Phase I Trial of a New Schedule of Romidepsin in Patients with Advanced Cancers

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Statement of Translational Relevance

Romidepsin has clinical activity in T-cell lymphomas but is minimally active in solid tumors. Like other histone deacetylase inhibitors, romidepsin activity in cancer cells can be classified as either pro-apoptotic or as differentiation induction. We had previously shown in pre-clinical studies that prolonged exposure of thyroid cancer cell lines with low, non-toxic doses of romidepsin induced expression of thyroglobulin and the sodium/iodide symporter (NIS). This resulted in an enhanced uptake of radioactive iodine that could potentially re-sensitize thyroid cancers to radioiodine. In this dose escalation phase I study, we assessed the safety and efficacy of a new schedule of romidepsin in patients with solid tumors, with the goal of using romidepsin to induce expression of targets for anticancer agents, such as the sodium iodine symporter to increase uptake of radioiodine in patients with thyroid cancer.
ABSTRACT

Purpose: Romidepsin is a potent histone deacetylase inhibitor (HDI) with activity in T-cell lymphoma. Given preclinical data demonstrating greater induction of gene expression with longer exposures to HDIs, a phase I study of a days 1, 3, and 5 romidepsin schedule was evaluated. A secondary objective was to assess the effect of romidepsin on radioactive iodine (RAI) uptake in thyroid cancers.

Experimental Design: Open label, single arm, phase I, 3 + 3 dose escalation study. Romidepsin was administered as a four-hour infusion on days 1, 3 and 5 of a 21-day cycle. Pharmacokinetics (PK) and pharmacodynamics (PD) were assessed, including histone acetylation in peripheral blood mononuclear cells (PBMCs); RAI uptake in refractory thyroid cancer; and HDI-related ECG changes.

Results: 28 patients with solid tumors, including eleven patients with thyroid cancer were enrolled. Six dose levels were explored and 7 mg/m^2 on days 1, 3, and 5 was identified as tolerable. No RECIST-defined objective responses were recorded although nine patients had stable disease a median 30 weeks (range 21 – 112) including six with thyroid cancer a median of 33 weeks. PD studies detected acetylated histones in PBMCs and ECG changes beginning at low dose levels. Follow-up RAI scans in patients with RAI refractory thyroid cancer did not detect meaningful increases.

Conclusions: A romidepsin dose of 7 mg/m^2 administered on days 1, 3, and 5 was found tolerable and resulted in histone acetylation in PBMCs. Although there were no objective responses with romidepsin alone, this schedule may be useful for developing combination studies in solid tumors.
INTRODUCTION

Romidepsin (FK228, FR901228, NSC630176, depsipeptide) is a potent, natural product histone deacetylase inhibitor (HDI) (1, 2). HDIs prevent the activity of histone deacetylases (HDACs), leading to unrestricted histone acetyltransferase activity and increased gene transcription (3). HDI exposure effects a global increase in histone acetylation as well as gene expression changes that lead to p21 induction and cell cycle arrest (4); increased expression of markers of differentiation such as fetal hemoglobin, P-glycoprotein, and the sodium-iodide symporter (5-7); and alterations in the expression of apoptotic proteins (8). The mechanism of action of HDIs is likely to be model specific, as several mechanisms have been suggested (9).

Romidepsin is effective in T-cell lymphoma. In two Phase II trials, 167 patients with cutaneous T-cell lymphoma (CTCL) treated with romidepsin had a 34-35% overall response rate (ORR), supporting approval in CTCL (10, 11). Similarly, an ORR of 25-38% in peripheral T-cell lymphoma (PTCL) in two Phase II trials supported approval for that indication (12, 13). Despite the clinical success of romidepsin in T-cell lymphoma, it has been disappointing that clinical trials conducted in solid tumors have failed to show significant clinical activity (14-18). The reason for this lack of activity is not well understood and studies that define mechanisms of resistance to HDIs are still early. It has been suggested that increased thioredoxin levels, increased levels of antiapoptotic proteins such as Bcl-XL or Bcl-2, or increased MAPK signaling may play a role (3, 19-21).

One potential strategy for developing romidepsin, or other HDIs in solid tumors is to exploit the “differentiation” capacity of the agents. While synergy can be observed
between HDIs and cytotoxic agents (22-24), another approach is to use the HDIs to amplify a therapeutic target via their ability to increase gene expression. We previously demonstrated in preclinical studies that the sodium-iodide symporter (NIS), expressed at high levels in the normal thyroid, is induced in thyroid cancer cells with exposure to nontoxic doses of romidepsin for 48 - 72 hours (7). In vitro, romidepsin induced expression of thyroglobulin and NIS in both differentiated and anaplastic thyroid cancer cell lines. In turn, this increased radioactive iodine uptake suggesting that romidepsin could augment or induce radioactive iodine uptake in patients with thyroid cancer.

Earlier phase I studies of romidepsin tested two schedules of administration of romidepsin - day 1 and 5 every 21 days and day 1, 8, and 15 every 28 days; more dose intense schedules, such as daily dosing, were not developed due to greater toxicity in preclinical models. Because the day 1, 8, and 15 schedule that is active in T-cell lymphoma has not shown significant clinical activity in solid tumors, we sought an alternate schedule that might be more readily combined with other therapies and that would provide more continuous HDAC inhibition, even if for a limited time. We thus conducted an open label single arm phase I escalation study to establish the MTD and associated toxicities of romidepsin when administered as a four-hour infusion on days 1, 3 and 5 of a 21-day cycle. With the short half-life of romidepsin, no accumulation of the drug would be expected, but we hoped the more frequent exposure would augment the epigenetic effects of the agent and thereby increase NIS function and radioiodine uptake in thyroid cancers. Histone acetylation, ECG changes, and uptake of radioactive iodine in thyroid cancer were examined as pharmacodynamic markers of romidepsin effect.
PATIENTS AND METHODS

Patient Eligibility Criteria: The study was approved by National Cancer Institute Intuitional Board Review, and registered at www.clinicaltrials.gov NCT00048334. All patients were required to give a written informed consent. Eligibility criteria included Eastern Cooperative Oncology Group (ECOG) performance status ≤2, measurable disease, and an ejection fraction of >50% by echocardiogram or cardiac MRI, or 45% by MUGA scan. Patients with cardiac risk factors were excluded. Patients with thyroid cancer could not have medullary histology and had to have evidence of no or minimal (“faint”) radioactive iodine (RAI) uptake on RAI whole body scan, no RAI therapy within 3 months prior to study entry, and no history of administration of IV iodinated contrast or other large iodine loads (i.e. CT, amiodarone, Super Saturated Potassium Iodide (SSKI)) during the previous 3 months.

Trial Design and Dose Escalation: This was an open label, single arm, phase I, 3+3, dose escalation trial; infusion doses ranged from 1 to 9 mg/m². Romidepsin was administered as a four-hour infusion on days 1, 3 and 5 of a 21-day cycle. Dose modifications and dose escalation beyond cycle 1 were allowed. MTD was to be defined as the highest dose level that resulted in a dose limiting toxicity (DLT) in fewer than 2/6 patients.

Toxicity Evaluation: All adverse events in this trial were graded using the NCI Common Toxicity Criteria version 2.0. DLT was defined as a hematologic toxicity of
absolute granulocyte count (AGC) of <500 (Grade 4) for ≥5 days; platelet count of
<10,000 (Grade 4) (both in patients without bone marrow involvement); Grade 3 or
greater non-hematologic toxicity in patients without disease involvement of that
particular organ system, excluding potassium (K), magnesium (Mg), calcium,
phosphate, uric acid, nausea and vomiting. The latter were considered DLT if scored as
grade 4 or if occurring despite maximal prophylaxis.

**Efficacy Evaluation:** Disease assessments (imaging) were performed every two cycles
(each cycle being 21 days). The primary efficacy measure was objective disease
response (complete responses + partial responses) according to the Response
Evaluation Criteria in Solid Tumors Version 1 (RECIST v.1).

**Cardiac Evaluation:** Serum electrolytes (K, and Mg) were checked prior to treatment
and repleted if K <4.0 mmol/L or Mg <0.85 mmol/L (25). Standard 12-lead
electrocardiograms (ECGs) were obtained prior to the first dose of each cycle, within 1
hour before, 4 hours after each infusion, and on the day after each infusion. ECG
abnormalities were assessed according to the AHA/ACCF/HRS recommendation for the
standardization and interpretation of the electrocardiogram (26). T wave and ST
segment abnormalities were graded based on definitions in the NCI Common Toxicity
Criteria, version 2: grade 0 was a normal ECG, grade 1 was defined as nonspecific T-
wave flattening or changes, and grade 2 was defined as ST segment or T wave
changes suggestive of ischemia.
Pharmacokinetics: Blood for pharmacokinetic analysis was collected prior to the dose, immediately prior to the end of the 4-hr infusion, and 0.5, 7, and 14 hours post-infusion. Plasma was stored frozen at -80°C until analysis. Plasma concentrations of romidepsin were determined by liquid chromatography with mass spectrometric (LC/MS) detection with a lower limit of quantitation of 2 ng/ml (27). Pharmacokinetic parameters were obtained using WinNonlin v5 (Pharsight Corp, Mountain View, CA), as previously reported.

Pharmacodynamics: Heparinized blood was obtained: prior to treatment (days 1, 3, and 5); at the end of infusion (days 1 and 5); and on the day following the last infusion. Histone acetylation was measured in peripheral blood mononuclear cells (PBMCs), using an immunodot-blot method previously validated (28). Radiolabeled sodium iodide (RAI) scans were performed in patients with thyroid cancer. Scans were performed using standard 2 mCi doses of 123I at baseline, and if disease was stable, after the third cycle and thereafter every 2 or 3 cycles.
RESULTS

Romidepsin Dose Escalation and Safety:

Twenty-eight patients with solid tumors were enrolled. Table 1 summarizes their baseline characteristics. Patients had multiple prior therapies: 10 had four or more regimens; among 11 patients with thyroid cancer, 8 had two or more radioiodine ablations. Cycle 1 toxicities, shown in Table 2, were similar to those described in the previous phase I trial of romidepsin (29). Grade 3 toxicities included leucopenia (2), lymphopenia (4), neutropenia (2), thrombocytopenia (2), anorexia (4), nausea (3), and vomiting (2). Few grade 4 toxicities were observed, none in cycle 1. Of note, hematologic toxicities were transient and there were no episodes of febrile neutropenia.

Dose escalation proceeded according to protocol guidelines (Supplementary Table 1), with dose-limiting toxicities defined in the first cycle. Six dose levels were explored and 414 doses administered. Dose level 2 (2 mg/m²) was expanded after a patient with advanced renal cell cancer experienced grade 3 hypoxia and grade 3 atrial fibrillation, thought due to disease progression and increased pleural effusion. No additional DLTs were observed at the 2 mg/m² dose level. Grade 3 nausea and vomiting observed on dose level 4 (5 mg/m²) was not considered dose-limiting because it corrected with antiemetic therapy. However, at dose level 6 (9 mg/m²), one patient experienced difficult-to-treat grade 3 nausea and anorexia and this prompted the enrollment of an additional six patients at this dose level. While no other patient had a DLT at 9 mg/m², we did not attempt further dose escalation because the toxicities were consistent with those observed in patients treated with the approved romidepsin dose and schedule, and because most patients did not tolerate prolonged dosing at 9 mg/m².
(Table 3 and Supplementary Table 2). Specifically, 7 of 9 patients enrolled at 9 mg/m² required dose reduction; completing eighteen cycles at 9 mg/m² and twenty cycles at 7 mg/m². Although a recommended phase II dose (RP2D) was not a defined protocol endpoint, we concluded that 7 mg/m² with the option to increase to 9 mg/m² if tolerable would be considered a RP2D.

**Efficacy**

Although no patient met criteria for RECIST-defined objective response, nine patients had stable disease a minimum of six cycles (18 weeks) with a median of 30 weeks (range 18-112) including six with thyroid cancer with a median of 33 weeks (range 26-112). The outcome in patients with thyroid cancer is summarized in Table 4. Three patients were considered non-evaluable: one had hypoxia, atrial fibrillation and disease progression (#6); one refused further therapy (#22); and another developed a thrombus at the site of the PICC line and refused further therapy (#24).

**Pharmacokinetics**

Romidepsin pharmacokinetics demonstrated rapid clearance at all dose levels. Figure 1A depicts the log₁₀-transformed mean plasma concentration vs. time (C X T) data at each dose level for cycle 1, day 1 (C1D1; n=28). Romidepsin demonstrated biphasic elimination, characterized by a fast distribution phase followed by a slower terminal elimination phase. Both Cₘₐₓ and exposure (AUCₗₐˢᵗ) increased with dose, suggesting linear pharmacokinetics (Figure 1B and 1C). In most patients, plasma romidepsin concentrations were below the lower limit of quantitation 14 hours after
completing the infusion, preventing accurate calculations of terminal elimination rates ($\lambda_Z$), $AUC_{INF}$, volume of distribution and clearance.

Mean cycle 1 pharmacokinetic parameters at each dose level were determined (Supplementary Table 3). Inter-day patient variability (%CV) for 27 of the 28 patients over days 1, 3, and 5 of cycle 1 ranged from 5-68% for $C_{MAX}$ and 2-59% for $AUC_{LAST}$. Intra-patient comparisons of day 1 and day 3 or 5 showed no significant change in plasma exposure (Friedman Test, $p=0.29$) (Figure 1D); thus, as expected, romidepsin did not accumulate on this dosing schedule (29).

**Pharmacodynamics**

Evaluation of histone acetylation in PBMCs has been used as a surrogate marker to confirm that romidepsin or other HDIs block deacetylase activity (28, 30-34). Using a previously validated histone acetylation assay in PBMCs (28) we examined samples collected before and after each dose, and 24 hours after the final dose. As shown in Figure 2A and 2B, comparing the 4hr post-romidepsin samples in cycle 1 with blood concentrations ($n = 45$), a modest correlation with romidepsin exposure (Spearman, $r = 0.34$, $p = 0.024$) was observed for the 24 hour samples in the Phase II study. Fold-increases in acetylation over pre-dose levels are grouped according to cohort in Figure 2C. The data suggested an apparent threshold at 3.3 mg/m$^2$, and no accumulation in acetylation over the 5 days of treatment.

Follow-up RAI scans were performed in six of eleven patients with thyroid cancer. None demonstrated significant uptake, although disease was detectable on FDG-PET scan (Table 3). Two patients showed faint or trace uptake in the post-
romidepsin scans, one in mid-lung and one in the hilum, but neither increase was sufficient to merit therapy with radioactive iodine.

Another effect of romidepsin that can be viewed as a pharmacodynamic marker is reversible ST-T wave flattening and inversion without associated evidence of ischemia (10, 13, 35). Figure 2D plots a subset of and Supplementary Table 4 lists the graded ST segment and T wave changes in 905 ECGs obtained in 120 cycles. Although grade 1 ST and T wave changes were observed in some pre-romidepsin ECGs, the frequency increased after romidepsin administration. At dose levels above 5 mg/m², grade 2 changes were observed frequently, demonstrating that ST-T wave changes occurred commonly and increased with increasing dose.
DISCUSSION

Promising pre-clinical data for HDIs and a mechanistic rationale prompted us to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of a new schedule of romidepsin administered on days 1, 3, and 5 of a 21-day cycle. We wished to explore a schedule that would provide more continuous HDAC inhibition and be easier to use in combinations. The cycle one toxicities included fatigue, nausea, vomiting, anorexia, and cytopenias. Dose escalation was discontinued when 9 mg/m\(^2\) was reached, as 7 of 9 patients enrolled at that dose level ultimately required a dose reduction to 7 mg/m\(^2\), mainly for prolonged grade 2 toxicities. We concluded that 7 mg/m\(^2\) could be sustained and can be considered, with dose escalation as tolerable, as the recommended Phase II dose for inclusion in combination therapies.

The data suggest that, relative to the day 1, 8, and 15 schedule, the day 1, 3 and 5 schedule gives comparable dose-normalized pharmacokinetic effects, and comparable pharmacodynamic effects including similar effects on histone acetylation and EGC changes. In the day 1, 8 and 15 schedule we found a median increase in histone acetylation in PBMCs of 3-fold at four hours, 1.85-fold at 24 h and 1.46-fold at 48 h (28). In the current study at the 4h time-point, an increase in histone acetylation of 2-fold or greater was consistently observed in PBMCs obtained from patients treated with a dose of 3.3 mg/m\(^2\) or higher; with a median 3-fold increase over baseline at 9.9 mg/m\(^2\). Furthermore, the data suggest an apparent threshold at 3.3 mg/m\(^2\), and no accumulation in acetylation over the 5 days of treatment. This magnitude of histone acetylation in PBMCs is comparable to that reported with other HDIs (31-34) and suggest histone acetylation may be a relatively sensitive indicator of drug effect, but that
there may be a plateau in the magnitude of global histone acetylation induced by HDI therapy.

We also utilized the ECG changes, a class effect of the HDIs developed to date, as a pharmacodynamic marker (36). Grade 1 changes were noted from the first dose level onward, and Grade 2 changes were observed at the 24-hour timepoint in over half of patients treated at dose level 5 (7 mg/m²) and onward. This frequency is consistent with, if not greater than, that observed on the day 1, 8, and 15 schedule (35).

On the day 1, 8, and 15 schedule, there was evidence of a romidepsin-mediated differentiating effect with induction of fetal hemoglobin over time (28). However, in vitro studies suggested lower doses/longer exposures were needed to optimize the differentiating effects of HDIs. These in vitro studies also showed good induction of NIS in thyroid cells (7). Thus we had hoped that the day 1, 3 and 5 schedule of romidepsin would induce NIS in thyroid cancer cells leading to improved RAI uptake (7). However, serial RAI scans in six of our eleven patients with thyroid cancer did not demonstrate a significant increase in RAI uptake, with only small increases in areas of lung metastases in two patients after romidepsin. Thus we were unable to confirm the hypothesis that romidepsin would induce NIS in thyroid cancer. However, we would note that a recent report showed an increase in RAI avidity in 2 of 16 patients scanned following romidepsin (37) and that an earlier report described a patient in whom RAI in tumor tissue increased following vorinostat (38). We would note that the in vitro studies suggested longer exposures were needed to optimize the differentiating effects and that the day 1, 3 and 5 romidepsin schedule leaves two weeks in each cycle without histone deacetylase inhibition. The schedule was selected in part because the safety of
romidepsin doses in close approximation was not known. And while the safety of administering romidepsin on alternating days was demonstrated, daily administration of an HDI orally or subcutaneously might be better at achieving the longer exposure that may be required for gene induction.

We would also note that eight of the eleven patients with thyroid cancer enrolled on this study had variant subtypes that often lose radioiodine uptake, a manifestation of de-differentiation. This patient population was selected because treatment options are lacking and radioiodine is inactive in these patients. However, inclusion of these tumor types may not have allowed a fair test of the hypothesis that HDIs can increase or re-express NIS in thyroid cancer. The hypothesis should be tested in cancers with reduced rather than absent uptake of radioiodine, and not in a Phase I setting.

Laboratory and clinical observations suggest the activities of romidepsin and other HDIs can be divided into two classes. One is rapid induction of apoptosis that seems likely to be due to acetylation and replication-mediated DNA damage with an acute change in proliferation signaling (39). The other is the gene induction and differentiation effect that constituted some of the earliest observations with this class of agents. Recent studies in our laboratory suggest the susceptibility of T-cell lymphomas results from apoptosis induction rather than differentiation, a finding consistent with the rapid destruction of malignant Sezary cells in treated patients (13, 19). If this hypothesis is correct, the tested schedule would not provide additional benefit in the T-cell lymphoma setting, and we would not recommend its study in T-cell lymphoma. This may also explain why the “low dose/longer exposure” schedule inherent in the oral daily
dosing of vorinostat does not have increased efficacy over romidepsin in T-cell lymphoma.

But the question that remains is how best to attempt to exploit HDIs in the therapy of solid tumors. Numerous ongoing trials combine HDIs with other anticancer agents (http://www.clinicaltrials.gov). Some trials attempt to exploit the ability of HDIs to relax chromatin or impair the DNA damage response, so as to increase the access and activity of drugs that target DNA. Other trials exploit the differentiating activities of HDIs to alter target expression, just as we attempted to do with the NIS in the study reported here. However, it is increasingly apparent that HDACs work in concert with histone methyltransferases or DNA methyltransferases to induce gene silencing and that this may constrain the response of genes to HDIs. In this regard we would note observations in clinical samples that the NIS promoter is frequently methylated in thyroid cancer, and that this may be associated with loss of mRNA expression and absence of radioiodine uptake (40, 41). Consequently, clinical approaches that attempt to alter gene expression will likely require a combined epigenetic approach, that administers an HDI with a demethylating agent or with novel agents in development such as inhibitors of the H3K27 methyltransferase, EZH2 (42). We feel that the schedule reported here is both safe and tolerable and could lend itself to such a combined epigenetic approach.
Acknowledgements:

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Table 1. On-study characteristics, gender, age, performance status and tumor types

<table>
<thead>
<tr>
<th>Baseline Characteristics (N= 28)</th>
<th>Patients, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, years</td>
<td>56.6</td>
</tr>
<tr>
<td>Prior therapy*</td>
<td>28 (100)</td>
</tr>
<tr>
<td>≥ 4 systemic therapies</td>
<td>10 (36)</td>
</tr>
<tr>
<td>Performance Status</td>
<td></td>
</tr>
<tr>
<td>ECOG 0</td>
<td>3</td>
</tr>
<tr>
<td>ECOG 1</td>
<td>24</td>
</tr>
<tr>
<td>ECOG 2</td>
<td>1</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>17 (60.7)</td>
</tr>
<tr>
<td>Female</td>
<td>11 (39.3)</td>
</tr>
<tr>
<td>Primary disease site</td>
<td></td>
</tr>
<tr>
<td>Thyroid</td>
<td>11 (39)</td>
</tr>
<tr>
<td>Kidney</td>
<td>7 (25)</td>
</tr>
<tr>
<td>Adrenal</td>
<td>3 (11)</td>
</tr>
<tr>
<td>Lung</td>
<td>2 (7)</td>
</tr>
<tr>
<td>Skin</td>
<td>2 (7)</td>
</tr>
<tr>
<td>Cervical</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Ovarian</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Prostate</td>
<td>1 (4)</td>
</tr>
</tbody>
</table>

* 10/11 patients with thyroid cancer had prior $^{131}$I radioiodine, and 8/11 had ≥ 2 ablations
Table 2. Adverse events reported in Cycle 1 and occurring at all dose levels in greater than 10% of patients

<table>
<thead>
<tr>
<th>Adverse Event in C1</th>
<th># pts (%)</th>
<th># pts Gr1 (%)</th>
<th># pts Gr2 (%)</th>
<th># pts Gr3 (%)</th>
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<tbody>
<tr>
<td><strong>Clinical AE's</strong></td>
<td></td>
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<tr>
<td>Nausea</td>
<td>17 (61%)</td>
<td>10 (36%)</td>
<td>4 (14%)</td>
<td>3 (11%)</td>
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<tr>
<td>Anorexia</td>
<td>14 (50%)</td>
<td>8 (29%)</td>
<td>2 (7%)</td>
<td>4 (14%)</td>
</tr>
<tr>
<td>ECG Changes</td>
<td>13 (46%)</td>
<td>13 (46%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vomiting</td>
<td>9 (32%)</td>
<td>6 (21%)</td>
<td>1 (4%)</td>
<td>2 (7%)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>8 (29%)</td>
<td>4 (14%)</td>
<td>4 (14%)</td>
<td>0</td>
</tr>
<tr>
<td>Headache</td>
<td>9 (32%)</td>
<td>9 (32%)</td>
<td>0</td>
<td>0</td>
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<tr>
<td><strong>Laboratory AE's</strong></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Leukopenia</td>
<td>11 (39%)</td>
<td>1 (4%)</td>
<td>8 (29%)</td>
<td>2 (7%)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>13 (46%)</td>
<td>10 (36%)</td>
<td>1 (4%)</td>
<td>2 (7%)</td>
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<tr>
<td>Neutropenia</td>
<td>9 (32%)</td>
<td>0</td>
<td>7 (25%)</td>
<td>2 (7%)</td>
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<tr>
<td>Hypoalbuminemia</td>
<td>10 (36%)</td>
<td>3 (11%)</td>
<td>6 (21%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>11 (39%)</td>
<td>7 (25%)</td>
<td>4 (14%)</td>
<td>0</td>
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<tr>
<td>Lymphopenia</td>
<td>10 (36%)</td>
<td>3 (11%)</td>
<td>1 (4%)</td>
<td>6 (21%)</td>
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<tr>
<td>Hypocalcemia</td>
<td>5 (18%)</td>
<td>2 (7%)</td>
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<tr>
<td>Bilirubin</td>
<td>5 (18%)</td>
<td>5 (18%)</td>
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<tr>
<td>Hyponatremia</td>
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<tr>
<td>SGOT</td>
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<tr>
<td>Creatinine</td>
<td>3 (11%)</td>
<td>3 (11%)</td>
<td>0</td>
<td>0</td>
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<tr>
<td>ALK</td>
<td>3 (11%)</td>
<td>3 (11%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hypomagnesemia</td>
<td>3 (11%)</td>
<td>2 (7%)</td>
<td>0</td>
<td>1 (4%)</td>
</tr>
</tbody>
</table>

*Clinical findings and laboratory abnormalities were reported as toxicities, regardless of clinical significance (n = 28)
### Table 3: Dose increase or reduction in patients enrolled at 7 and 9 mg/m² romidepsin on day 1, 3, and 5 schedule

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Entry Dose</th>
<th># Cycles at Entry Dose</th>
<th>Dose Change [Cycle/Day]</th>
<th>Final Dose</th>
<th># Cycles at New Dose</th>
<th>AE Prompting Dose Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>Thyroid</td>
<td>7 mg/m²</td>
<td>3</td>
<td>C4D1</td>
<td>9 mg/m²</td>
<td>6</td>
<td>--</td>
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<td>18</td>
<td>Thyroid</td>
<td>7 mg/m²</td>
<td>1</td>
<td>C2D1</td>
<td>9 mg/m²</td>
<td>9</td>
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<tr>
<td>19</td>
<td>Lung</td>
<td>7 mg/m²</td>
<td>6</td>
<td>C7D1</td>
<td>5 mg/m²</td>
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<td>G2 N&amp;V 17d</td>
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<td>20</td>
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<td>9 mg/m²</td>
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<td>G1 LFT’s and fever</td>
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<td>21</td>
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<td>4</td>
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<td>G3/4 Platelets, 11d</td>
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<td>22</td>
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<td>9 mg/m²</td>
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<td>--</td>
<td>G1 Anorexia, fatigue, 37d</td>
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<tr>
<td>23</td>
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<td>--</td>
<td>G1 Anorexia, 7d</td>
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<td>4</td>
<td>C5D1</td>
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<td>3</td>
<td>G3 N&amp;V</td>
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<td>C2D1</td>
<td>7 mg/m²</td>
<td>8</td>
<td>G2 Nausea 24d</td>
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<tr>
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<td>C7D1</td>
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<td>G3 Atrial fibrillation</td>
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<td>C2D1</td>
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<td>G2 Fatigue 23d</td>
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<td>Pt</td>
<td>Classification</td>
<td>Variant</td>
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<td>Time On Study (weeks)</td>
<td>Best Response</td>
<td>RAI Scan Obtained (weeks)a</td>
<td>Result</td>
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<td>PD</td>
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<td>Hürthle cell, poorly differentiated</td>
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<td>33</td>
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<td></td>
<td>RAI x3</td>
<td>3</td>
<td>NE</td>
<td>Off Study after cycle 1</td>
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<td>28</td>
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<td>6</td>
<td>PD</td>
<td>PD after cycle 2</td>
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</table>

a) Time when RAI scan obtained after study enrollment.

b) EB, External Beam radiotherapy also administered.

c) RAI scans were not performed in patients with disease progression.

d) ND, Not done. Patient with abdominal mass unable to tolerate low iodine diet.

e) Patient refused further therapy.
FIGURE LEGENDS

Figure 1: Pharmacokinetics of romidepsin. The area under the concentration-time curves up to the last quantifiable point (AUC\text{LAST}) was calculated in WinNonlin v5 using the linear trapezoidal rule. Maximum plasma concentrations (C_{\text{MAX}}) at the end of the 4-hr infusion were recorded as observed values. (A) Log-transformed C1D1 concentration-time profiles of all 28 patients. Relationship between dose and (B) C_{\text{MAX}} or (C) exposure, defined as AUC_{\text{LAST}}. A linear regression analysis was performed to determine the significance of the linear relationship. (D) Dose proportionality of romidepsin in dose-normalized romidepsin plasma exposure on C1D1, C1D3, and C1D5.

Figure 2. Pharmacodynamic endpoints of romidepsin. The fold-increase in acetylated histone H3 at the 4 h and 24 h timepoints compared to baseline in patient PBMCs was determined by an immunodot-blot. A linear regression analysis was used to detemine the relationship between fold-increase and (A) C_{\text{max}} or (B) AUC_{\text{LAST}}. (C) Fold-increase relative to baseline in acetylated histone H3 at the 4 and 24 h time points grouped by dose level as noted. Bar represents median value. Five of six patients studied at dose level 1 or 2 had no measurable increase in AcH3 (although increases over 2-fold were noted in these patients on subsequent cycles when higher doses were administered, data not shown). In contrast, 14 of 16 patients treated at 3.3 mg/m\text{2} or greater had > 2-fold increase in histone acetylation. (D) Stacked bar graph denoting incidence of ST segment and T wave changes in 650 ECGs obtained in 120 cycles, according to dose administered. These are a subset of the ECGs reported in Supplementary Table 4. ECG
data from patients whose dose was escalated to a higher dose level or de-escalated to a lower dose level than their entry dose are included at the actual dose administered, not at the entry dose. “Pre” indicates ECGs obtained prior to the first dose of a cycle, “4 h” and “24 h” indicate ECGs obtained at infusion end or on the following day, respectively, on days 1, 3 or 5. White segment depicts grade 0, gray segment depicts grade 1 and black segment depicts grade 2 ST and T wave changes.
REFERENCES


Phase I Trial of a New Schedule of Romidepsin in Patients with Advanced Cancers


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