Purpose: Romidepsin is a potent histone deacetylase inhibitor (HDI) with activity in T-cell lymphoma. Given preclinical data showing greater induction of gene expression with longer exposures to HDIs, a phase I study of a day 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 4-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 (PBMC), RAI uptake in refractory thyroid cancer, and HDI-related ECG changes.

Results: Twenty-eight patients with solid tumors, including 11 patients with thyroid cancer were enrolled. Six dose levels were explored, and 7 mg/m² on days 1, 3, and 5 was identified as tolerable. No Response Evaluation Criteria In Solid Tumors–defined objective responses were recorded although 9 patients had stable disease a median 30 weeks (range, 21–112) including 6 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/m² 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.
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 showed in preclinical studies that prolonged exposure of thyroid cancer cell lines with low, nontoxic 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 resensitize 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 NIS, to increase uptake of radiiodine in patients with thyroid cancer.

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 proapoptotic or as differentiation induction. We had previously shown in preclinical studies that prolonged exposure of thyroid cancer cell lines with low, nontoxic 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 resensitize 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 NIS, to increase uptake of radiiodine in patients with thyroid cancer.

Patients and Methods

Patient eligibility criteria

The study was approved by the National Cancer Institute Institutional Review Board and registered at www.clinicaltrials.gov NCT0048334. All patients were required to give 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 before study entry, and no history of administration of intravenous iodinated contrast or other large iodine loads [i.e., computed tomography (CT), amiodarone, super saturated potassium iodide] 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 4-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 of 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 more nonhematologic 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 conducted every 2 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 before treatment and repeated if K < 4.0 mmol/L or Mg < 0.85 mmol/L (25). Standard 12-lead ECGs were obtained before 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 American Heart Association/American College of Cardiology Foundation/Heart Rhythm Society (AHA/ACCF/HRS) recommendation.
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 4 or more regimens; among 11 patients with thyroid cancer, 8 had 2 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 (n = 2), lymphopenia (n = 6), neutropenia (n = 2), thrombocytopenia (n = 2), anorexia (n = 4), nausea (n = 3), and vomiting (n = 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 S1), with DLTs defined in the first cycle. Six dose levels were explored and 414 doses were 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 6 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 S2). Specifically, 7 of 9 patients enrolled at 9 mg/m² required dose reduction; completing 18 cycles at 9 mg/m² and 20 cycles at 7 mg/m². Although a recommended phase II dose (RP2D) was not a defined 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, 9 patients had stable disease a minimum of 6 cycles (18 weeks) with a median of 30 weeks (range, 18–112) including 6 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 nonevaluable: 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 showed rapid clearance at all dose levels. Figure 1A depicts the log₁₀-transformed mean plasma concentration versus time (C × T) data at each dose level for cycle 1, day 1 (C1D1; n = 28). Romidepsin showed biphasic elimination, characterized by a fast
distribution phase followed by a slower terminal elimination phase. Both $C_{\text{max}}$ and exposure (AUC$_{\text{last}}$) increased with dose, suggesting linear pharmacokinetics (Fig. 1B and C). 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 ($\lambda_2$), AUC$_{\text{INF}}$, volume of distribution, and clearance.

Mean cycle 1 pharmacokinetic parameters at each dose level were determined (Supplementary Table S3). Interday patient variability (% CV) for 27 of the 28 patients over days 1, 3, and 5 of cycle 1 ranged from 5% to 68% for $C_{\text{max}}$ and

### Table 2. Adverse events reported in cycle 1 and occurring at all dose levels in more than 10% of patients

<table>
<thead>
<tr>
<th>Adverse event in C1</th>
<th>No. of pts (%)</th>
<th>No. of pts grade 1 (%)</th>
<th>No. of pts grade 2 (%)</th>
<th>No. of pts grade 3 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical AEs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>17 (61%)</td>
<td>10 (36%)</td>
<td>4 (14%)</td>
<td>3 (11%)</td>
</tr>
<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>
</tr>
<tr>
<td>Laboratory AEs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukopenia</td>
<td>11 (39%)</td>
<td>1 (4%)</td>
<td>2 (9%)</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>
</tr>
<tr>
<td>Neutropenia</td>
<td>9 (32%)</td>
<td>0</td>
<td>7 (25%)</td>
<td>2 (7%)</td>
</tr>
<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>
</tr>
<tr>
<td>Lymphopenia</td>
<td>10 (36%)</td>
<td>3 (11%)</td>
<td>1 (4%)</td>
<td>6 (21%)</td>
</tr>
<tr>
<td>Hypocalcemia</td>
<td>5 (18%)</td>
<td>2 (7%)</td>
<td>3 (11%)</td>
<td>0</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>5 (18%)</td>
<td>5 (18%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hyponatremia</td>
<td>5 (18%)</td>
<td>5 (18%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AST</td>
<td>3 (11%)</td>
<td>2 (7%)</td>
<td>1 (4%)</td>
<td>0</td>
</tr>
<tr>
<td>Creatinine</td>
<td>3 (11%)</td>
<td>3 (11%)</td>
<td>0</td>
<td>0</td>
</tr>
<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>1 (4%)</td>
<td>0</td>
</tr>
</tbody>
</table>

NOTE: Clinical findings and laboratory abnormalities were reported as toxicities, regardless of clinical significance ($n = 28$). Abbreviations: AE, adverse events; ALK, alkaline phosphatase; AST, aspartate aminotransferase.

### Table 3. Dose increase or reduction in patients enrolled at 7 and 9 mg/m$^2$ romidepsin on day 1, 3, and 5 schedule

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Entry dose</th>
<th>No. of cycles at entry dose</th>
<th>Dose change, cycle/day</th>
<th>Final dose</th>
<th>No. of cycles at new dose</th>
<th>AE prompting dose change</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>Thyroid</td>
<td>7 mg/m$^2$</td>
<td>3</td>
<td>C4D1</td>
<td>9 mg/m$^2$</td>
<td>6</td>
<td>—</td>
</tr>
<tr>
<td>18</td>
<td>Thyroid</td>
<td>7 mg/m$^2$</td>
<td>1</td>
<td>C2D1</td>
<td>9 mg/m$^2$</td>
<td>9</td>
<td>—</td>
</tr>
<tr>
<td>19</td>
<td>Lung</td>
<td>7 mg/m$^2$</td>
<td>6</td>
<td>C7D1</td>
<td>5 mg/m$^2$</td>
<td>1</td>
<td>G2 N and V 17d</td>
</tr>
<tr>
<td>20</td>
<td>Prostate</td>
<td>9 mg/m$^2$</td>
<td>1</td>
<td>C2D1</td>
<td>7 mg/m$^2$</td>
<td>1</td>
<td>G1 LFT’s and fever</td>
</tr>
<tr>
<td>21</td>
<td>ACC</td>
<td>9 mg/m$^2$</td>
<td>4</td>
<td>C5D1</td>
<td>7 mg/m$^2$</td>
<td>4</td>
<td>G3/4 platelets, 11d</td>
</tr>
<tr>
<td>22</td>
<td>Cervix</td>
<td>9 mg/m$^2$</td>
<td>1</td>
<td>Off-study</td>
<td>—</td>
<td>—</td>
<td>G1 anorexia, fatigue, 37d</td>
</tr>
<tr>
<td>23</td>
<td>Ovarian</td>
<td>9 mg/m$^2$</td>
<td>1</td>
<td>C2D1</td>
<td>7 mg/m$^2$</td>
<td>1</td>
<td>G3 N and V</td>
</tr>
<tr>
<td>24</td>
<td>Thyroid</td>
<td>9 mg/m$^2$</td>
<td>1</td>
<td>Off-study</td>
<td>—</td>
<td>—</td>
<td>G1 anorexia, 7d</td>
</tr>
<tr>
<td>25</td>
<td>ACC</td>
<td>9 mg/m$^2$</td>
<td>4</td>
<td>C5D1</td>
<td>7 mg/m$^2$</td>
<td>3</td>
<td>G3 N and V</td>
</tr>
<tr>
<td>26</td>
<td>Thyroid</td>
<td>9 mg/m$^2$</td>
<td>1</td>
<td>C2D1</td>
<td>7 mg/m$^2$</td>
<td>8</td>
<td>G2 nausea, 24d</td>
</tr>
<tr>
<td>27</td>
<td>Thyroid</td>
<td>9 mg/m$^2$</td>
<td>6</td>
<td>C7D1</td>
<td>7 mg/m$^2$</td>
<td>2</td>
<td>G3 atrial fibrillation</td>
</tr>
<tr>
<td>28</td>
<td>Thyroid</td>
<td>9 mg/m$^2$</td>
<td>1</td>
<td>C2D1</td>
<td>7 mg/m$^2$</td>
<td>1</td>
<td>G2 fatigue, 23d</td>
</tr>
</tbody>
</table>

Abbreviation: AE, adverse event.
2% to 59% for AUC_{last}. Intrapatient comparisons of day 1 and day 3 or 5 showed no significant change in plasma exposure (Friedman test, \( P = 0.29 \); Fig. 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

<table>
<thead>
<tr>
<th>Pt</th>
<th>Classification</th>
<th>Variant</th>
<th>Prior radiotherapy, (^{131}I) ablations</th>
<th>Time on study, wk</th>
<th>Best response</th>
<th>RAI scan obtained,(^a) wk</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Papillary</td>
<td>Tall cell variant</td>
<td>RAI ( \times ) 2, EB(^b)</td>
<td>10</td>
<td>PD</td>
<td>PD after cycle 3(^c)</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>Papillary</td>
<td>Follicular variant</td>
<td>RAI ( \times ) 2, EB</td>
<td>12</td>
<td>PD</td>
<td>ND(^d)</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>Papillary</td>
<td>Tall cell variant</td>
<td>RAI ( \times ) 3</td>
<td>112</td>
<td>SD</td>
<td>28, 53, 103</td>
<td>Neg</td>
</tr>
<tr>
<td>10</td>
<td>Papillary</td>
<td>Tall cell variant</td>
<td>RAI ( \times ) 2</td>
<td>39</td>
<td>SD</td>
<td>36</td>
<td>Faint</td>
</tr>
<tr>
<td>11</td>
<td>Papillary</td>
<td>Tall cell, poorly differentiated</td>
<td>EB only</td>
<td>3</td>
<td>PD</td>
<td>PD after cycle 1(^c)</td>
<td>—</td>
</tr>
<tr>
<td>17</td>
<td>Follicular</td>
<td>Hürthle cell, poorly differentiated</td>
<td>RAI ( \times ) 1, EB</td>
<td>33</td>
<td>SD</td>
<td>31</td>
<td>Neg</td>
</tr>
<tr>
<td>18</td>
<td>Papillary</td>
<td>Tall cell, with Hashimoto thyroiditis</td>
<td>RAI ( \times ) 1, EB</td>
<td>33</td>
<td>SD</td>
<td>14, 30</td>
<td>Neg</td>
</tr>
<tr>
<td>24</td>
<td>Papillary</td>
<td>Tall cell variant</td>
<td>RAI ( \times ) 3</td>
<td>3</td>
<td>NE</td>
<td>Off-study after cycle 1(^e)</td>
<td>—</td>
</tr>
<tr>
<td>26</td>
<td>Follicular</td>
<td>Hürthle cell</td>
<td>RAI ( \times ) 3</td>
<td>30</td>
<td>SD</td>
<td>16, 31</td>
<td>Neg</td>
</tr>
<tr>
<td>27</td>
<td>Follicular</td>
<td>Hürthle cell</td>
<td>RAI ( \times ) 3</td>
<td>27</td>
<td>SD</td>
<td>14</td>
<td>Faint</td>
</tr>
<tr>
<td>28</td>
<td>Papillary</td>
<td>Tall cell variant</td>
<td>RAI ( \times ) 3, EB</td>
<td>6</td>
<td>PD</td>
<td>PD after cycle 2(^c)</td>
<td>—</td>
</tr>
</tbody>
</table>

Abbreviation: SD, stable disease.  
\(^a\)Time when RAI scan obtained after study enrollment.  
\(^b\)External beam (EB) radiotherapy also administered.  
\(^c\)RAI scans were not conducted 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 1. Pharmacokinetics of romidepsin. The area under the concentration–time curves up to the last quantifiable point (AUC_{last}) was calculated in WinNonlin v5 using the linear trapezoidal rule. Maximum plasma concentrations (C_{max}) at the end of the 4-hour infusion were recorded as observed values. A, log-transformed C1D1 concentration–time profiles of all 28 patients. Relationship between dose and (B) C_{max} or (C) exposure, defined as AUC_{last}, A linear regression analysis was conducted to determine the significance of the linear relationship. D, dose proportionality of romidepsin in dose-normalized romidepsin plasma exposure on C1D1, C1D3, and C1D5.
we examined samples collected before and after each dose and 24 hours after the final dose. As shown in Fig. 2A and B, comparing the 4-hour 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 Fig. 2C. The data suggested an apparent threshold at 3.3 mg/m², and no accumulation in acetylation over the 5 days of treatment.

Follow-up RAI scans were conducted in 6 of 11 patients with thyroid cancer. None showed 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 S4 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, showing that ST–T wave changes occurred commonly and increased with increasing dose.

Discussion

Promising preclinical 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 1 toxicities included
schedule was selected in part because the safety of romidepsin leaves 2 weeks in each cycle without HDAC inhibition. The effects and that the day 1, 3, and 5 romidepsin schedule exposures were needed to optimize the differentiating effects of HDIs. These suggested lower doses/longer exposures were needed to constrain the response of genes to HDIs relative to the day 1, 8, and 15 schedule reported here is both safe and tolerable and could lend itself to such a combined epigenetic approach.

In contrast, 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 4 hours, 1.85-fold at 24 hours, and 1.46-fold at 48 hours (28). In the current study at the 4-hour 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/m² or higher, with a median 3-fold increase over baseline at 9.9 mg/m². Furthermore, the data suggest an apparent threshold at 3.3 mg/m², and no accumulation in acetylation over the 5 days of treatment. This magnitude of histone acetylation in PBMCs is comparable with that reported with other HDIs (31–34) and suggests that 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 used 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 time point in more than 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 6 of our 11 patients with thyroid cancer did not show a significant increase in RAI uptake, with only small increases in areas of lung metastases in 2 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 that longer exposures were needed to optimize the differentiating effects and that the day 1, 3, and 5 romidepsin schedule leaves 2 weeks in each cycle without HDAC 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 shown, 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 8 of the 11 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 1 setting.

Laboratory and clinical observations suggest the activities of romidepsin and other HDIs can be divided into 2 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 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.
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