Epstein–Barr virus reactivation in extranodal natural killer/T-cell lymphoma patients: a previously unrecognized serious adverse event in a pilot study with romidepsin


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Background: Romidepsin, a histone deacetylase (HDAC) inhibitor, has been approved for the treatment of relapsed and refractory peripheral T-cell lymphoma. However, the efficacy and safety of romidepsin has never been studied in patients with relapsed or refractory extranodal natural killer (NK)/T-cell lymphoma (ENKTL).

Patients and methods: We conducted an open-label, prospective pilot study to evaluate the efficacy and feasibility of romidepsin in the treatment of patients with ENKTL. The treatment was intravenous infusion of romidepsin (14 mg/m²) for 4 h on days 1, 8, and 15 of a 28-day cycle, and was repeated until disease progression or the occurrence of unacceptable toxicity.

Results: A total of five patients enrolled on to this pilot study. However, three patients developed fever and elevated liver enzyme and bilirubin levels immediately after their first administration of romidepsin. We suspected that these events were associated with Epstein–Barr virus (EBV) reactivation because of the rapidly elevated EBV DNA titers in blood from these patients. An in vitro study with the ENKTL cell line SNK-6 cells also showed that HDAC inhibitors including romidepsin increased the copy number of EBV DNA in a dose-dependent manner. These findings suggested that romidepsin-induced histone acetylation reversed the repressed state of the genes required for EBV reactivation and that romidepsin treatment may have caused EBV reactivation in EBV-infected tumor cells in ENKTL patients. Therefore, we discontinued the enrollment of patients into this pilot study.

Conclusions: Our study suggests that the use of romidepsin may cause severe EBV reactivation in patients with ENKTL.

Key words: extranodal NK/T-cell lymphoma, romidepsin, EBV reactivation
introduction

Romidepsin, a histone deacetylase (HDAC) inhibitor, was approved by the United States Food and Drug Administration for relapsed and refractory cutaneous T-cell lymphoma (CTCL) and peripheral T-cell lymphoma (PTCL) based on its proven efficacy in previous phase II trials [1, 2]. The overall response rates to relapsed or refractory CTCL and PTCL in those phase II trials were 34% and 38%, respectively. A pivotal phase II study with 130 patients also reported a 25% overall response rate for relapsed or refractory PTCL, and similar response rates have been reported for nodal subtypes of PTCLs such as angioimmunoblastic T-cell lymphoma and anaplastic lymphoma kinase-negative anaplastic large-cell lymphoma [3]. However, this latter study did not report sufficient data about the efficacy of romidepsin in extranodal natural killer (NK)/T-cell lymphoma (ENKTL) because only one patient with this disease was recruited. ENKTL is one of the most common subtypes of PTCL in far eastern Asia and its resistance to anthracycline because of its expression of p-glycoprotein is well known [4, 5].

Although the treatment outcomes of ENKTL have improved with the use of nonanthracycline-based chemotherapy incorporating l-asparaginase [6–8], the prognosis for relapsed or refractory patients is still extremely poor because of the limited treatment options. We conducted an open-label, prospective pilot study to evaluate the efficacy and feasibility of using romidepsin in the treatment of relapsed or refractory ENKTL patients. However, we found serious adverse events in this pilot study. We thought that these events may have been related to romidepsin-induced Epstein–Barr virus (EBV) reactivation because tumor cells of ENKTL are invariably infected with EBV, and romidepsin-induced EBV reactivation has been suggested to occur in EBV-infected lymphocytes or cell lines [9, 10]. Here, we report on three cases of EBV reactivation in patients treated with romidepsin in this pilot study.

patients and methods
design of the pilot study

The study was conducted at two institutes belonging to the Consortium for Improving Survival of Lymphoma [11]. Patients with histologically confirmed ENKTL who satisfied the following criteria were enrolled: (i) relapsed or refractory disease after salvage chemotherapy or autologous stem-cell transplantation; (ii) performance status ≤ grade 2 in the Eastern Cooperative Oncology Group scale; (iii) aged 20–80 years; (iv) ≥1 measurable lesion; (v) serum creatinine concentration up to 1.5 times the upper limit of normal (ULN); (vi) serum transaminases up to 2.5 times the ULN and serum bilirubin up to 1.5 times ULN; and (vii) absolute neutrophil count ≥1500/mm³, platelets ≥75 000/mm³, and hemoglobin ≥9.0 g/dl. The primary end point was the disease control rate, which was defined as the percentage of patients who had a complete or partial response or stable disease. The treatment was intravenous infusion of romidepsin (14 mg/m²) for 4 h on days 1, 8, and 15 of a 28-day cycle, and was repeated until disease progression or the occurrence of unacceptable toxicity or withdrawal of informed consent. The response to treatment was evaluated after the completion of every two cycles based on the revised response criteria for malignant lymphoma [12]. As a biomarker of the tumor burden of ENKTL, EBV DNA titer in ethylenediaminetetraacetic acid-anticoagulated whole blood was also quantified using the Qiang Artus EBV RB real-time polymerase chain reaction (PCR) assay (Qiagen GmbH, Hilden, Germany) according to the manufacturer’s instructions, as described previously [13]. The institutional review boards of the participating institutes approved the study, and this study was registered at www.clinicaltrials.gov as NCT01913119.

Table 1. Clinical characteristics of patients at enrollment and treatment outcomes

<table>
<thead>
<tr>
<th>No.</th>
<th>Clinical characteristics at enrolment</th>
<th>Cycle 1</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>EBV DNA</td>
<td>Day 1</td>
<td>Day 8</td>
<td>Day 15</td>
<td>Response</td>
<td>Adverse events</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>titer in blood</td>
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<td></td>
</tr>
<tr>
<td>#1</td>
<td>M/48 1st: CCRT + VIDL 2nd: SMILE 3rd: GDP 4th: ASCT</td>
<td>IE (nasal cavity)</td>
<td>Not detectable</td>
<td>Done</td>
<td>Not done</td>
<td>Done</td>
<td>Stable disease</td>
<td>Fever, sepsis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#2</td>
<td>M/50 1st: MIDLE 2nd: ASCT</td>
<td>IV (nasal cavity, liver, lung, spleen)</td>
<td>392 copies/µl</td>
<td>Done</td>
<td>Not done</td>
<td>Not done</td>
<td>Not evaluated</td>
<td>Fever, hyperbilirubinemia, elevated liver enzyme levels, suspicious EBV reactivation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#3</td>
<td>M/64 1st: CCRT + VIDL 2nd: SMILE 3rd: ASCT</td>
<td>IV (nasal cavity, back muscles)</td>
<td>Not detectable</td>
<td>Done</td>
<td>Done</td>
<td>Done</td>
<td>Not evaluated</td>
<td>Fever, sepsis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#4</td>
<td>F/63 1st: CCRT 2nd: SMILE 3rd: ASCT</td>
<td>IE (nasal cavity)</td>
<td>Not detectable</td>
<td>Done</td>
<td>Not done</td>
<td>Not done</td>
<td>Not evaluated</td>
<td>Fever, hyperbilirubinemia, elevated liver enzyme levels, suspicious EBV reactivation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#5</td>
<td>M/62 1st: CCRT + VIDL 2nd: SMILE 3rd: GDP</td>
<td>IV (liver, bone marrow)</td>
<td>Not detectable</td>
<td>Done</td>
<td>Not done</td>
<td>Not done</td>
<td>Not evaluated</td>
<td>Fever, hyperbilirubinemia, elevated liver enzyme levels, EBV reactivation</td>
<td></td>
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</tbody>
</table>

EBV, Epstein–Barr virus; M, male; F, female; CCRT, concurrent chemoradiotherapy; VIDL, etoposide, ifosfamide, dexamethasone, l-asparaginase; SMILE, steroid, methotrexate, ifosfamide, l-asparaginase, etoposide; GDP, gemcitabine, dexamethasone, cisplatin; MIDLE, methotrexate, ifosfamide, dexamethasone, l-asparaginase, etoposide; ASCT, autologous stem-cell transplantation.
in vitro study with an ENKTL cell line

The EBV-positive ENKTL cell line, SNK-6 was obtained from Dr Y. K. Jeon (Seoul National University Hospital, Seoul, Korea). Cells were cultured in RPMI 1640 (Life Technologies, Inc., Gaithersburg, MD) supplemented with 10% heat-inactivated human serum and 700 U/ml recombinant human interleukin 2 (PeproTech, Inc., Rocky Hill, NJ), penicillin and streptomycin (Gibco-BRL, Grand Island, NY) in a 5% CO2-containing atmosphere. We used three HDAC inhibitors: romidepsin and vorinostat purchased from Selleck Chemicals (Houston, TX), and phenylbutyrate from Enzo Life Sciences, Inc. (Farmington, NY). The concentrations of each HDAC inhibitor for in vitro treatment with SNK-6 were determined according to cell viability, which was assessed using the Trypan blue exclusion assay. The EBV DNA titer was also measured in the supernatants from HDAC inhibitor-treated and control cells. DNA was extracted and analyzed using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA) and QIAcube instrument. The concentration of circulating EBV DNA is expressed as copies of EBV genome/µl. For the prototypic assay for EBV, the standard curves obtained were linear from 10^3 to 10^6 copies per reaction with an average slope of −3.37. For western blot assay, whole cell lysates were prepared using ice-cold RIPA buffer [0.5% sodium deoxycholate, 1% Nonidet P-40, 150 mM NaCl, 50 mM Tris (pH 7.5), 0.1% sodium dodecyl sulfate (SDS) and 1 mM phenylmethylsulfonyl fluoride ]. Samples were cleared by microcentrifugation (14 000 rpm, 30 min, 4°C) and assessed for protein concentration. After electrophoresis on a 12% SDS-polyacrylamide gel, proteins were transferred on to nitrocellulose membranes. After 1 h of incubation in blocking solution (5% non-fat milk), membranes were exposed to the primary antibody for LMP1 (DAKO, Denmark) overnight at 4°C. The blot was exposed to horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature. Proteins were visualized using enhanced chemiluminescence reagents (Amersham Pharmacia Biotech, Arlington Heights, IL).

results

EBV reactivation in patients enrolled in the pilot study

We started this pilot study in August, 2013 with the target number of 16 and expected follow-up duration of 2 years. However, a total of five patients participated in the study: the first patient was enrolled on the 4 October 2013, and the last patient was on the 24 July 2014. The characteristics and treatment courses of the five patients enrolled into this pilot study are summarized in Table 1. The first patient received romidepsin on days 1 and 15, and the treatment was skipped on day 8 because of fever. Although our early evaluation of disease using computed tomography (CT) and positron emission tomography/CT scans showed stable disease and his EBV DNA titer was not detectable, he was withdrawn from the study because of the sepsis developed after day 15. The second patient developed fever and elevated liver enzyme levels after receiving romidepsin on day 1. We repeated the blood tests, including the EBV DNA titer, and found a relationship between the deterioration in liver function and the high EBV DNA titer (Figure 1A). Because we thought that the hyperbilirubinemia might be related to EBV reactivation given the rapid increase in the EBV DNA titer, we administered 4 mg of dexamethasone i.v. every 6 h and etoposide at a dosage of 100 mg/m^2 for the next 3 days. The patient’s liver function improved as the EBV DNA titer decreased. The third patient received three doses of romidepsin on days 1, 8, and 15. However, he was withdrawn from the study because of sepsis after the third administration of romidepsin. The fourth patient developed fever and progression of neutropenia and anemia after she received romidepsin on day 1. Serial monitoring of her liver function showed elevations in the bilirubin and liver enzyme levels (Figure 1B). The EBV DNA titer increased up to 106 691 copies/µl even though her last EBV DNA titer was less than the detectable level. Finally, she died from multiple organ failure related to the rapid progression of liver failure.

![Figure 1](https://example.com/figure1.png)

Figure 1. (A–C) Serial monitoring shows an association between laboratory findings and EBV titers in blood in patients #2, #4, and #5.
The Data and Safety Monitoring Board (DSMB) reviewed these four cases and discussed the probability of romidepsin-induced EBV reactivation in patients #2 and 4 (Table 1). The DSMB then recommended continuation of enrollment because the elevated EBV DNA titer and liver function deterioration might have been a manifestation of disease progression of ENKTL. We treated the fifth patient, a 62-year-old man with ganciclovir prophylaxis. We also monitored his EBV DNA titer every 2 days even though his EBV DNA titer was less than the detectable level before treatment. After day 1, the EBV DNA titer increased together with the bilirubin and liver enzyme levels (Figure 1C). We administered etoposide 100 mg/m² i.v. on day 10, and his EBV DNA titer decreased and his general condition improved. Finally, we stopped any further enrollment of patients into this study after considering the poor feasibility and association of the treatment with EBV reactivation.

romidepsin-induced EBV reactivation in an ENKTL cell line

On the basis of our clinical experience in this pilot study, we decided to examine whether romidepsin could induce EBV reactivation in the ENKTL cell line, SNK-6. First, we treated SNK-6 cells with three HDAC inhibitors (romidepsin, phenylbutyrate, and vorinostat) for 24, 48, or 72 h. The EBV DNA copy number was measured in the cell culture supernatants using quantitative PCR. Each of the HDAC inhibitors significantly increased EBV copy number in dose-dependent manner after treatment for 48 or 72 h (Figure 2A). Among three HDAC inhibitors, romidepsin caused a significantly greater increase in the number of EBV DNA copies compared with phenylbutyrate and vorinostat (romidepsin: $4.7 \times 10^3$ copies/µl; phenylbutyrate: $2.6 \times 10^3$ copies/µl; vorinostat: $3.7 \times 10^3$ copies/µl). We also analyzed the expression of EBV-major protein, LMP1 in HDAC inhibitor-treated cells by western blot analysis. HDAC inhibitor treatment led to a dose-dependent increase in LMP1 protein levels (Figure 2B).

discussion

EBV infects more than 90% of the population and mostly exhibits latency following infection. During latency, the EBV genome is bound by histone proteins into a chromatin structure [14]. However, EBV can reactivate from the latent phase to the lytic phase and produce infectious viral progeny through the activation of Zta and Rta, the products of the viral BZLF1 and BRLF1 genes that are known to play key roles in initiating EBV reactivation [10]. Zta and Rta are tightly repressed by chromatin, and the hyperacetylation of the histone tails allows the activation of the Zta and Rta promoters, which leads to the transcription of BZLF1 and BRLF1 during the switch from latency to the lytic cycle [15]. Thus, the HDAC inhibitor-mediated chromatin remodeling can induce EBV reactivation. This HDAC
inhibitor-mediated induction of lytic genes and gene products were also demonstrated in a previous in vitro study using arginine butyrate, another HDAC inhibitor and EBV-infected lymphoma cells exposed to arginine butyrate were susceptible ganciclovir [16]. Thus, a previous phase I/II trial of arginine butyrate and ganciclovir in patients with EBV-associated lymphoid malignancies could support the close association of HDAC inhibitor and EBV reactivation [17].

In this pilot study, three patients developed fever and elevated liver enzyme and bilirubin levels immediately after their first administration of romidepsin. Although we initially considered these to be infectious complications, we suspected that these serious adverse events could be associated with EBV reactivation because of the rapidly increased EBV DNA titers in the blood from these patients. Clinical manifestations of EBV reactivation are nonspecific. Thus, symptoms and signs such as fever and laboratory abnormalities such as elevated liver enzyme levels and cytopenia are similar to those seen with other viral infections. However, the high titer of EBV DNA in these patients was unusual and suggested the possibility of EBV reactivation.

This elevation of EBV DNA titer might have been related to disease progression because the level of circulating EBV DNA in blood is related to the tumor burden in ENKTL [18]. However, two patients (#4 and #5) did not have detectable levels of EBV DNA before the start of treatment with romidepsin (Table 1), and their EBV DNA titers increased immediately after the first administration of romidepsin (Figure 1A–C). Thus, these cases seem to be explained by EBV reactivation rather than disease progression. Serial monitoring of EBV DNA titer in patient #5 showed a strong relationship between EBV viral load and deterioration of liver function (Figure 1C). In addition, the magnitude of increase in EBV DNA titer seemed to be less than in two previous patients (#2 and #4), which may have been related to the use of ganciclovir prophylaxis in patient #5.

Consistent with our clinical findings, our in vitro study showed that HDAC inhibitors including romidepsin increased the copy number of EBV DNA of ENKTL cells in a dose-dependent manner (Figure 2). Taken together, these findings suggest that romidepsin-induced histone acetylation might reverse the repressed state of genes required for EBV reactivation and that romidepsin treatment in ENKTL patients might cause EBV reactivation from EBV-infected tumor cells. In a previous report, romidepsin-induced EBV reactivation was introduced in two patients with relapsed PTCL who were enrolled in a phase II trial. One patient with PTCL developed EBV-positive NK cell malignancy after 15 cycles of treatment, and the other patient with CTCL developed EBV-related lymphoproliferative disorder after six cycles [19]. Although the clinical presentations and onset times differed from those observed in our study, our experience with romidepsin is consistent with these earlier findings.

In our study, the remaining two patients were also withdrawn from the study after the first cycle because they developed fever and sepsis. Although they did not have an elevated EBV DNA titer, we reasoned that their fever and sepsis might have been related to EBV reactivation and that they might have shown definite evidence of EBV reactivation after they were treated for longer. The findings of our pilot study suggest that romidepsin might not be a feasible treatment of ENKTL patients because of the risk of EBV reactivation. On the basis of our experience, we suggest that the use of romidepsin should be avoided in patients with ENKTL because EBV reactivation may cause fatal complications. We also suggest that other HDAC inhibitors should be used more cautiously in treating other lymphomas, especially in cases of EBV-associated lymphoid malignancy.

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disclosure
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references
Is it possible to encourage hope in non-advanced cancer patients? We must try

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Background: Data are lacking on the relationship between hope and other variables in non-advanced cancer patients. The study explored the relationship between hope, symptoms, needs, and spirituality/religiosity in patients treated in a supportive care unit (SCU).

Patients and methods: From September 2013 to March 2014, the consecutive patients who accepted to complete: (i) Needs Evaluation Questionnaire (NEQ), (ii) the Edmonton Symptom Assessment System (ESAS), (iii) Hope Herth Index (HHI), and (iv) the System of Belief Inventory (SBI) were enrolled. Moreover, clinical/demographic data were collected and the findings were analyzed.

Results: A total of 276 patients who completed the HHI questionnaire (participation rate 276/300 = 92%) were included; 131 reported HHI total score >37 (median value). The majority of patients had a Karnofsky performance status >80; 71% were on cancer therapies, and only 29 patients had metastases or relapse. Patients with higher HHI scores were less educated (\(P = 0.012\)), reported lower ESAS total score (15.4 versus 22.6, \(P < 0.001\)), and had less often been referred to a psychologist previously to the study (\(P = 0.002\)); patients with a higher HHI score also reported higher spirituality (\(P < 0.001\)). Some NEQ items resulted significantly associated with HHI score after adjustment for other variables: the need to have sincere clinicians (\(\beta = -2.7\)), better dialogue (\(\beta = -2.1\)), and more reassurance from the clinicians (\(\beta = -2.5\)); better attention (\(\beta = -4.4\)) and respect for intimacy (\(\beta = -3.3\)) from nurses; to speak with people who have the same illness experience (\(\beta = -2.5\)), to be more reassured by relatives (\(\beta = -3.3\)) and to feel less abandoned (\(\beta = -4.3\)). Higher SBI scores were independently associated with higher HHI scores (\(\beta = 1.7\) for 10 points increase).

Conclusions: In cancer patients, hope can be encouraged by clinicians through dialogue, sincerity, and reassurance, as well as assessing and considering the patients’ needs (above all the psycho-emotional), symptoms, psychological frailty, and their spiritual/religious resources.

Key words: hope, needs, symptoms, spirituality/religiousness, supportive care

introduction

In the holistic and personalized approach of cure and care of cancer patients, early attention to their ‘physical, emotional, spiritual/existential/religious well-being, search of meaning,