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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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SUMMARY

*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-h 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.



## 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

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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 to 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 $\geqslant$18 years, normal organ and marrow function and Eastern Cooperative Oncology Group performance status of 0–2. 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-h 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-h 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 h 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 5 μm 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, 400× 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,