Phase II Trial of the Histone Deacetylase Inhibitor Romidepsin in Patients With Recurrent/Metastatic Head and Neck Cancer

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Phase II trial of the histone deacetylase inhibitor romidepsin in patients with recurrent/metastatic head and neck cancer

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Abstract

Objectives—Patients with advanced squamous cell carcinoma of the head and neck (SCCHN) have limited treatment options. Inhibition of histone deacetylases (HDACs) represents a novel therapeutic approach warranting additional investigation in solid tumors.

Methods—A phase II trial of single agent romidepsin, an HDAC inhibitor, was performed in 14 patients with SCCHN who provided consent for pre- and post-therapy samples of accessible tumor, blood and uninvolved oral mucosa. Romidepsin was administered at 13 mg/m^2 as a 4-hour

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intravenous infusion on days 1, 8 and 15 of 28 day cycles, with response assessment by RECIST every 8 weeks.

Results—Objective responses were not observed, although 2 heavily pretreated patients had brief clinical disease stabilization. Observed toxicities were expected, including frequent severe fatigue. Immunohistochemical analysis of 7 pre- and post-treatment tumor pairs demonstrated induction of $p21^{Waf1/Cip1}$ characteristic of HDAC inhibition, as well as decreased Ki67 staining. Exploratory microarray analyses of mucosal and tumor samples detected changes in gene expression following romidepsin treatment that were most commonly associated with regulation of transcription, cell cycle control, signal transduction, and electron transport. Treatment with romidepsin did not alter the extent of DNA methylation of candidate gene loci (including *CDH1* and *hMLH1*) in SCCHN tumors.

Conclusions—Single agent romidepsin has limited activity for the treatment of SCCHN but can effectively achieve tumor-associated HDAC inhibition. Although tolerability of romidepsin in this setting may be limiting, further evaluation of other HDAC inhibitors in combination with active therapies may be justified.

Keywords

romidepsin; head and neck cancer; squamous cell carcinoma; histone deacetylase (HDAC) inhibitors; phase II trial

Introduction

Of the 40,000 new cases of squamous cell carcinoma of the head and neck (SCCHN) diagnosed in the United States annually, approximately 30% of patients will ultimately have either unresectable locally recurrent or metastatic disease [1]. Most combination cytotoxic chemotherapy regimens, though improving response rates as compared to single agents in randomized trials, have historically failed to increase overall survival beyond 6–8 months [2–6]. Although cetuximab is active as a single agent[7] and improves survival in combination with platinum-based chemotherapy [8], very limited options exist for patients experiencing disease progression, and new therapeutic approaches are clearly needed.

Romidepsin (FK 228, FR 901228, depsipeptide), a unique bicyclic peptide originally isolated from *Chromobacterium violaceum* strain 968, is a potent inhibitor of the enzyme histone deacetylase (HDAC). Deregulated acetylation of histones is thought to play an important role in the pathogenesis of hematological as well as solid tumors by changing the chromatin structure and transcription of genes involved in cell cycle control, differentiation and apoptosis. Overexpression of HDAC proteins (HDAC 2 and HDAC 6) has been observed in oral SCCHN tumors, correlating with advanced tumor stage [9,10] and poor patient survival [10]. Romidepsin has recently received FDA approval indications in cutaneous and peripheral T-cell lymphoma and has shown preclinical activity and potent radiosensitization properties in a broad range of solid tumors, including SCCHN [11–13].

We conducted an NCI/CTEP-sponsored phase II trial of romidepsin in SCCHN patients to assess the anticancer activity of this agent in a solid tumor and to critically evaluate its in vivo mechanisms of action and effects on both tumor and normal adjacent tissues.

Patients and methods

Patient selection and eligibility

This single arm phase II study was initiated at Montefiore Medical Center, Weill Cornell Medical College, Beth Israel Medical Center (NY) and the Dana-Farber Cancer Institute and

was conducted through the New York Cancer Consortium (trial registration number NCT 00084682). The institutional review board of each institution approved the trial, and from June 2005–October 2008, 14 patients provided informed consent and were enrolled.

Eligible patients had squamous cell carcinoma of the head and neck, excluding nasopharyngeal carcinoma, that was recurrent or metastatic and incurable with surgery or radiation therapy. Patients could have received any number of prior chemotherapy regimens for unresectable, recurrent or metastatic disease that must have been completed at least 4 weeks prior to study entry. Additional criteria included age ≥18 years, normal organ and marrow function and Eastern Cooperative Oncology Group performance status of 0 to 2. Due to concerns regarding potential for romidepsin-induced cardiotoxicity, patients were excluded if they had prior history of arrhythmias, cardiac hypertrophy, myocardial infarction or congestive heart failure.

Treatment plan

Romidepsin was administered at a dose of 13 mg/m^2 as a 4-hour intravenous infusion on days 1, 8, and 15 of each 28-day cycle. As romidepsin is moderately emetogenic, prophylactic antiemetics were administered. Patients were assessed with radiographic disease measurements at two cycle (8 week) intervals. Toxicities were graded using National Cancer Institute CTCAE v.3.0.

Statistical considerations

The primary objective of this phase II trial was to determine clinical efficacy of romidepsin in patients with SCCHN. For this objective, the primary endpoint was disease control (i.e., achievement of complete response, partial response, or stable disease) at 8 weeks using RECIST. Simon's optimal two-stage design was used, providing early termination of the trial given a sufficiently inactive regimen. The target disease control rate (defined as CR + PR + SD, without evidence of clinical deterioration) at 8 weeks was assumed to be 60%, and a rate less than 40% was considered to be clinically unimportant. Based on these assumptions, first stage accrual of 18 patients was planned, with an additional 28 patients if 8 or more patients achieved response or stable disease. The power of the study was 0.90.

Samples

Peripheral blood samples (10 mL) were collected before and after the initial 4-hour romidepsin administration (Cycle #1, day #1 only) using a blue-speckled top Vacutainer CPT cell separation tube containing sodium citrate gel and density gradient media, and peripheral blood mononuclear cells (PBMCs) were subsequently collected by centrifugation.

Pre- and post-treatment oral mucosa specimens for RNA isolation were obtained from patients using the OralCDx system (CDx Laboratories, Suffern, NY) on day 1 of the first cycle of therapy. The brush samples were taken from separate mucosal surfaces not involved with obvious cancer or mucositis.

Tumor biopsies were obtained from primary lesions or from an involved metastatic site. The baseline biopsy was obtained within 4 weeks prior to starting romidepsin, and all efforts were made to take the post-treatment biopsy from the same anatomic site within 24 hours following the administration of the third (day 15) dose of romidepsin on the first cycle. A fresh portion of each tumor biopsy was immediately flash-frozen in liquid nitrogen and stored at −80 °C for further analysis. Tumor samples were also fixed in formalin and embedded in paraffin for immunohistochemical analysis, and all were histologically confirmed for presence of SCCHN.

Histone acetylation in PBMCs

Histone proteins were isolated from PBMCs by acid extraction and histone H3 acetylation was assessed by western blot analysis with a rabbit-anti-acetylated histone H3 antibody (Upstate Bioscientific, Lake Placid, NY), as previously described [14,15].

Tumor immunohistochemistry

Immunohistochemical analysis for $p21^{Waf1/Cip1}$ (Signal Transduction, Lexington, KY) and Ki67 was performed on formalin-fixed, paraffin embedded 5μm tumor samples. The immunostained slides were examined by light microscopy by a pathologist (LRC) who had no information about the treatment status or outcome of patients. Ki-67 and p21 were recorded as the number of Ki-67 or p21-positive tumor cells per 1000 tumor cells counted in five high-power fields (1 mm^2, Olympus BX41 microscope, 400x magnification). In cases with considerable intratumoral heterogeneity and irregular distribution of Ki-67 and p21 positive tumor cells, we selected for evaluation the fields with the greatest number of tumor cells staining for these markers (hot spots). For each patient, we recorded both Ki-67 and p21 as average values per 200 cells. The staining intensity was recorded according to the manufacturer recommendations as: 0, if tumor cells had complete absence of staining; 1+, if tumor cells had faint, barely perceptible staining or weak heterogeneous staining; 2+, if tumor cells had strong staining. Tumors with 1+ and 2+, expression were interpreted as positive for Ki-67 or p21 expression, and tumors with no expression (0 score) were interpreted as negative.

cDNA microarray analyses

In order to rule out gene expression alterations due to stromal cell contamination, each tumor specimen used in our studies contained greater than 70% cancer cells by analysis of corresponding hematoxylin and eosin stained sections. Following RNA extraction, linear amplification of tissue mRNA and subsequent fluorescent labeling of corresponding cDNA was carried out using the MessageAmp T7 linear amplification kit (Ambion, Austin, TX) and cDNA labeling protocols developed at the Albert Einstein College of Medicine. Hybridization to 19,297 gene cDNA microarrays was carried out overnight at 50°C in a buffer containing 30% formamide, 3X SSC, 0.75% SDS and 100 ng of human Cot-1 DNA. Slides were scanned using the GenePix 4000A microarray scanner. Red (Cy5) and green (Cy3) signal intensities for each element on the array were calculated using the GenePix Pro 3.0 software package.

After the microarray data was filtered based on signal to noise ratio and lowess-normalized, pretreatment levels were compared to post-romidepsin treatment expression levels by computing the empirical Bayes moderated t-statistic, and the Benjamini-Hochberg method was applied to correct for multiple testing. Significant genes were identified by P-value ≤ 0.05 and fold-difference in mean expression ≥ 2.

DNA methylation analyses

We applied the technique of bisulfite sequencing [16] to study tumor DNA methylation status of *CDH1* (encoding for E-cadherin) and *hMLH1* (encoding a DNA mismatch repair protein), frequently hypermethylated loci in SCCHN tumors [17,18], as well as *H19* and *SNRPN*, normally imprinted loci, pre- and post-romidepsin treatment.

Results

Patient characteristics

Fourteen patients were enrolled in the study and received treatment, 13 of whom were evaluable for response. One patient withdrew consent after the first dose of romidepsin due to toxicity and was not evaluable for response evaluation. All patients were evaluable for toxicity.

Patient demographics and characteristics are presented in Table 1. The patient population was heavily pretreated. All patients had prior chemoradiotherapy as part of curative-intent therapy, 5 patients had prior re-irradiation to the head and neck, and 1 patient had prior palliative radiotherapy to the sole site of measurable disease (chest). Although the median of prior systemic palliative therapies was 1 (range 0–4), 5 patients (36%) had at least 2 prior systemic therapies for recurrent or metastatic disease.

Safety and tolerability

A total of 68 doses of romidepsin were administered to the 14 patients enrolled in this study. Adverse events at least possibly related to romidepsin (worst grade seen, at any timepoint) are presented in Table 2. Common toxicities associated with romidepsin were nausea, vomiting, constipation, and fatigue. Although most adverse events were moderate and were grade 1 or 2 in severity, grade 3 or 4 fatigue developed in 5 patients (36%) and was clinically associated with disease progression. Hematological toxicities were generally mild, including thrombocytopenia (2 patients, one with grade 4 toxicity) and exacerbation of pre-existing anemia; leukopenia was not observed. Aside from grade 3 hypotension in 1 patient and grade 4 thrombosis (pulmonary embolism) in 1 patient, no clinically significant cardiovascular toxicity was observed.

Tumor responses

Thirteen patients were evaluable for response to romidepsin. Although no partial responses were observed, 2 patients had a best response of stable disease, resulting in a disease control rate of 15.4% (95%CI: 1.9% – 45.5%). However, 9 patients experienced disease progression, including 2 patients with progressive disease after one cycle of therapy. Symptomatic deterioration was noted in 2 other patients, following 1 and 2 cycles of therapy, which was counted as evidence of lack of clinical benefit; although measurable disease was stable according to RECIST, clinical deterioration was associated with rapid demise. As the first stage goal of 8 patients with disease control was not achievable, patient accrual to the protocol was terminated early at 14 patients for lack of efficacy.

Three heavily pretreated patients had best objective response of stable disease after 2 cycles of therapy, though one was associated with symptomatic deterioration. One of these patients (patient #6, laryngeal cancer) had clear disease progression to palliative chemotherapy (docetaxel) prior to enrollment. The patient was removed from the trial for clinical deterioration after 4 cycles of therapy. Another patient with disease stabilization (patient #3, base of tongue cancer, Fig. 1A) had experienced frank disease progression to four prior palliative chemotherapy regimens prior to enrollment, including cytotoxic chemotherapy (docetaxel, methotrexate), gefitinib, and a phase I trial of an investigational agent. This patient experienced disease progression on romidepsin after 4 cycles of therapy. Patient #10, a patient with recurrent supraglottic laryngeal cancer with prior disease progression after palliative combination of docetaxel, carboplatin and cetuximab, experienced symptomatic deterioration immediately after 2 cycles of romidepsin, necessitating removal from the study and referral to hospice care.

Analysis of PBMCs for histone hyperacetylation

Six patients had sufficient yield of PBMCs for pre- and post-therapy comparisons. Immediately following the initial 4 hour infusion of romidepsin, variable histone H3 hyperacetylation, with increase ranging from 10% to 490%, was observed in all tested samples (Fig. 1B) but did not correlate with response assessments. As a control, ex vivo exposure of PBMCs from a non-cancer volunteer (MH) to micromolar concentrations of romidepsin demonstrated histone H3 hyperacetylation at 4 hours (Fig. 1C).

Tumor immunohistochemistry

Seven pairs of pre-therapy and cycle 1, day 16 tumor biopsies were available for immunohistochemical analysis of $p21^{Waf1/Cip1}$ and Ki67 expression. Increased $p21^{Waf1/Cip1}$ staining post-romidepsin treatment was observed in all tested tumors (Fig. 2A), and in parallel, all post-treatment samples had reduced or stable levels of Ki67 staining (Fig. 2B).

cDNA microarray analyses

Post-amplification RNA yields from four pairs of pre-therapy and post-treatment (cycle 1, day 16) tumor biopsies and four pairs of pre- and post-treatment (cycle 1, day 1) oral mucosal samples were sufficient for hybridization to 19,297 element cDNA microarrays for gene expression analysis. Of 641 differentially expressed genes identified in the SCCHN tumors, 253 genes were significantly up-regulated, while 388 genes were significantly down-regulated. In mucosal specimens, 151 genes were significantly up-regulated, while 368 genes were significantly down-regulated (Supplemental Table 1).

DNA methylation studies

Analysis of extent of DNA methylation in individual CpG dinucleotide positions in the *CDH1* and *hMLH1* tumor suppressor genes and the normally imprinted *H19* and *SNRPN* loci was conducted from pre- and post-therapy (cycle 1, day 16) DNA obtained from 7 tumor specimens (Fig. 3). Interpatient variability in baseline DNA methylation was noted at each CpG site tested for both tumor suppressor and normally imprinted loci, although the extent of DNA methylation for the tumor suppressor promoter loci was lower at baseline than for the imprinted *H19* and *SNRPN* loci, as expected (data not shown). No consistent change in DNA methylation extent was observed for the evaluated loci post-romidepsin therapy.

Discussion

Although disease stabilization was observed in a few heavily pretreated patients, the efficacy of romidepsin in this study was inadequate to justify its further clinical development in SCCHN as a single agent. This outcome appears to be a consistent clinical observation of romidepsin and other HDAC inhibitors as single agents in SCCHN and other solid tumors [19–26].

Nevertheless, HDAC inhibitors have been successfully combined with cytotoxic chemotherapy and biological agents with evidence of enhanced anticancer activity. Romidepsin synergistically enhances the sensitivity of non-small cell lung cancer cells to erlotinib [27] and increases cytotoxicity of docetaxel in preclinical models of androgen-independent prostate cancer [28]. Ramalingam et al. [29] recently published results of a randomized phase II trial of vorinostat, a class I and II HDAC inhibitor, or placebo in combination with paclitaxel and carboplatin for patients with advanced non-small cell lung cancer in which treatment with vorinostat was associated with significantly improved response rate and a trend toward improved median progression-free and overall survival. HDAC inhibitors have additionally demonstrated preclinical evidence of

radiosensitization [30], and several ongoing clinical trials are testing this hypothesis. While single agent activity is poor, additional, hypothesis-driven investigation may be warranted to establish a role for romidepsin and other HDAC inhibitors in SCCHN and other solid tumors.

The FDA recently approved romidepsin at a similar dose (14 mg/m^2) and same schedule for patients with cutaneous or peripheral T-cell lymphoma who have received at least one prior therapy, and romidepsin is associated with frequent and durable objective tumor response in these settings [31–33]. Treatment is associated with frequent gastrointestinal toxicities, anorexia, and fatigue, with grade 3 or 4 fatigue occurring in up to 19% of patients [34]. In our study, the frequency of observed grade 3 or 4 fatigue was notably high, occurring in 36% of patients, but was always associated with disease progression.

Romidepsin is a potent inhibitor of HDAC activity. In a phase II trial in patients with cutaneous T-cell lymphoma, extent of histone H3 hyperacetylation in PBMCs at 24 hours following romidepsin treatment was significantly associated with clinical response [35]. Although we observed increased histone H3 acetylation in normal PMBCs of several SCCHN patients post-initial romidepsin infusion (Fig. 1B), it did not correlate with clinical response assessments.

One of the most commonly induced genes by HDAC inhibitors is the cdk inhibitor p21$^{Waf1/Cip1}$ [36]. Induction of p21$^{Waf1/Cip1}$ occurs in a p53-independent fashion and contributes to the cell cycle arrest and differentiation induced by members of this drug class. In all tested SCCHN tumors, p21$^{Waf1/Cip1}$ protein expression was up-regulated and correlated with a decrease in tumor cell proliferation as evaluated by Ki67 expression. The high frequency of Ki67 decline and reduced proliferation in tumors post-romidepsin suggests more disease stabilization than was clinically observed. However, post-therapy biopsies were obtained on day 16 of cycle 1, whereas tumor response assessment was 4–6 weeks later, suggesting the possibility of rapid development of tumor resistance following the first cycle of therapy. Romidepsin is a known P-glycoprotein substrate, and resistance to romidepsin may be related to reversible induction of histone acetylation and chromatin changes leading to enhanced *MDR-1* transcription [37].

As an HDAC inhibitor, romidepsin is thought to act primarily by altering transcriptional activity. Although our exploratory cDNA expression microarray data indicate both up-regulated and down-regulated genes with at least a 2-fold change in normal mucosa and SCCHN tumors following romidepsin exposure, the tumor and mucosal samples demonstrate a preponderance of down-regulated genes (Supplemental Table 1). The observed gene expression changes in tumor and mucosal cells represent a broad range of biological processes, including transcriptional regulation, cell cycle control, signal transduction and electron transport. Tumor genes noted to be up-regulated following romidepsin exposure included *IGFBP3*, a gene involved in negative regulation of cell growth that is characteristically up-regulated following HDAC inhibition [38]. Down-regulated tumor cell cycle genes include *E2F2* and cyclin B1 (*CCNB1),* a known HDAC-regulated gene [39]. Interestingly, the expression of *HDAC6*, associated with tumor aggressiveness in SCCHN [9], and *EGFR* was down-regulated in SCCHN tumors following romidepsin, the mechanism and clinical significance of which warrants further study. Of note, recent preclinical studies of the HDAC inhibitor valproic acid in combination with hydroxyurea have shown downregulation of EGFR protein expression and triggering of apoptosis in SCCHN cells and xenografts [40].

Although increased p21 protein expression was observed in all post-treatment tumor specimens, no significant change in p21 mRNA expression was observed in the microarray

studies. As our post-treatment biopsies were obtained at 24 hours following romidepsin administration, p21 transcription may have returned to baseline, while p21 protein, which is longer lasting, was still up-regulated. This hypothesis is supported by known in vivo effects of romidepsin on p21 promoter region histone acetylation by chromatin immunoprecipitation studies in chronic lymphocytic leukemia; a maximal increase in p21 protein expression and promoter acetylation was observed at 4 hours following romidepsin administration, while acetylation returned to baseline at 24 hours [41].

The gene expression changes in mucosal samples, sampled immediately prior and at two hours following the initial romidepsin infusion (cycle 1, day 1), may reflect early effects of HDAC inhibition. Up-regulated genes in the mucosal specimens include annexin A1 (*ANXA1*), a cell cycle regulatory gene known to be induced by romidepsin [42]. Of note, the *MYCN* oncogene was found to be up-regulated in mucosal specimens. Interestingly, the tumor suppressor *DAPK1* was also found to be down-regulated. The observation of romidepsin-induced changes in gene expression in normal mucosal specimens reflect changes in the mucosal location of head and neck carcinogenesis, some of which may predispose to oncogenesis; this observation may have implications for studies of HDAC inhibitors in SCCHN prevention.

Although HDAC inhibitors as single agents have not been known to reverse the hypermethylated state of tumor suppressor genes in tumor cell lines, they may have indirect effects on DNA methylation and other epigenetic processes. Of note, activator-protein 2-alpha (AP-2α), overexpressed in approximately 70% of SCCHN tumors, has recently been demonstrated to target methylation of *hMLH1* in SCCHN cells via HDAC recruitment, resulting in microsatellite instability; treatment with trichostatin A, an inhibitor of class I and II HDACs, results in decreased *hMLH1* methylation [43]. It is known that HDAC inhibitors including romidepsin synergistically increase the activity of hypomethylating agents [44], and several clinical trials of romidepsin and decitabine have been initiated. We studied four loci, including 2 tumor suppressor genes (*CDH1* and *hMLH1*) and 2 imprinted genes (*H19* and *SNRPN*), each with a CpG island at its transcription start site, using pyrosequencing as a *quantitative* measure of methylation pre- and post-romidepsin therapy. Our preliminary analysis indicates that romidepsin does not alter the extent of tumor DNA methylation for any of the evaluated genes.

This study illustrates that the model of SCCHN, associated with frequent anatomical accessibility, can be used for extensive translational research in solid tumors. Although the clinical efficacy of romidepsin as a single agent in incurable SCCHN is limited, our observations provide evidence of HDAC inhibitor-induced mechanistic activity in solid tumors that may justify further study of HDAC inhibitors in combination with existing active therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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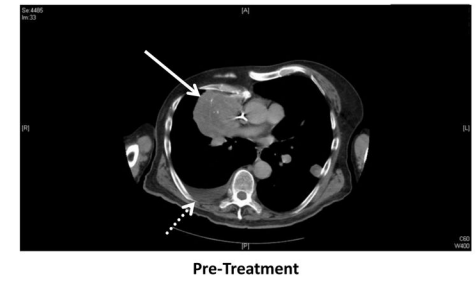
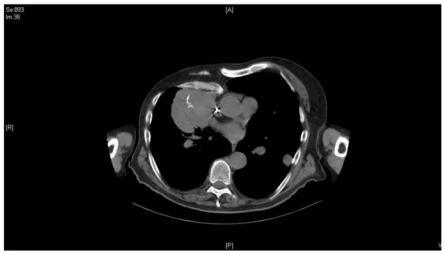
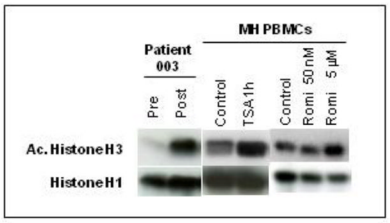




A.

Patient	Fold Change	Best Response
1	1.3	PD
2	1.1	PD
3	2.4	SD
5	1.2	PD
8	4.1	PD
9	4.9	PD

B.



C.

Fig. 1.

A. Stable large lung metastases (arrow) following the initial 2 cycles of romidepsin in a patient (patient #3) with prior chemoradiotherapy and disease progression on four palliative lines of systemic therapy. The pleural effusion seen prior to therapy (dotted arrow) had resolved. *B.* Results of densitometric analysis indicating modest increase in PMBC histone H3 acetylation following romidepsin administration, and individual responses to therapy. *C.* Representative western blot of PBMC histone proteins obtained pre-treatment and immediately after the first 4-hour romidepsin infusion, demonstrating histone H3 hyperacetylation (arrow). Positive controls include ex vivo exposure of PBMCs from a non-cancer volunteer (MH) to the HDAC inhibitors trichostatin A (TSA) and romidepsin (Romi). Histone H1 is used as a loading control.

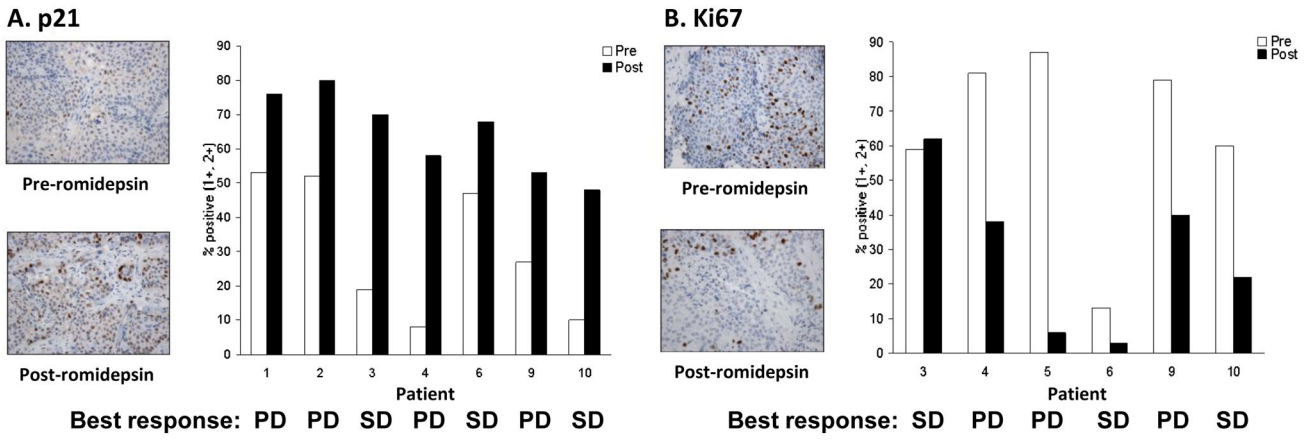


Fig. 2.

Formalin-fixed, paraffin embedded pre- and post-treatment tumor biopsy pairs were stained with anti-p21$^{Waf1/Cip1}$ (*A*) or Ki67 (*B*). Tumor cells were scored as 0, 1+ or 2+. The percent positively staining cells is plotted on the y-axis.

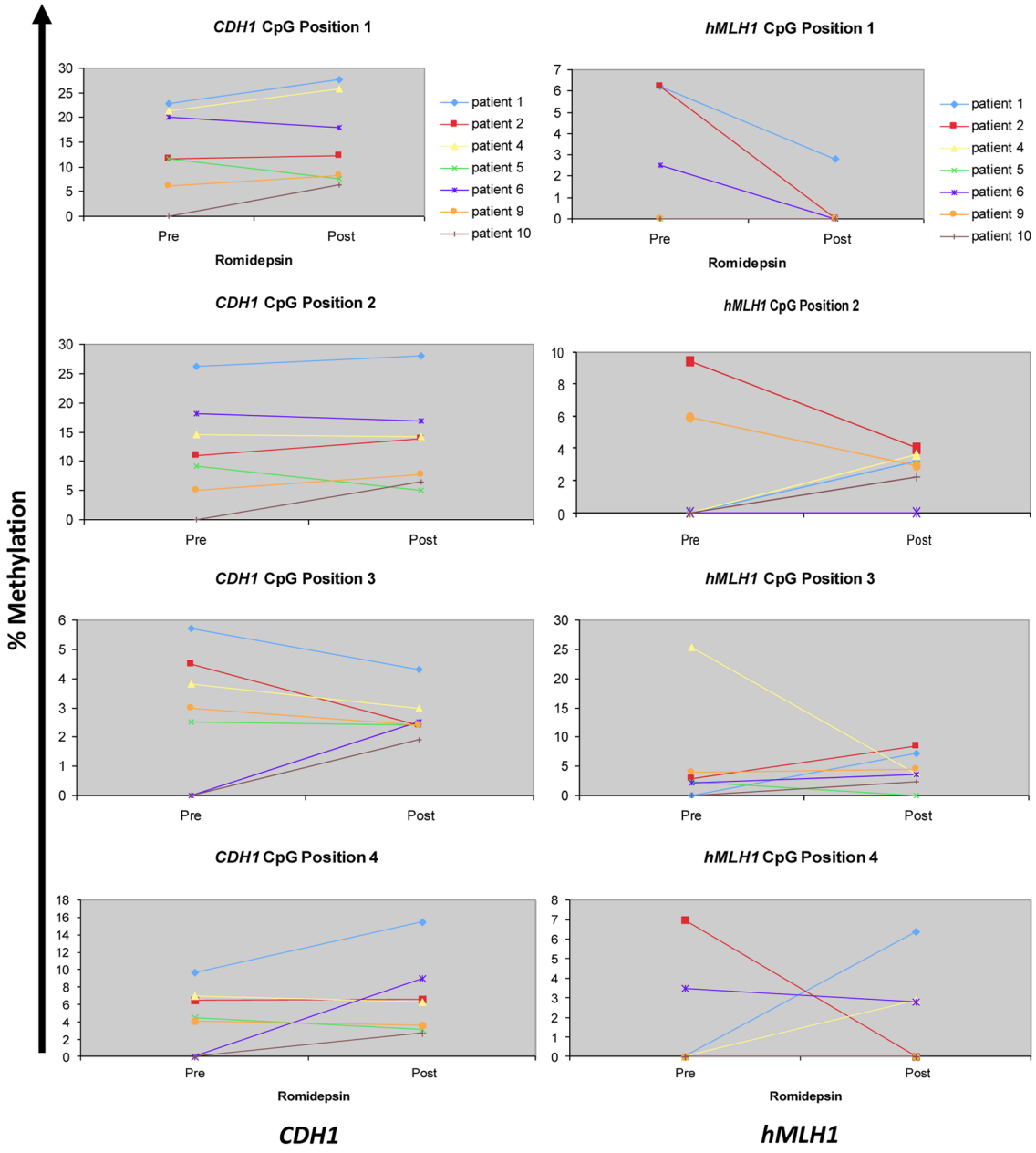


Fig. 3.

Extent of methylation of individual CpG positions in promoter elements of *CDH1* and *hMLH1* in baseline and post-romidepsin treated SCCHN tumors as determined by bisulfite PCR followed by pyrosequencing.

Table 1

Patient characteristics

No. patients	14
Gender	
M	11
F	3
Age	
Median	59
Range	46–75
Race/Ethnicity	
Black	1
Caucasian/Hispanic	6
Caucasian/Non-Hispanic	7
ECOG PS	
0	2
1	8
2	4
Primary tumor site	
Oral cavity	2
Oropharynx	6
Hypopharynx	2
Larynx	4
Recurrence status	
Locoregional	9
Metastatic	5
Prior RT	
chemoradiotherapy	14
re-irradiation	5
Palliative	1
Prior palliative systemic therapies	
0	2
1	7
2	3
3	1
4	1

Table 2

Incidence of romidepsin-related adverse events during therapy (N = 14 patients).

	Worst Grade (%)			
Adverse event	**1**	**2**	**3**	**4**
Blood/bone marrow				
Anemia	2 (14)	0	2 (14)	0
Leukopenia	0	0	0	0
Thrombocytopenia	1 (7)	2 (14)	1 (7)	1 (7)
Cardiac				
Hypertension	1 (7)	0	0	0
Hypotension	0	0	1 (7)	0
Constitutional symptoms				
Fatigue	4 (28)	2 (14)	4 (28)	1 (7)
Gastrointestinal				
Anorexia	0	0	2 (14)	0
Constipation	5 (36)	1 (7)	0	0
Diarrhea	2 (14)	0	0	0
Dyspepsia	1 (7)	0	0	0
Nausea	5 (36)	5 (36)	1 (7)	0
Vomiting	5 (36)	3 (21)	2 (14)	0
Infection				
Lung	0	0	3 (21)	0
Soft tissue	0	0	1(7)	0
Pain	1 (7)	0	0	0
Vascular				
Thrombosis	0	0	0	1 (7)