Molecular and Clinical Assessment in the Treatment of AIDS Kaposi Sarcoma with Valproic Acid

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The AIDS Malignancy Consortium undertook a pilot trial of valproic acid among patients with AIDS-associated Kaposi sarcoma (KS). Treatment was associated with low toxicity, but the KS clinical response and KS herpesvirus lytic induction rates were not sufficiently high to meet predefined criteria for efficacy.

Kaposi sarcoma (KS) is an AIDS-defining neoplasm that may occur even when human immunodeficiency virus (HIV) RNA levels are effectively suppressed by antiretroviral therapy [1, 2]. KS is consistently associated with the KS herpesvirus (KSHV or HHV-8) [3]. In tumors, latent viral gene expression is detected consistently, whereas lytic viral genes are infrequently expressed. Histone deacetylase (HDAC) inhibitors are important laboratory tools for activating KSHV lytic cycle gene expression [4]. Viral lytic induction in vivo might directly lead to death of latently infected tumor cells or lead to expression of antigenic viral proteins that would render tumor cells more susceptible to T cells. Valproic acid (VA), an agent used to treat seizure and mood disorders, activates lytic gene expression in KSHV-infected cell lines [5].

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VA has properties of a HDAC inhibitor [6] and the literature suggests a variety of possible effects in patients with AIDS and KS. VA has been variably reported to rescue replication-competent HIV from resting CD4$^+$ T cells and thus reduce the size of the HIV latency reservoir [7, 8].

Induction of KSHV lytic infection also raised concerns that VA treatment could lead to KS progression or development of KS in seropositive patients without tumor [5]. With these rationales and concerns in mind, the AIDS Malignancy Consortium (AMC) in conjunction with the AIDS and Cancer Specimen Resource (ACSR) undertook a pilot clinical study to determine the safety and efficacy of VA in AIDS-KS patients.

Patients and methods. Eligible patients had biopsy-confirmed KS and documented HIV infection. Patients with visceral or rapidly progressive KS were excluded, as were those with a Karnofsky performance status <60 or life expectancy <3 months. Patients who were receiving antiretroviral therapy were required to be receiving a stable regimen for ⩾4 weeks before enrollment. Because VA and zidovudine have been associated with lactic acidosis, patients with a history of lactic acidosis and those receiving zidovudine-containing regimens were excluded [9].

We prospectively specified 4 criteria be met to conclude that VA was safe, effective, and likely to be working according to the hypothesized mechanism: a low toxicity-related discontinuation rate (<35%), a low rate of accelerated KS progression (<10%), a high clinical response rate (>30%), and a high rate of induction of lytic viral gene expression (>60%). Eighteen patients were sufficient to evaluate these 4 measures. No adjustment for multiple testing was made.

This was a prospective, open-label pilot study to determine the safety of VA in patients with AIDS-KS and evaluate the effect of VA on KSHV gene expression. Secondary endpoints included evaluation of the effects of VA on HIV and KSHV in blood and clinical response. After giving written informed consent, patients received VA for 28 days followed by a 2-week taper. VA (250-mg capsules) was administered twice daily. The dose was escalated over the first 6 days from 500 mg to 1000 mg. Thereafter dosing was adjusted to achieve the therapeutic range established for epilepsy (50–100 mg/L). Although VA treatment ended after 6 weeks, patients were monitored for 24 weeks or until KS progression.

Clinical assessments, including history and physical examinations, tumor assessments, complete blood count, serum elec-

Table 1. Selected Patient and Tumor Characteristics

Characteristic	Patients
Male sex	18 (100)
Age, median years (range)	37 (29–60)
Absolute CD4+ cell count, median cells (range)	286 (82–1119)
Undetectable HIV load	13 (72)
⩾50 lesions	9 (50)
No. of patients with raised lesions (median no. of lesions)	6 (33)
No. of patients with flat lesions (median no. of lesions)	13 (72)
Oral lesions present	4 (22)
Tumor-associated edema	10 (56)

NOTE. Data are no. (%) of patients, unless otherwise indicated. HIV, human immunodeficiency virus.

trolytes, and renal and liver function tests were performed at baseline; on days 8, 15, 29; and monthly thereafter. CD4+ T lymphocyte counts were obtained at baseline. HIV RNA was measured at baseline; on days 45–50; and every 3 months thereafter. Tumor punch biopsies were obtained at baseline and on days 8 and 29. Plasma and peripheral blood mononuclear cells (PBMCs) for KSHV copy number were obtained at baseline; on days 8, 15, and 29; and 1 month after baseline. Tumor assessments were performed at baseline; on days 8, 15, and 29; and monthly thereafter.

Punch biopsy specimens (3 mm in diameter) were snap frozen in liquid nitrogen for RNA studies or formalin fixed for immunohistochemistry. Specimens forwarded to the ACSR were coded and batched before transfer to laboratories for blinded evaluation. KSHV protein expression (LANA, vIL6, ORF8.1, ORF59) was categorized as (−), (+/−), (+), (++), (+++) or nonevaluable on the basis of the number of positive-staining cells and stain intensity. KSHV transcription was profiled using real-time quantitative polymerase chain reaction (PCR), as previously described [10]. RNA quality was ascertained using an Agilent Bioanalyzer. Data were normalized to the mean of 3 housekeeping messenger RNAs (mRNAs) to yield dCT, a log-transformed measure of relative RNA abundance.

KSHV copy number in plasma and PBMCs was assessed by real-time PCR as previously described [11]. Blood was collected into heparin tubes, transported at ambient temperature, and processed within 30 h after collection. DNA was isolated using the Qiagen Blood Kit (Qiagen).

Tumor assessments and grading of responses as complete, partial, stable, or progression were performed as previously described [12]. Adverse events were classified as possibly, probably, or definitely related to VA, and their severity was graded using the National Cancer Institute Common Toxicity Criteria, version 3.0.

The Wilcoxon signed rank test was used to evaluate changes from baseline for laboratory correlates. The Spearman correlation coefficient was used to evaluate bivariate correlations.

Results. Nineteen patients were enrolled. One patient withdrew before receiving treatment. The remaining 18 patients completed the planned 28 days of therapy. All patients were men. At entry, most were receiving antiretroviral therapy, had an undetectable HIV load, had previously received treatment for KS, and had tumor-associated edema. One-half of the patients had ⩾50 lesions. Patient and tumor characteristics are summarized in Table 1.

The only adverse event that occurred in >1 patient was mild diarrhea, which occurred in 2 patients. No patient discontinued treatment as a result of toxicity.

One patient achieved a partial response that persisted without further intervention at last follow-up 5 months after therapy. Two other patients achieved partial responses 4 months and 6 months after treatment without any intervening KS treatment. One patient showed an ~30% increase in the size of marker lesions in the 4 weeks of therapy, while at the same time the total number of lesions decreased from 128 to 99. No patients developed rapid KS progression.

Viral gene expression in tumor biopsies was assessed by immunohistochemical staining. Latent (LANA) and lytic (vIL6, ORF8.1, and ORF59) antigens were detected in most specimens at baseline and following VA treatment (Table 2). Paired *t* tests showed no statistically significant differences in expression between baseline and either day 8 or day 29 for any of these antigens. Using real-time quantitative PCR to assess KSHV mRNA level in biopsy specimens, significant changes were not detected by *t* test. We hypothesized that higher VA trough levels might be associated with more-robust lytic induction. Although we saw no correlation between day 8 VA trough levels and antigen expression assessed by immunohistochemical staining, the VA trough level on day 8 correlated with quantities of several KSHV mRNAs (Figure 1). The top 5% of KSHV mRNAs were ordered by individual regression coefficients to build a multiple regression model. Orf 48 (unknown function), orf8 (gB) and orf 22 (gH, 2 primers) correlated with VA levels at day 8 with $P \leqslant .02$ (12 patients).

Baseline and day 29 HIV RNA levels were available for 16 patients. Two patients (13%) had detectable HIV viremia at baseline but not at day 29. One patient (6%) had HIV detected at baseline and day 29. Thirteen patients (81%) had no detectable HIV at baseline or day 29. No patients developed detectable HIV viremia during therapy. KSHV plasma and PBMC copy numbers were available for 18 patients. No significant

Table 2. Antigen Expression in 18 Treated Patients

This table is available in its entirety in the online version of *Clinical Infectious Diseases*

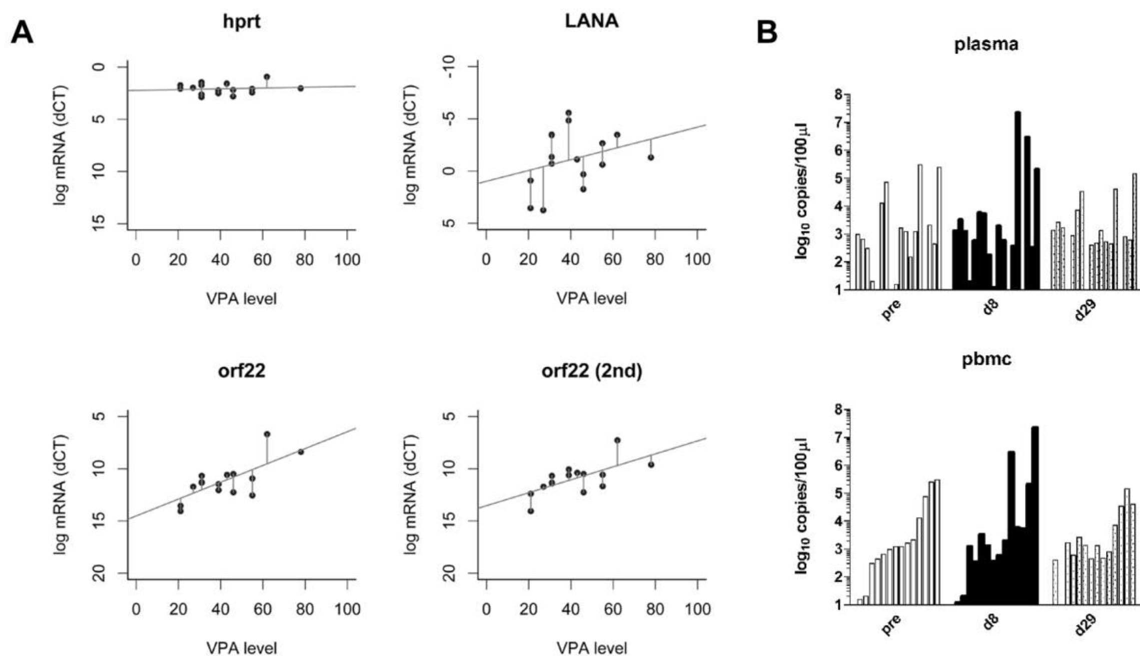

Figure 1. Kaposi sarcoma herpesvirus (KSHV) messenger RNA (mRNA) and DNA. *A,* correlation of KSHV mRNA levels with valproic acid (VA) trough levels after treatment. The VA trough level is shown on the horizontal axis, and relative mRNA levels (dCT) are shown on the vertical axis. The human housekeeping mRNA hprt, KSHV latent mRNA LANA, KSHV orf22, which exhibited a linear trend ($P \leqslant .05$), and orf22 as measured by a second independent primer are shown. Also shown are the linear regression lines and residuals. *B,* KSHV DNA copy number in blood. Vertical bars corresponding to each patient are grouped as pretreatment, day 8, and day 29. The Y axis shows the $\log_{10}$ of KSHV copy number. hprt, hypoxanthine phosphoribosyltransferase; pbmc, peripheral blood mononuclear cell.

differences were observed between baseline and either day 8 or day 29 copy numbers in either plasma or PBMCs. A graphical presentation of this data is in Figure 1. There was also no correlation between day 8 VA levels and KSHV copy numbers in plasma or PBMCs at day 8.

Discussion. VA did not meet the prespecified criteria for clinical response or KSHV lytic induction. However, insofar as none of the patients discontinued VA because of toxicity, no patients developed accelerated KS progression, and there were no detected adverse effects on HIV viremia, we believe that the study offers reassurance about using VA in HIV-infected patients with seizure or mood disorders who are seropositive for KSHV or have KS.

Whether induction of KSHV lytic infection would lead to the hoped-for therapeutic effect or, as some have feared, lead to tumor progression remains unsettled. The finding that expression of lytic viral mRNAs correlated with VA levels hints at the possibility that dose escalation might lead to increased lytic expression. However, the modest changes seen and lack of corresponding changes in either viral antigen expression assessed by immunohistochemistry or viral DNA copy number in blood suggest that robust induction of lytic viral gene expression may not be readily achievable and suggest consideration of alternative lytic activation strategies. Vorinastat, 5-azacytidine, and bortezomib have all been recognized as potent lytic inducers in vitro [13] and may be more promising for future investigations.

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Potential conflicts of interest. All authors: no conflicts.

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