary site
Submit serology history
Submit sign and symptom list
No concurrent therapy with cytotoxic chemotherapy or interferon
Immunosuppression (documented) - CSA, FK506, steroids, etc, with levels if available

5.2 Eligible patients will require central venous access via Port-a-Cath or Hickman line because of the hyperosmolarity of the arginine butyrate solution.

5.3 A pregnancy test should be performed within 2 days of beginning therapy, unless the patient is postmenopausal or not fertile for medical reasons.

5.4 On days -1, treatment with ganciclovir at standard doses (5 mg/kg IV over 1 hour bid) will begin (unless already on-going) and will continue throughout the cycle. On day 0, infusion of arginine butyrate will begin at a total starting dose of 500 mg/kg/day to be infused continuously. In the absence of intolerable toxicity, dose escalation of arginine butyrate will be according to the following scheme:

Level 1: 500 mg/kg/day IV (20.8 mg/kg/hr) for 2 days
Level 2: 1000 mg/kg/day IV (41.6 mg/kg/hr) for 2 days
Level 3: 1250 mg/kg/day IV (62.5 mg/kg/hr) as tolerated, and continued until day 21.If no lethargy or decreased level of consciousness is reported at the dose of 1250mg/kg/day for three patients, then the Level 3 dose will be increased to 1500mg/kg/day. 1500mg/kg/day will then be the maximum dose.

For doses of 1000mg/kg or higher, antiemetics (i.e.zofran) may be administered prior to arginine butyrate infusions.
If patient becomes lethargic or has decreased level of consciousness, stop the drug until recovered. Then resume at the last dose tolerated. Continue Gancuclovir, do not interrupt.
If headache occurs, Tylenol 650 mg may be administered pretreatment and every 6 hours.

5.5 Serum will be obtained pretreatment and 4 hours into infusion on days 2, 4, 6, 8, 14, 21 of treatment for measurement of butyrate levels. Serum samples will also be obtained between 4-6 hours and 12-18 hours after each dose escalation, and after

discontinuation. Pretreatment and days 2, 4, 6 and 8 treatment urines will collected for measurement of butyrate levels.

5.6 Arginine butyrate infusion and ganciclovir will continue until day 21 (last treatment day in cycle)

5.7 Patients will be examined on day 21, and serum will be obtained for renal, hepatic and hematologic function.

5.8 Repeat staging and/or biopsy (if tumor peripherally accessible) 1 month from first biopsy

5.9 Cycle 2 of therapy may begin starting on day 29. For this cycle and for all future cycles, arginine butyrate and ganciclovir will be administered at the highest dose tolerated in the previous cycle. This dose schedule will continue unless there is evidence of intolerable toxicity (see below), or obvious disease progression.

5.10 Patients will undergo restaging at day 50. If patient demonstrates stable disease or response, cycle 3 can begin on day 57. If there is evidence of disease progression, or intolerable toxicity, the patient will be removed from the protocol.

5.11 Patients will continue to receive the above total of three cycles of therapy as long as their tumor responds, or until they meet one of the criteria for removal from study.

5.12 Therapy in individual patients will be discontinued at any dose level if unacceptable drug-related toxicity occurs. Adverse drug reaction guidelines will be observed for reporting toxicity.

6.0 Dose Modifications/Toxicities To Be Monitored

Any abnormalities in pulmonary, renal, hepatic or hematologic function which occur while patient is on study must return to baseline before going on to the next course.

Arginine Butyrate:

If grades 0, 1, or 2 toxicity is encountered, the patient may continue on protocol. If grade 3 toxicity is encountered, the principal investigator will be informed, and the patient may be continued at the discretion of the investigator once the toxicity had resolved. If the patient is continued on treatment, the dose of arginine butyrate will be lowered by 25%. If the patient tolerates this (reversible grade 2 toxicity or less), the patient may continue at this dose level. Otherwise, the dose may be reduced further, or treatment may be stopped, depending upon the decision of the investigator and the patient. If grade 4 toxicity is encountered, protocol therapy will be discontinued, and the principal investigator informed. The drug monitor at the Investigational Drug Branch will be notified immediately.

Ganciclovir:

Renal impairment:

Creatinine Clearance (mL/min)	Ganciclovir (mg/kg)	Dosing Interval (hours)
> 80	5.0	12
50-79	2.5	12
25-49	2.5	24
< 25	1.25	24

Creatinine Clearance can be related to serum creatinine by the following formulae:

For Males: $\frac{(140 - \text{age (yrs)})(\text{body wt (kg)})}{(72)(\text{serum creatinine (mg/dL)})}$

For Females: $\frac{(140 - \text{age (yrs)})(\text{body wt (kg)})}{(72)(\text{serum creatinine (mg/dL)})} \times 0.85$

7.0 Clinical Study Parameters

	Daily	Every other Day	Every 3 Weeks Course	Prior to Each
H&P	x			x
Tumor Measure*			x	x
Weight	x			x
Performance Status	x			x
Urinalysis				x
Serum Chemistries		x		x
Creatinine Clearance				x
Liver Function		x		x
Chest X-ray*				x
CT scan of Site of primary lesion*				x
Bone Scan*				x

*These tests, if indicated, will be repeated after each cycle, or at any time dictated by clinical findings.

8.0 Clinical Pharmacology

8.1 All patients entered will undergo a pharmacokinetics study. This is to detect whether dose-dependent pharmacokinetics occur and whether drug levels are under the curve correlate with toxicity.

8.2 Blood samples of approximately 2 ml will be collected in heparinized tubes. These samples will be collected at pretreatment and during the first cycle of treatment after 4 hours of infusion on days 2, 4, 6, 8, 14, 21, and at 4-6 hours and 12 - 18 hours after each dose escalation and post-treatment. Plasma from these samples will be separated by centrifugation, immediately frozen, and stored at -20^{o}C until analyzed.

8.3 Aliquots of urine (approx. 25 ml) will be collected during the first cycle pretreatment, and days 2, 4, 6 and 8 of treatment. Urine will be promptly frozen and stored at -20^{o}C until analyzed.

9.0 Tumor Tissue Samples

At the discretion of the principal investigator, tumor tissue samples from effusions, malignant ascites, blood or easily accessible tumor tissue will be obtained pretreatment and post-treatment (as standard care for routine evaluation and staging of patients with cancer), and some of these materials may be used for Special Investigative Laboratory Studies. If a more invasive procedure is necessary in order to obtain adequate tissue samples, then the patient will not be required to undergo such a procedure and imaging studies will be used for disease response. A separate informed consent will be obtained for pleuracentesis, pericentesis or tumor biopsy.

9.1 Special Investigative Laboratory Studies

Establish cell line from tumor
EBV immunohistochemistry / *in situ* hybridization
EBV flow cytometry
EBV PCR
Ganciclovir-sensitivity induced by Arginine Butyrate

10.0 Evaluation Criteria

10.1 Patients with reversible grade 3 toxicity may continue treatment, but at a reduced dose.

10.2 An evaluable drug course shall include any course of drug administration given in compliance with all aspects of prescribed drug administration and schedule in a patient who fulfills all eligibility requirements. Any exclusions from evaluation must be specified by the principal investigator in the flow sheets.

10.3 The minimum follow-up time period required shall be 42 days unless the patient dies prematurely from disease or drug-related toxicity. Any drug-related death will be reported immediately to the principal investigator, and the drug monitor of the Investigational Drug Branch at the NCI.

11.0 Response Criteria

11.1 Since this is a Phase I study and patients will have a few indications, we did not intend to calculate a response rate based on numbers of responders compared to number evaluable. However, we appreciate (and hope for) the possibility that a high enough response rate might occur in lymphoproliferative disease to possibly merit a submission for a pivotal study, if there is indeed a high enough response rate.

If toxicity results in disruption of treatment, we would like administer the next dose at 25% below the toxic dose for the remainder of the cycle, if the patient is willing. Otherwise, one cycle of at least 5 days of dosing will be the minimum duration of treatment required for evaluation.

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11.2 All tumor measurements must be recorded in centimeters and should consist of the longest tumor diameter and the longest perpendicular diameter. Liver size measurements should be recorded at the xiphoid line and in the right MCL.

11.3 Definitions of response:

Complete response (CR): Disappearance of all evidence of active disease for a minimum of 4 weeks. The patient must be free of any symptoms related to cancer. All lytic lesions must have remineralized. No new lesions can have occurred.

Partial response (PR): 50% or greater decrease in the sum of the products of perpendicular diameters of measurable lesions for a minimum of 4 weeks. *No* simultaneous increase in size of any lesion or appearance of any new lesion may occur. For tumors which can only be measured in one dimension, a greater than 50% shrinkage in largest dimension will qualify as a PR.

Minor response (MR): Objective regression of measurable lesions of greater than 25%, but less than 50% for a minimum of 4 weeks. No simultaneous increase in size of any lesion or appearance of any new lesion may occur.

Stable disease (SD): Steady state of disease; less than minor response, without progression. There may be no appearance of new lesions or worsening of cancer-related symptoms. This state must be maintained for a minimum of eight weeks to qualify for disease stability.

Progressive disease (PD): Unequivocal increase of at least 25% in the size of measurable lesions, or any clearly progressive skeletal involvement manifested by new lytic lesions. For patients in partial remission, an increase of 25% or more in the sum of the products of the diameters of all measured tumors over that which was obtained at the time of maximum response. Appearance of any new lesion.

Relapse: The development of any new lesions or regrowth of old lesions in patients who achieved a complete response.

11.3 Duration of response will be measured from the beginning of treatment to the first sign of progression or relapse.

11.3 Duration of survival shall be determined from the time of the tissue diagnosis.

12.0 Evaluation of Toxicity:

12.1 Organ specific toxicity will be recorded as outlined in the Appendix. The principal investigator and attending physician will decide if a toxicity is most likely drug or disease related. Toxicities must be immediately reported to the principal investigator.

12.2 Any drug-related death will be reported immediately to the principal Investigator. All life-threatening events (grade 4) which may be due to drug administration, and all fatal events must be reported by phone to the Investigational Drug Branch of the NCI within 24 hours. A written report is to follow within 10 working days.

12.3 Attempts will be made to obtain consent to perform postmortem examinations on every patient who dies.

13.0 Criteria for Removal from Study:

13.1 Disease progression as defined above. Also, any new symptoms which require the use of radiation therapy will be criteria for removal from study.

13.2 Unacceptable drug-related toxicity.

13.3 Patient refusal to continue treatment. The reason must be documented in the research chart, the clinical chart, and in the flow sheets.

13.4 A patient may receive a total of 3 courses of treatment, and then stop unless there is continued response with each cycle.

14.0 Data Analysis

The phase I trial described here (and the subsequent planned trial using Isobutyramide), are primary designed as simple dosing studies to analyze toxicity and tolerability. The number of patients to be treated in Phase I studies is low. As documented above, there is no currently accepted or effective treatment for this disease (in the case of EBV-LPD), or patients have not responded to accepted therapies (criteria for entry into the trial for the other EBV-associated malignancies), and these diseases are fatal. It is therefore not ethical to have a control, untreated arm of the trial.

It is anticipated that we can treat and analyze, at most, 5 patients per year on a single treatment regimen, and that this Phase I trial will require two years to establish enough evidence to ultimately design pivotal Phase II trials of these compounds. Statistical analysis and design of the planned Phase II trials will be carried out by Dr. Theodore Colton, Director of Epidemiology and Statistics, Boston University School of Public Health.

15.0 Registration information, Data Management and Reporting Requirements

15.1 All patients must have an On-Study Form filled out, including the dose. The form must be signed by the principal investigator before the patent enters the study.

15.2 All flow sheets and summaries will be reviewed by the principal investigator at least every other week.

15.3 Any life-threatening and/or unpredictable toxicity will be reported immediately to the principal investigator and to the drug monitor. The Institutional Review Board will be kept informed of toxicities noted by investigators.

15.4 Completed study summaries will be submitted to CTEP, IDB within one month of study completion. The interim status reports will be presented at Phase I working group meetings as requested.

16.0 Termination of Study:

16.1 Disabling or life-threatening toxicity which precludes further drug administration.
16.2 The patient may withdraw from the study at any time for any reason.

17.0 References:

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