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Romidepsin in the Treatment Of Hematologic Malignancies

A Compendium of Case Studies

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The Potential of Histone Deacetylase Inhibitors for the Treatment of Lymphoma And Multiple Myeloma

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Overview

Histones comprise a family of nuclear proteins that interact with DNA, resulting in DNA being coiled around a histone octameric core within the nucleosome. Histone deacetylase (HDAC) enzymes catalyze the deacetylation of lysine residues in the histone N-terminal tails and are also found in large multiprotein complexes with transcriptional co-repressors. Human HDACs are classified into 4 classes based on their similarity to yeast factors, with 18 different HDACs identified to date.[1] The physiologic counterparts of HDACs are histone acetyltransferases (HATs).

The organization of chromatin into either a relatively open or condensed form affects key molecular processes such as transcription, DNA repair, recombination, and replication, and there is clear evidence that gene expression controlled by epigenetic changes can play an important role in cancer onset and progression.[2,3] Increased acetylation of histones H3 and H4 is associated with open and active chromatin and increased transcription, whereas deacetylation of these histone residues is associated with condensed chromatin and transcriptional repression.[4] Alteration in the balance of activity between these enzymes, in association with aberrant gene transcription, has been observed in cancer cells in both hematologic and solid malignancies.[5-8]

Moreover, both HDACs and HATs activities are recruited to target genes in complexes that contain sequence specific transcription factors. Several different transcription factors are assembled with these complexes including Bcl-6, Mad-1, PML, and ETO.[9] Indeed, there are several examples in which HDACs are functionally involved in oncogenic translocation products in cancer development.[10-13] For example, Bcl-6, a transcription factor overexpressed in approximately 40% of diffuse large B-cell lymphomas (DLBCLs), recruits HDAC2 to repress growth through regulating target genes. In vitro treatment of DLBCL cells with HDAC inhibitors causes hyperacetylation of Bcl-6, release of HDAC2 from the complex, resulting in reactivation of repressed target genes and subsequent apoptosis of lymphoma cells.[13]

HDAC inhibitors, a new class of chemotherapeutic agents, have been shown to have potent anticancer activities in preclinical studies and are currently in various stages of clinical development.[1]

Increased acetylation of specific residues in histones H3 and H4 is associated with open and active chromatin and increased mRNA transcription; however, the exact final pathways that lead to the anticancer effects observed

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in various tumor types remain to be fully elucidated. There is nevertheless clear evidence that key activities of HDAC inhibitors include:

1. induction of tumor cell apoptosis and suppression of cell proliferation by activation of cell cycle checkpoints at G1/S or G2/M;
2. induction of cellular differentiation;
3. suppression of angiogenesis;
4. enhancement of host immune surveillance.

Induction of apoptosis by HDAC inhibition has been linked to alterations in gene expression resulting in upregulation of proapoptotic genes (*Bax, Bak, Bim, TP2, Apaf-1, Trail, DR4, DR5, Bmf*) and downregulation of antiapoptotic genes (*Bcl-2, Bcl-XL, XIAP, Survivin, Akt, c-FLIP, c-RAF, Mcl-1*).[14-16] Moreover, HDAC inhibitors can induce a cell-cycle checkpoint at the G1/S transition[17] through transcriptional activation of *CDKN1A* encoding the cyclin-dependent kinase inhibitor $p21^{waf1/cip1}$.

Furthermore, the molecular basis of the anticancer effects of HDAC inhibitors may well go beyond inhibition of histone acetylation. A growing list of nonhistone targets involved in regulation of cell proliferation, cell death, and cell migration have been identified, including p53, Ku70, alpha tubulin, and Hsp-90. Thus, acetylation-dependent changes in the activities of such proteins may play an equally important role in mediating the anticancer effects of HDAC inhibitors.[15,18,19]

Several families of HDAC inhibitors can be discerned, divided by their chemical structure, with varying ability to inhibit the various classes of HDACs. These compounds have demonstrated in vivo and in vitro activity against both hematologic and nonhematologic malignancies alone or in combination with traditional chemotherapeutic agents.[1]

Table 1. Clinical Trials Involving HDAC Inhibitors in Patients With Lymphoma

Study	Disease	Phase	N	Response
Vorinostat				
Duvic[29]	CTCL	II	33	PR 8 (4 SS)
Olsen[30]	CTCL	IIb	74	CR 1, PR 21
O'Connor[28]	HL/DLBCL/ CTCL/Other	I	31	CR 1, PR 4, SD 3
Crump[41]	DLBCL	I	18	CR 1, SD 1
Kirschbaum[49]	Indolent B-cell NHL	II	15	CR 1, PR 1, SD 3
Romidepsin				
Piekarz[35]	PTCL	I/II	39	CR 3, PR 8, SD 5
Piekarz[35]	CTCL	I/II	71	CR 4, PR 18, SD 9
Lerner[36]	CTCL	I/II	45	CR 1, PR 11, SD 17
Panobinostat				
Prince[62]	CTCL	I	11	CR 2, PR 3, SD 2

CR=complete remission; CTCL=cutaneous T-cell lymphoma; DLBCL=diffuse large B-cell lymphoma; HL=Hodgkin lymphoma; NHL=non-Hodgkin lymphoma; PR=partial remission; PTCL=peripheral T-cell lymphoma; SD=stable disease; SS=Sézary Syndrome.

Clinical Studies of HDAC Inhibitors In Lymphoma

Cutaneous T-cell Lymphoma

Vorinostat The most studied HDAC inhibitor in cutaneous T-cell lymphoma (CTCL) is suberoylanilide hydroxamic acid (vorinostat; Table 1). Vorinostat is an orally bioavailable pan-HDAC inhibitor.[20] Early in vitro studies showed vorinostat to facilitate the transcription of genes governing growth arrest, differentiation, and both caspase-dependent and -independent apoptosis.[21] Mechanisms of action include increased expression of the cell cycle–arrest inducer $p21^{waf1/cip1}$, which is independent of the tumor suppressor p53, abrogation of Bcl-2/Bcl-XL, activation of Bid and Bim,[22-24] increase in the proapoptotic protein Bax, and activation of caspases 3 and 8.[23,25,26] Activity in CTCL cell lines has been demonstrated at concentrations of 1–5 µm, with selective induction of apoptosis in CTCL cell lines and greater apoptosis of peripheral blood lymphocytes from patients with CTCL than the peripheral blood lymphocytes of healthy donors. These proapoptotic changes occur selectively despite the accumulation of acetylated histones and the induction of cell-cycle arrest occurring in both normal and tumor cells.[27]

Favorable preclinical studies and the response of a CTCL patient in a phase I trial[28] led to the initiation of a single-center phase II dose-finding trial of oral vorinostat to determine response rate and duration in patients with relapsed or refractory stage IA–IVB CTCL. Thirty-three patients, 28 with advanced-stage CTCL, with a median of 5 prior therapies (range, 1–15), were enrolled in 1 of 3 sequential dosing cohorts. Eight patients achieved a

partial response (PR), including 7 with advanced-stage disease and 4 with Sézary syndrome, the leukemic form of CTCL. The median time to response (TTR) and time to progression (TTP) for responders was 11.9 and 30.2 weeks, respectively. Moreover, 14 of 31 evaluable patients had relief from pruritis. Responses were observed in all stages of the disease, including patients with early-stage refractory mycosis fungoides, tumors with large-cell transformation, and nodal and blood involvement. The most common drug-related side effects of fatigue, thrombocytopenia, and gastrointestinal upset were generally well-tolerated, and the regimen of 400 mg daily appeared to have the most favorable safety profile.[29]

Another single-arm open-label phase IIb trial in 74 heavily pretreated patients, whose previous treatments had included bexarotene, has also been performed. Of all patients, 61 patients had at least stage IIB disease. The overall response rate (ORR) was 29.7% and 29.5% in patients with stage IIB or higher disease. The median duration of response was not reached but estimated to be greater than 185 days, and the TTP was 4.9 months overall, and 9.8 months for stage IIB or higher responders.[30]

Consequently, vorinostat was recently approved by the US Food and Drug Administration (FDA) for patients who have progressive, persistent, or recurrent CTCL following 2 prior systemic treatments.[31]

Romidepsin Romidepsin (depsipeptide, FK228) is a cyclic peptide with inhibitory activities against class I and II HDACs. Early in vitro studies of romidepsin in T-cell lymphomas demonstrated significant apoptosis in the HUT78 human CTCL cell line. Investigations revealed induction of histone acetylation, increased $p21^{waf1/cip1}$ expression, detection of markers of apoptosis and cell-cycle arrest.[32] In 2001, responses in 4 patients with T-cell lymphoma were reported. These patients, on a phase I trial conducted at the National Cancer Institute (NCI), were treated with 12.7 or 17.8 mg/m^2 of romidepsin on days 1 and 5 of a 21-day cycle. Two patients had refractory Sézary syndrome and experienced a rapid fall in Sézary cells with improvement in skin erythema and edema. Another patient with extensive tumors achieved a response. One patient with refractory peripheral T-cell lymphoma (PTCL) and heavy skin infiltration achieved complete remission (CR) after 8 cycles of therapy.[33]

The achievement of CRs in patients with Sézary syndrome was confirmed in a multicenter NCI study in which investigators administered romidepsin as a 4-hour infusion on days 1, 8, and 15 of a 28-day cycle with a starting dose of 14 mg/m^2. The preliminary results of 71 patients with CTCL showed an ORR of 31%, with CR observed in 4 patients, PR in 18, and stable disease (SD) in 9. The median duration of response was 17, 5.5+, and 6+ months, respectively. One patient remained in an ongoing CR off therapy after 5 years, and 1 patient who achieved PR continues to receive romidepsin for more than 69 months. RNA analysis of both normal and malignant circulating peripheral mononuclear cells in this trial demonstrated increased histone acetylation. In addition, changes in a recognized gene signature for CTCL[34] were also detected when analyzed by quantitative polymerase chain reaction (PCR).[35] This signature is composed of the 5 genes *STAT4, GATA-3, PLS3, CD1D,* and *TRAIL.* Of note, PCR of peripheral blood mononuclear cells for these genes demonstrated high sensitivity and specificity for Sézary syndrome, rather than aleukemic CTCL, and it has been suggested that these genes can be utilized for a molecular diagnosis of Sézary syndrome.[34]

Favorable responses in CTCL have been confirmed in a transatlantic study of patients with stage IB–IVA CTCL/Sézary syndrome. In this study of 31 evaluable patients who had received a median of 3 prior systemic therapies including prior chemotherapy, an ORR of 39%, with a CR rate of 8%, was observed. Moreover, a further 58% had stabilization of their disease and 53% of all patients demonstrated relief of pruritis. The median TTR for these patients was 2 months.[36]

Panobinostat Panobinostat (LBH589) is an orally or intravenously available hydroxamic acid derivative with pan-HDAC inhibitory activity. It too has demonstrated activity in phase I studies in CTCL, with 10 relapsed patients treated with 20 or 30 mg orally 3 times per week, with 2 of these patients achieving CR, as well as 4 PRs and 2 SD. Microarray data on punch biopsies of CTCL-affected skin at 0, 4, 8, and 24 hours postadministration demonstrated a distinct gene-expression response profile, with a variety of genes altered, including those involved in cell cycle, cell proliferation, angiogenesis, apoptosis, and immune regulation.[37] In a further phase I trial of weekly intravenous (IV) panobinostat given on days 1, 8, and 15 of a 4-week cycle, 1 patient with CTCL achieved CR and 1 patient with PTCL achieved PR.[38]

Other HDAC Inhibitors In a large phase I trial of MS-275, 2 of the patients had lymphoma and, of those, 1 patient with CTCL achieved SD.[39]

Peripheral T-cell Lymphomas

Most of the early clinical trials of HDAC inhibitors included small numbers of patients with PTCL, with occasional responses recorded.[38] The large phase II trial of romidepsin also reported results in patients with PTCL, with a response rate of 28% in a group of 39 patients, with 3 CRs, 8 PRs, and 5 SD. The median duration of response for these groups was 12, 12, and 6 months, respectively.

Responses were observed independent of prior therapy, with some patients having undergone prior stem cell transplantation. Responses were observed across a variety of subtypes of PTCL, including PTCL (not otherwise specified), ALK-negative anaplastic large-cell lymphoma, and enteropathy-associated T-cell lymphoma.[35] A patient with a CD4- and CD56-positive hermatodermic neoplasm enrolled on a phase I/IIa trial of oral panobinostat achieved PR at a dose of 45 mg 3 times per week.[40]

Diffuse Large B-cell Lymphoma And Burkitt Lymphoma

Twelve patients with relapsed or refractory DLBCL were treated with single agent IV or oral vorinostat in 2 combined phase I trials. Three patients achieved an objective response, including 2 patients with a Richter's transformation of chronic lymphocytic leukemia (CLL) who achieved ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET)-negative CR and PR, respectively.[28] Of the 18 patients treated on a phase II trial of vorinostat in multiply relapsed or refractory DLBCL, 1 patient achieved ongoing CR after 3 months, with remission lasting 225+ days; another had SD for 301 days.[41] A phase I trial of dose-escalating IV belinostat (PXD101) given on days 1–5 of a 21-day cycle in 11 patients with refractory hematologic tumors included 5 patients with non-Hodgkin lymphoma (NHL). Of these, 1 patient with a Richter's transformation of CLL had ongoing SD for over 6 months.[42]

Follicular Lymphoma, Mantle Cell Lymphoma, and Other Indolent B-cell Lymphomas

In vitro data of trichostatin A and sodium butyrate on follicular NHL cells has shown induction of cell-cycle arrest and downregulation of Bcl-2 expression. Western blot analysis and chromatin immunoprecipitation assays showed that despite increased overall acetylation, localized H3 histone deacetylation occurred at both Bcl-2 promoter areas.[43] Furthermore, it seems romidepsin in particular has the unique ability to overcome the block in apoptosis mediated by prosurvival Bcl-2 proteins. This observation is unlike others seen with HDAC inhibitors such as vorinostat and panobinostat, whose effects in vitro and in vivo appear to be completely suppressed by overexpression of these proteins (Newbold and Johnstone, unpublished data). Considering that romidepsin is a prodrug, it is possible that in addition to inhibiting HDAC activities, this compound may affect other molecular processes that lead to apoptosis even in the presence of overexpressed prosurvival Bcl-2 proteins.

A phase I study undertaken in Japan where oral vorinostat was given at 200 mg twice daily for 14 days every 3 weeks to patients with NHL included 4 patients with follicular NHL, 2 of whom showed PR and another tumor reduction of greater than 30%.[44]

In mantle cell lymphoma lines with t(11;14)(q13,32), romidepsin was able to induce H3 acetylation and downregulate levels of Bcl-2, Bcl-XL and Mcl-1, inducing apoptosis via the mitochondrial pathway, in a dose-dependent fashion.[45] Vorinostat is able to cause a marked decrease in cyclin D1 in mantle cell lymphoma lines, despite mRNA levels and protein stability of cyclin D1 remaining minimally affected. It appears that the translation of cyclin D1 is blocked by inhibiting the PI3/Akt/mTOR/eIF4E-BP pathway.[46] Other studies show that, in addition, vorinostat upregulates $p21^{waf1/cip1}$ and $p27^{kip1}$ and inhibits vascular endothelial growth factor, leading to apoptosis in a dose-dependent manner.[47] Following from this finding, synergy has been demonstrated between the small-molecule mTOR inhibitor temsirolimus and vorinostat, with subsequent ERK dephosphorylation and caspase 3 activation in 3 mantle cell lymphoma lines,[48] allowing the prospect of clinically relevant synergy with tolerable and nonoverlapping side effects.

Investigators in a phase II study using the 200-mg twice-daily dose of vorinostat treated 15 patients with relapsed or refractory follicular, marginal zone, and mantle cell lymphoma. Ongoing CR was observed in a patient with follicular NHL after treatment discontinuation; PR was seen in another. Three patients with marginal zone lymphoma had ongoing SD beyond 9 cycles of treatment.[49]

Significant survival advantage has been demonstrated using romidepsin in Epstein-Barr virus–transformed lymphoblastoid cell tumors in mice, where 90% survival was seen at 30 days, compared to 20% in the control group. Romidepsin-mediated apoptosis was associated with a 12-fold increased level of active caspase 3, but some apoptosis persisted despite z-VAD-fmk treatment to inhibit caspase activity. Romidepsin-resistant cells expressed higher levels of latent membrane protein 1 and nuclear factor (NF)-κβ than romidepsin-sensitive cells; apoptosis was able to be induced by NF-κβ inhibition. These data imply that the apoptosis induced by romidepsin is via caspase-dependent and -independent pathways.[50] There are no published results on the use of romidepsin in B-cell lymphomas.

Adult T-cell Leukemia/Lymphoma

Adult T-cell leukemia/lymphoma (ATL) is a highly aggressive chemotherapy-resistant disease associated with HTLV-1 infection, with an exceptionally poor prognosis. A recent study looked at the effects of vorinostat, MS-275, and panobinostat on both HTLV-1–infected (MT-1, MT-2, MT-4, and HUT102) and human ATL cells. All of the HDAC inhibitors induced G2/M cell-cycle arrest and apoptosis and appeared to cause

accumulation of NF-κβ by blocking its nuclear translocation (Newbold and Johnstone, Peter MacCallum Cancer Center, Australia, unpublished data). Given the dire prognosis of this particular, albeit uncommon, disease, HDAC inhibitors deserve to be tested in the clinical setting with chemotherapy as well as antiviral therapies.[51]

Clinical Studies of HDAC Inhibitors in Multiple Myeloma

Several investigators have demonstrated that as single agents, HDAC inhibitors have activity against multiple myeloma cells in vitro,[52-56] inducing growth arrest and apoptosis in both cell lines and primary patient cells in a dose-dependent manner. This effect is associated with downregulation of Bcl-2, Bcl-XL and Mcl-1 and upregulation of p21 and p53, with these effects seemingly IL-6–independent.

The first clinical trial of HDAC inhibition specifically in myeloma patients was by Niesvizky and colleagues using the cyclic peptide romidepsin. In this phase II trial, romidepsin was well-tolerated, with fatigue, nausea, and transient thrombocytopenia being the major adverse events. Twelve patients were treated at the same dose used successfully to treat CTCL and PTCL, 14 mg/m^2 IV on days 1, 8, and 15 of a 28-day cycle.[57] Patients were generally heavily pretreated, with an average of 3 prior lines of therapy (range, 2–4) and a mean disease duration of 6.25 years (range, 2–9). In this trial, using a stringent response criteria, 2 patients were withdrawn after the first cycle with SD. Six patients progressed during the first 2 cycles and 4 patients remained stable after 2–7 cycles. There were no PRs observed, although no definition of minimal response was used (unlike the Richardson trial[58]). Moreover, although SD was the best response, rapidly increasing paraprotein levels were generally observed during the prior treatment with plateauing of the paraprotein level with romidepsin therapy.[59]

Richardson and associates reported on 13 patients with multiple myeloma treated with vorinostat in a dose-escalating phase I design. The study was prematurely terminated because of sponsor withdrawal and the maximum tolerated dose was not determined. Of note, the dose intensity administered in the various cohorts was somewhat lower than that generally used for the treatment of CTCL (400 mg/day continuous dosing).[58]

ITF2357 is an orally effective member of the hydroxamic family of HDAC inhibitors with in vivo activity against multiple myeloma cells.[60] A recent phase II study administered 150 or 100 mg of ITF2357 every 12 hours for 4 consecutive days followed by 3 days of rest every week of a 28-day cycle. Up to 12 weeks of treatment with ITF2357 were scheduled. Concomitant oral dexamethasone was given at a maximum dose of 20 mg every week. Sixteen patients with multiple myeloma with a median of 3 prior therapies (range, 2–8) were treated. The most common grade 3–4 toxicities were gastrointestinal side effects, neutropenia, and thrombocytopenia. Five patients achieved SD and 1 achieved PR.[61]

Panobinostat, another orally available hydroxamic acid derivative, is also currently being evaluated in phase I and II studies in patients with multiple myeloma. In an ongoing phase I study of 7 evaluable patients treated to date, 3 heavily pretreated patients have achieved SD, with improvements in constitutional symptoms and plateauing of the monoclonal protein observed (A. Spencer, Alfred Hospital, Melbourne, personal communication).[40]

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Romidepsin in Multiple Myeloma

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A 60-year-old man was initially diagnosed with immunoglobulin G lambda multiple myeloma in February 2006 when he presented to a dermatologist with eczema. Protein electrophoresis demonstrated a paraprotein level of 40 g/L, with a plasma-cell bone marrow infiltration of 50%. Conventional cytogenetics were normal but fluorescence in situ hybridization demonstrated t(4;14) with no associated 13q deletion. He had a number of asymptomatic bone lesions visible on skeletal survey. He commenced 4 cycles of infusional vincristine, doxorubicin, and dexamethasone (VAD) chemotherapy but failed to achieve PR, with the M band falling only to 23 g/L. Marrow infiltration was reduced to 13%. He subsequently underwent successful stem cell mobilization and collection following 1 course of cyclophosphamide (4 gm/m^2) with filgrastim. Given his lack of response he was observed over 6 months without treatment, with his M band climbing to 51 g/L, with 90% marrow infiltrate on biopsy.

He was referred to Peter MacCallum Cancer Centre and entered a clinical trial of bortezomib with dexamethasone, which involved maintenance therapy. Specifically, induction consisted of 8 3-week treatment cycles: bortezomib 1.3 mg/m^2 as a single bolus IV dose on days 1, 4, 8, 11 and oral dexamethasone 20 mg once daily on the day of bortezomib injection and the day thereafter (days 1, 2, 4, 5, 8, 9, 11, 12) followed by a 10-day break between doses of bortezomib (days 12–21). Consolidation consisted of 3 5-week treatment cycles of bortezomib 1.3 mg/m^2 as a single bolus IV dose on days 1, 8, 15, 22 and oral dexamethasone 20 mg once daily on the day of bortezomib injection and the day thereafter (days 1, 2, 8, 9, 15, 16, 21, 22) followed by a 13-day break between doses of bortezomib (days 23–35). Maintenance consisted of bortezomib 1.3 mg/m^2 as a single bolus IV dose on days 1 and 15 and oral dexamethasone 20 mg once daily on the day of bortezomib injection and the day thereafter (days 1, 2, 15, 16) of 4-week cycles until disease progression.

By the end of cycle 2 of induction, the patient's M band had fallen from 51 to 14 g/L and reached a nadir of 5 g/L by the end of cycle 5. It remained at this level through the consolidation phase, with marrow showing 10% involvement with malignant plasma cells. However, by the end of the third month of maintenance it started to climb to 12 g/L and then 16 g/L (total of 11 months on bortezomib therapy). Staging marrow showed approximately 30% involvement, with no evidence of renal or bone disease. Adverse events of the bortezomib/dexamethasone combination included grade 1 sensory peripheral neuropathy affecting both feet and grade 1 constipation. Platelet count at the commencement of each cycle was approximately 230 $\times$ 10^9/L, with cycle nadir approximately 170 $\times$ 10^9/L.

At the time of this progression on the bortezomib/dexamethasone maintenance phase, he was immediately entered into a subsequent clinical trial consisting of romidepsin with bortezomib. In this trial, patients received bortezomib (1.3 mg/m^2 on days 1, 4, 8, 11) with dexamethasone (20 mg on days 1, 2, 4, 5, 8, 9, 11, 12). This patient's dexamethasone dose was capped at the same level he was receiving on his prior bortezomib maintenance therapy (ie, 12 mg on the days of and after bortezomib). Romidepsin was administered at 10 mg/m^2 IV on days 1, 8, and 15 every 28 days.

After 1 cycle (day 28) the patient's paraprotein level had fallen to 11 g/L and remains with SD after 5 cycles. He remains free of disease elsewhere. He has tolerated the treatment well, with the only adverse events being grade 1 fatigue. With each cycle he developed thrombocytopenia, with a nadir of approximately 45 $\times$ 10^9/L occurring from days 8–15 and rebounding above baseline before the commencement of each cycle (Figure 1).

Discussion

Patients with myeloma harboring t(4;14) have a generally poor prognosis, with alkylating agents failing to induce prolonged remissions. Even high-dose therapy with autologous stem cell transplantation results in remission

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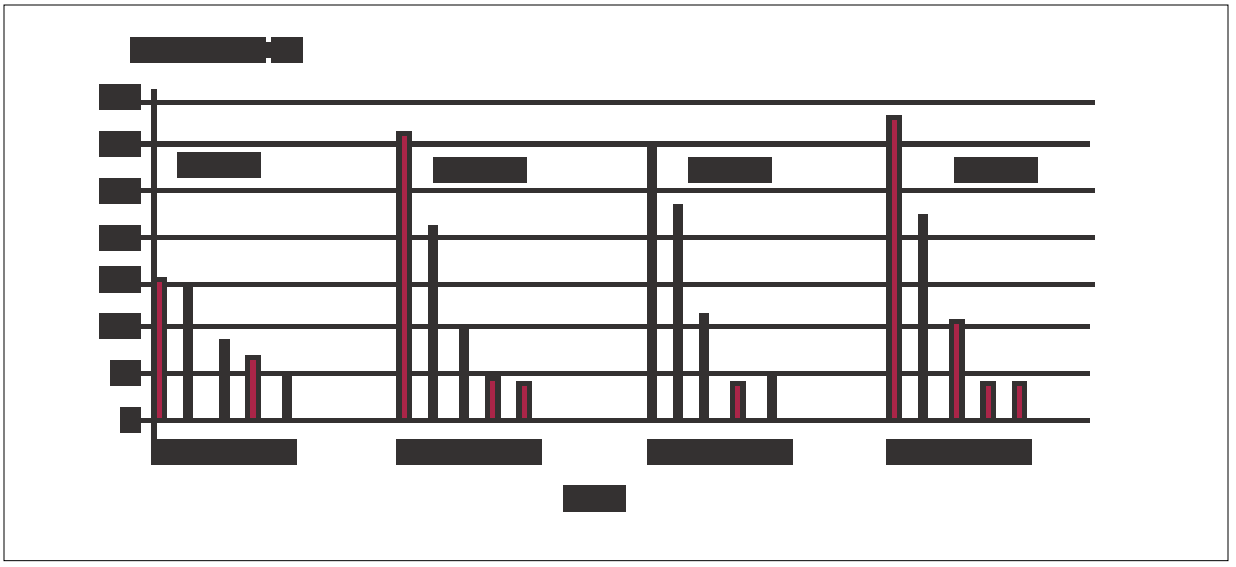

Figure 1. Platelet kinetics of patient receiving bortezomib and romidepsin.

generally shorter than 12 months.[1] Thus, novel strategies are required in such patients. Bortezomib appears to overcome some of the adverse characteristics of this subtype of myeloma, with response rates and response duration observed similar to those patients without this translocation.[2] Thus, bortezomib therapy seemed the most appropriate treatment for our patient. Indeed, he achieved a response with a durable progression-free interval of 11 months. The particularly novel aspect of the initial clinical trial he entered was the use of maintenance bortezomib. The impact of this strategy on remission duration is unknown and full analysis of this trial (follow-up ongoing) will give some insight as to whether remission duration is improved with a maintenance bortezomib strategy.

At the time of progression on bortezomib we had a number of choices of therapy. Waiting for symptomatic progression was 1 option, but given the tempo of the climb in the M band we elected to treat immediately. Although we decided to enroll him on a clinical trial to see if the addition of the HDAC inhibitor romidepsin would result in a further drop in his M band, we discussed other treatment options with the patient. These included high-dose melphalan with autologous stem cell transplantation (with or without thalidomide maintenance) and thalidomide- or lenalidomide-based therapy.

Tolerance of the combination was excellent, with no additional nonhematopoietic toxicity except for grade 1 fatigue. Of particular interest was the pattern of thrombocytopenia (Figure 1). Indeed, transient thrombocytopenia is relatively common with most HDAC inhibitors[3] and is usually mild, although it has been dose-limiting in some studies.[4,5] Typically, platelet count nadir occurs during the second week of therapy and is self-limiting. The pathophysiology of thrombocytopenia is not clearly understood; however, the finding of normal number of megakaryocytes with hypolobular nuclei during platelet nadir may suggest impairment in megakaryocytic differentiation/budding.[6,7] Anemia and neutropenia are uncommon, although septic complications have been rarely reported.[8] As both bortezomib and romidepsin cause transient thrombocytopenia, we were particularly interested in this study to examine the kinetics and depth of thrombocytopenia.

Of particular note in this case study was the fall in the M band with the introduction of the romidepsin to the ongoing bortezomib. This observation supports the hypothesis that romidepsin subverts some of the escape pathways that develop in bortezomib resistance. Several groups studied HDAC inhibitors preclinically in combination with the proteasome inhibitor bortezomib, demonstrating that this combination synergistically induces apoptosis, mitochondrial injury, radical oxygen species generation, and oxidative injury in multiple myeloma cells.[9-11] In addition, inhibition of aggresome formation (a key escape mechanism for survival of malignant plasma cells following bortezomib therapy) appears to be crucial. When the proteasome is inhibited by bortezomib, aggregates of misfolded proteins are directed along acetylated alpha-tubulin fibrils toward a single perinuclear region, forming an aggresome. This process allows the cell to survive and requires tubulin deacetylase activity.

Catley and colleagues demonstrated that panobinostat induces alpha tubulin hyperactylation. Thus, when panobinostat is administered with bortezomib, hyperacetylated alpha-tubulin form bundles, with diminished aggresome formation, and the cell undergoes apoptosis.[10] Moreover, Kawaguchi and colleagues demonstrated that HDAC6, a microtubule-associated deacetylase, is a component of the aggresome and has the capacity to bind both polyubiquitinated misfolded proteins and dynein motors, thereby acting to recruit misfolded protein cargo to dynein motors for transport to aggresomes. Cells deficient in HDAC6 fail to clear misfolded protein aggregates from the cytoplasm, cannot form aggresomes properly, and are rendered hypersensitive to the accumulation of misfolded proteins.[12] Finally, romidepsin and bortezomib have been shown to interact synergistically in CD138-positive primary human bone marrow multiple myeloma, but not in their normal CD138-negative counterparts, with romidepsin also enhancing the lethality of bortezomib in bortezomib-resistant U266 multiple myeloma cells. (S. Grant, Virginia Commonwealth University, unpublished data).

Consequently, investigators are now examining the combination of HDAC inhibitors with bortezomib in clinical trials. These studies are still immature but do demonstrate that such combinations are tolerable with near-maximum single-agent doses of both drugs deliverable, with promising response rates.[13-15] The study our patient entered was initially a phase I study of progressive dose escalation of romidepsin in combination with standard bortezomib/dexamethasone dosing. Response was assessed after every 2 cycles according to International Myeloma Working Group criteria (with minimal response defined as >25% but <50% reduction in M protein). Toxicities were assessed using NCI-CTCAE version 3. In total, 25 patients have been enrolled, of which 18 have completed more than 2 cycles and are evaluable for response. The median number of prior regimens is 2 (range, 2–5). The majority of patients have been treated previously with autologous stem cell transplantation (n=11) and neurotoxic regimens: VAD (n=10), thalidomide (n=12), bortezomib (n=6), and lenalidomide (n=4). The median number of treatment cycles delivered was 4 (range, 1–8) and maintenance cycles 6 (range, 3–15). Ten patients entered the phase 1 study. No dose-limiting toxicities occurred at 8 mg/m^2 (n=1) or 10 mg/m^2 (n=6) of romidepsin. At 12 mg/m^2 (n=3), dose-limiting toxicities of thrombocytopenia (grade 4, n=3), febrile neutropenia (n=1), peripheral neuropathy (n=1), and constipation (n=1) were observed. Of note, 2 patients with grade 4 thrombocytopenia had platelet counts of 50–100 $\times 10^9$/L prior to commencing therapy. The maximum tolerated dose for this regimen was determined to be romidepsin 10 mg/m^2 with bortezomib 1.3 mg/m^2. Other drug-related toxicities observed included grade 3 fatigue (n=2), neutropenia (n=1), sepsis (n=2), and peripheral neuropathy (n=1) and grade 2 peripheral neuropathy (n=6) and nausea (n=1). Five patients required dose reductions of bortezomib due to peripheral neuropathy (n=4). Two patients required a dose reduction in romidepsin because of fatigue (n=1) and abnormal liver function tests (n=1). The ORR is 67% (12/18; 5 CR/near CR, 3 very good PR, 4 PR) with an additional 5 (28%) patients achieving a minimal response. Four patients have progressed, after C1 (n=2), C4 (n=1), and C8+3Mx (n=1). Of note, 3 patients entered this trial after having progressed on the bortezomib maintenance trial as described earlier. These 3 patients were receiving fortnightly bortezomib and progressing; on the introduction of bortezomib/romidepsin, the M band has fallen from 41 to 26 g/L (C2); 16 to 11 g/L (C2); 1 patient has not completed C1. We found that this combination of a proteasome inhibitor and dexamethasone with romidepsin is well-tolerated and demonstrates substantial efficacy in a heavily pretreated group of patients. The high response rate (ORR, 67%), impressive depth of response (44% CR + very good PR), durable responses, and the observation of a drop in M band in patients progressing on bortezomib as their immediate prior therapy all indicate that this HDAC inhibitor has synergistic activity with bortezomib/dexamethasone.

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Romidepsin in Non-Hodgkin Lymphoma

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A 59-year-old woman presented in January 2005 with atypical symptoms of pruritus, then peripheral small lymphadenopathies, fever, and cough, leading to the diagnosis of PTCL, an angioimmunoblastic T-cell lymphoma (AITL) subtype in January 2006. At the time she was referred to our department, she had fever (38.5°C), weight loss (-6 kg), abundant night sweats, severe pruritus, and cough. Performance status was 2. Enlarged lymph nodes were found in cervical, axillary, and inguinal areas. Computed tomography (CT) scan did not show any thoracic or abdominal lymph nodes or splenomegaly. Hemoglobin level was 11.6 g/dL, with blood infiltration by the lymphoma. C-reactive protein level was 30 mg/L, lactate dehydrogenase 513 UI/L (normal, <450). Coombs test and other biologic examinations were normal. Lymph node, skin, and bone marrow biopsies showed a typical AITL.

The patient was included in a prospective study of doxorubicin, cyclophosphamide, vindesine, bleomycin, and prednisone (ACVBP) combined with bortezomib (LNH05-1T study, Groupe d'Etude des Lymphomes de l'Adulte, not yet published). She received 4 induction courses followed by sequential consolidation from January to August 2006. After induction, she was in very good PR with only a small bone marrow infiltration. At the end of treatment, she was in CR.

Four months later (December 2006), pruritus reappeared without any other symptoms indicating a relapse. Skin lesions reappeared in February 2007 and a biopsy showed a relapse of AITL. No other location was found and the patient was treated with corticosteroids only. In June 2007, peripheral lymph nodes reappeared. The patient was included in a phase II study of pralatrexate run by Allos Therapeutics (not yet published). At the end of the first cycle, her performance status improved, peripheral lymph nodes notably disappeared (<1 cm in diameter for the largest), and skin lesions decreased. However, at time of the second cycle, PET scan showed persistence of active lymph node fixation in different areas, and pruritus and skin lesions had increased. This finding was considered progression of the lymphoma and the patient left the study.

In October 2007, she entered a phase II study of romidepsin (Gloucester Pharmaceuticals, ongoing study). Before treatment, she presented severe pruritus with numerous skin lesions and cervical and axillary lymph nodes (Figure 2). PET scan showed active lesions in these lymph nodes. Bone marrow was normal. After the first cycle, pruritus, skin lesions, and lymph nodes improved. Tolerance was good, with a short episode of tachycardia 4 hours after the third infusion. At the end of cycle 2, she presented with grade 2 neutropenia and thrombocytopenia. During the second cycle she experienced nausea

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and/or vomiting after each infusion. Staging showed CR: no pruritus or skin lesion, no lymph node, and no fixation on PET scan. During the third cycle, she experienced nausea and/or vomiting, grade 3 thrombocytopenia (43,000/µL), and grade 4 neutropenia (480/µL). She also presented cutaneous papules different from previous skin lesions; a biopsy showed these lesions to be compatible with drug toxicity and absent of lymphoma cells. These lesions spontaneously disappeared.

During cycles 4–10, she experienced nausea or diarrhea after some of the infusions (grade 1 or 2). Platelet or neutrophil levels decreased after some of the infusions, usually with nadir near 70,000/µL for platelets and 700/µL for neutrophils. No infectious manifestations or hemorrhage occurred. Romidepsin infusions were regularly performed every week without delay. Staging at months 4, 6, 8, and 10 confirmed the persistence of CR.

She is currently in the twelfth cycle and plans to continue the treatment with a less intense schedule: romidepsin will be given once every 2 weeks during the next 12 months.

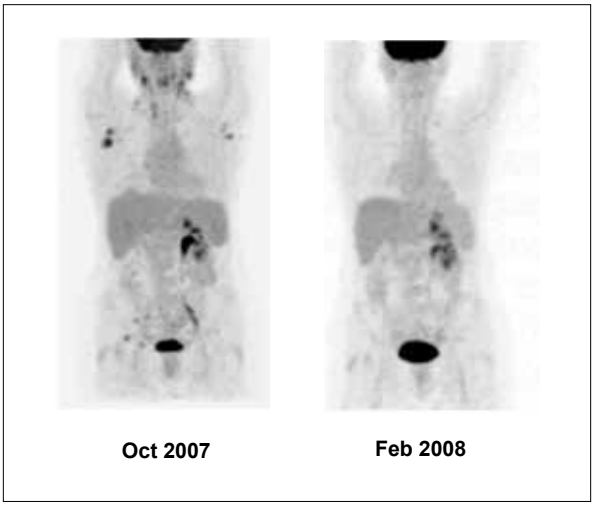

Figure 2. PET scan before treatment (Oct. 2007) showing cervical, axillary, and inguinal fixing lymph nodes and after 3 courses of romidepsin (Feb. 2008) without any pathologic fixation.

Discussion

AITL is one of the most frequent PTCLs in Europe and is a little less frequent in Asia and North America.[1] As in our case, diagnosis is often delayed because of atypical presentation. Symptoms at diagnosis are heterogeneous while the increase in lymph node volume might be small. Patients frequently present with poor performance status, fever, weight loss, small-enlarged peripheral lymph nodes, stage IV bone marrow involvement, anemia, and various biologic abnormalities. Diagnosis is usually easy to obtain once a lymph-node biopsy has been performed.[2] Median survival is usually short, approximately 2–3 years, with 30% of the patients surviving at 5 years.[1] No good treatment has been described;[1] in other PTCLs and cyclophosphamide, doxorubicin, prednisone, and vincristine (CHOP) is frequently used even if results are quite inferior to those obtained in DLBCL.[3]

A small number of studies have tried to identify prognostic factors in AITL, yielding controversial results.[4-7] Their findings might be hampered by the relatively limited number of patients and/or the heterogeneous therapies. The impact of international prognostic index score was rarely defined in the literature for AITL and it was curiously not predictive of survival at all. And, in contrast to what has been originally reported for PTCL not otherwise specified, a high prognostic index for PTCL-U score was not associated with a worse outcome in our AITL series.[8]

Several therapeutic possibilities have been proposed to improve on these results but usually failed to succeed. CHOP is the chemotherapy regimen most used but it is certainly not the best because its efficacy is low and not better than other classic regimens.[1] In one study in which patients received more intensive chemotherapy compared with previous reports, it was not possible to demonstrate any survival benefit for patients submitted to consolidation with autologous stem cell transplantation.[7] In another retrospective study, including 29 AITL patients treated with high-dose chemotherapy followed by autologous stem cell transplantation, CR increased from 45% before high-dose chemotherapy to 76% after, with 44% and 37% 5-year overall and progression-free survival, respectively.[9] Similar results have been recently reported in a large series of AITL patients treated with high-dose chemotherapy and autologous stem cell transplantation, with 59% and 42% overall and progression-free survival, respectively, at 4 years after transplantation.[10]

In a small phase II study, cyclosporine was associated with a good response rate: 8 of 12 treated patients responded with 3 CRs.[11] Among the new molecules tested in PTCL, zanolimumab, an anti-CD4 antibody has shown preliminarily interesting results: 1 CR and 2 PRs in the 4 AITL patients.[12,13] Alemtuzumab alone has little activity, but combined with CHOP its activity is increased: 6 of 6 patients responded in a phase II study.[14] However, this regimen was associated with a high rate of opportunistic infections or viral reactivation.

Another promising class, HDAC inhibitors, have shown high activity in CTCLs and are currently being tested in PTCL.[15] Preliminary results from a phase II

study showed a good response rate, with 3 CRs and 8 PRs among the 27 treated patients (30%).[16] Our patient confirms the good efficacy of romidepsin in such refractory patients.

In conclusion, the therapeutic possibilities for PTCL are beginning to change, and in the near future we may achieve better results than before. However, the definitive interest of these new drugs will only be demonstrated through randomized studies in the first-line setting. These studies will be undertaken only when better agents to be combined with CHOP have been identified.

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Romidepsin in Cutaneous T-cell Lymphoma

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CTCLs, a heterogeneous group of disorders, present primarily in the skin.[1] The most common CTCL subtypes are mycosis fungoides, which presents as skin patches, plaques, and tumors, and Sézary syndrome, in which patients present with erythroderma (diffuse erythema >80% of skin surface area) and leukemic blood involvement. Prognosis varies widely, with early-stage disease having indolent behavior, whereas patients with advanced disease have poor prognosis. Numerous skin-directed therapies (SDTs) and systemic agents are available, but durable responses are rare, particularly in patients with Sézary syndrome. Romidepsin (FK228), a novel HDAC inhibitor, is currently in development for T-cell malignancies. We present a case of Sézary syndrome that responded to romidepsin in clinical trial.

Case History

Our patient is a 52-year-old white woman with no significant past medical history, who in 1999 developed a pruritic truncal rash that progressed to involve her face. She was diagnosed with Sézary syndrome in 2003 (T4N0M0B2, 1979 NCI/MF Cooperative Group Stage III) and was referred to the Hospital of the University of Pennsylvania. She was treated with combination multimodality immunomodulatory therapy (various combinations of topical corticosteroids, topical nitrogen mustard ointment, PUVA phototherapy, oral bexarotene, isotretinoin, pegylated interferon-α, interferon-γ, and monthly extracorporeal photopheresis) and her skin lesions and itching resolved, but her peripheral blood disease did not improve. By May 2007, her skin lesions recurred and she developed enlarged axillary and inguinal lymphadenopathy; lymph-node biopsy confirmed nodal involvement by CTCL.

On physical exam, she was well-appearing but had diffuse moderate erythema, with scaling on her entire body surface area, including her scalp. She had mild scaling of her palms and soles but no keratoderma, alopecia, or ectropion. She had cervical, axillary, inguinal, and femoral lymphadenopathy (range, 1.5–2.5 cm^2 by palpation). Her peripheral blood white blood cell count was 17,900/μL, with 68% lymphocytes. Peripheral blood flow cytometry revealed an elevated CD4:CD8 ratio and an expanded population of aberrant CD3(dim)-, CD4(dim)-, and CD7-positive and CD26-negative T-cells (93% of lymphocytes, 11,338/μL). Her current disease stage was now T4N3M0B2, stage IVA.

In July 2008 she enrolled in a multicenter phase II open-label clinical trial (Gloucester Pharmaceuticals) at our site and received romidepsin 14 mg/m^2 as a 4-hour IV infusion on days 1, 8, and 15 of a 28-day cycle, for 6 cycles. As per protocol, she received granisetron or ondesatron as antiemetic premedication 30 minutes prior to romidepsin. Electrocardiograms (ECGs) were performed at baseline, after premedication, and at the end of romidepsin infusion to monitor QTc intervals. Disease assessments (erythroderma skin score, lymph-node diameter measurements, peripheral blood flow cytometry for Sézary count), clinical photographs, and peripheral blood flow cytometry were performed on day 1 of each cycle. CT scans of chest/abdomen/pelvis were performed at baseline and every 2 months.

At the start of the study, the patient had no systemic symptoms except for severe itch. Table 1 illustrates her baseline and subsequent peripheral blood indices. By the end of cycle 1 her previously severe itch had resolved, her skin erythroderma showed areas of clearing and her total white blood cell had normalized (17,900 to 8,400/μL). By cycle 6, she had complete resolution of her erythroderma (erythroderma skin score decreased 11 to 0; Figure 3), 97 % improvement in her peripheral blood malignant T-cell count (CD4+, CD26- population; Table 2), and 30% improvement in her lymphadenopathy (Figure 3). She experienced mild/moderate nausea, vomiting, taste disturbance, fatigue, diarrhea, and mild nonspecific ST-T wave ECG changes after each romidepsin infusion that would resolve after 3 days, but overall she tolerated treatment well.

She elected to stay on treatment beyond 6 cycles to maintain her response. By cycle 9, she experienced worsening nausea and fatigue with each infusion, and required

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Table 2. Patient's Laboratory Values at Baseline and Weeks 3, 5, and 7

Cycle	WBC (µL)	% Lymphs	CD4:CD8	% CD4+ CD26-	# CD4+ CD26- (µL)	LDH (313–681)
Baseline	17,900	68	64.6:1	93	11,338	667
3	6,900	29	18.9:1	75	1,510	450
5	9,000	11.8	4:1	42	448	491
7	10,000	11.2	1:1	17	188	426

LDH=lactate dehydrogenase; WBC=white blood cell count.

dose reduction from 14 to 10 mg/m^2. She developed a new nonspecific, mildly itchy, papular eruption on her face, trunk, and extremities by cycle 12, day 1. Skin biopsies revealed a nonspecific lichenoid lymphocytic infiltrate, suggestive of a lichenoid hypersensitivity reaction. Clinically and histologically, this new rash was different from her previous CTCL. Because the rash appeared to flare after each romidepsin infusion, she withdrew from the trial and her lichenoid skin eruption resolved within 2 months. She has been off therapy for 4 months and has maintained her clinical response.

Discussion

CTCLs ares rare extranodal NHLs that arise from skin-homing lymphocytes. Mycosis fungoides and Sézary syndrome comprise the majority of CTCL cases; the malignant T-cells in these conditions typically express cutaneous lymphocyte antigen (CLA)/CCR4/CD4 and lose CD7 and/or CD26 expression.[2] Histologically, mycosis fungoides shows an infiltrate of cerebriform lymphocytes with epidermotropism. Sézary syndrome is the leukemic form of the disease in which patients develop erythroderma and measurable blood involvement of over 1,000 Sézary cells/µL or an elevated blood CD4:CD8 ratio of over 10:1 and detectable aberrant T-cell populations (CD4-positive, CD7-negative >30%, CD4-positive, CD26-negative cells >40% of lymphocytes). As the disease progresses, the malignant cells may undergo large cell transformation, which is either CD30-positive or -negative. The malignant cells express a T-helper type 2 phenotype, producing increased interleukin (IL)-4, IL-5, and IL-10 cytokines, which results in attenuated Th1 responses and contributes to the endogenous immunosuppression observed in advanced patients.[3]

Current treatments depends on disease stage; recently the International Society of Cutaneous Lymphoma revised the original staging system proposed in 1979 (NCI/Mycosis Fungoides Cooperative Group). Treatment approaches for these conditions are distinct from that of other T-cell NHLs, as recently highlighted in the National Comprehensive Cancer Network (NCCN) Practice Guidelines in Oncology.[4] Limited patches and plaques can be treated with SDTs and managed primarily by dermatologists, whereas refractory skin disease or more advanced-stage disease (ie, tumors, erythroderma, patients with nodal, blood, or visceral involvement) may require systemic therapies, often in addition to the SDTs. Current SDTs include topical corticosteroids, topical

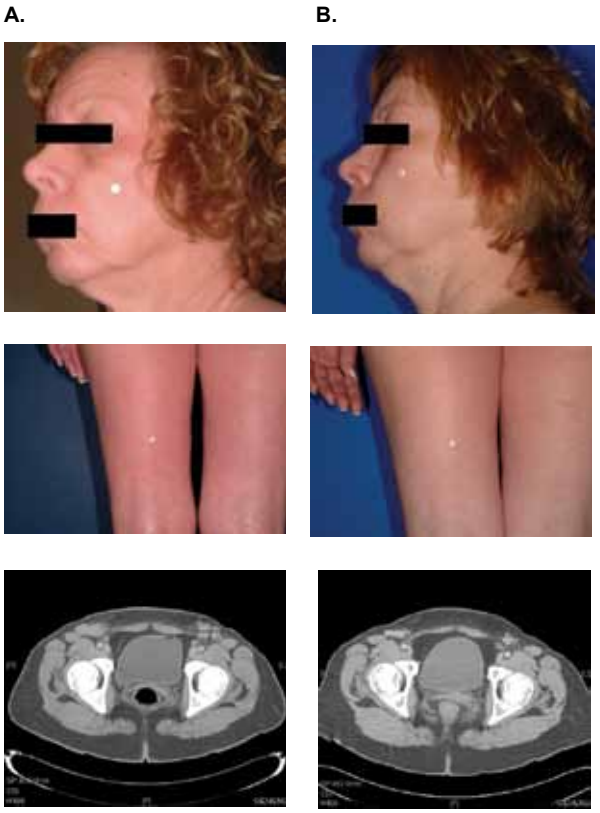

Figure 3. Column A: Baseline erythroderma (face, posterior thighs) and inguinal lymphadenopathy (computed tomography scan). Column B: After 6 cycles of romidepsin therapy, her skin has completely cleared and her inguinal lymphadenopathy improved 30%.

compounded chemotherapy, topical retinoids, imiquimod, phototherapy, and total skin electron beam radiation. Systemic therapies include interferon-α and -γ, bexarotene, extracorporeal photopheresis, denileukin diftitox, methotrexate, alemtuzumab, vorinostat, single-agent and combination chemotherapy, and hematopoietic stem cell transplantation. As in many other rare diseases, only a minority of these standard agents are FDA-approved for mycosis fungoides or Sézary syndrome, which can affect medical insurance prescription coverage.

HDAC inhibitors inhibit the deacetylation of histone proteins associated with DNA and appear to have potent anticancer effects on a number of cancer cell lines in vitro.[5] Their effects on gene expression result in alterations in cell proliferation, migration, differentiation, and apoptosis. In addition, they likely have myriad nonhistone effects; as reviewed by Bates and Piekarz, it remains to be seen which of these mechanisms are primarily responsible for their anticancer effects. HDAC inhibitors appear to have particular activity against T-cell lymphoid malignancies, and numerous agents are being tested in both CTCL and PTCL. Vorinostat, an oral pan-HDAC inhibitor, was FDA-approved for CTCL in 2006.[6] In the pivotal phase II multicenter clinical trial, an ORR of 29.5% was observed in 61 patients (stage IIB or higher) treated with 400 mg daily (1 patient achieved CR). In these patients, median time to treatment response was 56 days and duration of response ranged from 34 to 441 days. Median TTP for stage IIB or higher patients was at least 9.8 months. Nearly half of treated patients experienced grade 2 diarrhea, fatigue, and nausea. Grade 3 adverse events included fatigue (5%), pulmonary embolism (5%), thrombocytopenia (5%), and nausea (4%).

Romidepsin is an intravenously administered pan-HDAC inhibitor that has shown activity in CTCL and peripheral T-cell lymphoma in recent clinical trials.[7] In the recently completed pivotal multicenter phase IIB, single-arm, open-label trial, 23 of 48 (47.9%) refractory stage IIB–IVA CTCL patients had a clinical response as measured by a combination of a weighted scoring instrument to determine skin involvement (severity-weighted assessment tool or erythroderma skin score), imaging, and circulating Sézary cells (in all 72 evaluable patients, ORR was 42%, including 6 with a clinical CR).[8] The median follow-up of the cohort is only 5.3 months; thus, the median duration of response has not been reached.

The most common adverse events were nausea, fatigue, vomiting, anorexia, hypomagnesemia, and pyrexia. Several of these are likely class-related, given the similar profile observed with vorinostat. One third of patients experienced grade 3 or higher adverse events (fatigue, disease progression, pyrexia) and 22% had a serious adverse event (disease progression, pyrexia, sepsis, tumor lysis syndrome, and hypotension). In contrast to vorinostat, romidepsin did not cause thrombocytopenia. Notably, patients with significant cardiac history or who were taking QTc-prolonging or CYP3A4-inhibiting medications were excluded from the trial, given prior concerns of cardiac toxicity raised in earlier clinical trials.[9]

Our patient with Sézary syndrome had disease that had been refractory to 5 systemic therapies and had progressed to include nodal involvement. She demonstrated a significant clinical response to romidepsin, with rapid improvement of itch and peripheral blood Sézary count after the first cycle and subsequent complete resolution of her erythroderma by cycle 6. Our patient experienced progressive nausea and fatigue with subsequent cycles; alternative, less frequent dosing regimens, particularly in the maintenance phase could be explored. Given the broad effects of HDAC inhibitors on gene expression, there is great interest in whether they would enhance the activity of other systemic agents; combination trials in CTCL are ongoing.[5,10] Finally, the long-term immunologic effects of HDAC inhibitors are also an area of active research, with recent reports demonstrating anti-inflammatory effects and upregulation of T-regulatory cells.[11]

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