Phase I Trial of MS-275, a Histone Deacetylase Inhibitor, Administered Weekly in Refractory Solid Tumors and Lymphoid Malignancies

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Abstract

Purpose: MS-275 is a histone deacetylase inhibitor that has shown potent and unique anticancer activity in preclinical models. The aims of this phase I trial were to determine the dose-limiting toxicities and maximum tolerated dose of oral MS-275 in humans administered with food on a once weekly schedule and to study the pharmacokinetics of oral MS-275.

Experimental Design: Patients with refractory solid tumors and lymphoid malignancies were treated with oral MS-275 on a once weekly schedule for 4 weeks of a 6-week cycle. Samples for pharmacokinetic and pharmacodynamic analyses were collected during cycle 1. Protein acetylation in subpopulations of peripheral blood mononuclear cells was measured using a multivariable flow cytometry assay.

Results: A total of 22 patients were enrolled, and 19 were considered evaluable for toxicity. The maximum tolerated dose was 6 mg/m². No National Cancer Institute Common Toxicity Criteria grade 4 toxicities were observed. Dose-limiting grade 3 toxicities were reversible and consisted of hypophosphatemia, hyponatremia, and hypoalbuminemia. Non-dose-limiting grade 3 myelosuppression was also observed. The mean terminal half-life of MS-275 was 33.9 ± 26.2 h and the T1/2max ranged from 0.5 to 24 h. Although there was considerable interpatient variability in pharmacokinetics, the area under the plasma concentration versus time curve increased linearly with dose.

Conclusions: MS-275 is well tolerated at a dose of 6 mg/m² administered weekly with food for 4 weeks every 6 weeks. Drug exposure increases linearly with dose, and protein acetylation increased in all the subpopulations of peripheral blood mononuclear cells following MS-275 administration.

Histone deacetylases (HDAC) are critically important in the regulation of gene expression and may participate in cell cycle regulation (1, 2). Several classes of HDAC inhibitors have been identified: (a) short-chain fatty acids, such as phenylbutyrate (3); (b) hydroxamic acids, including trichostatin A (4), suberoylanilide hydroxamic acid (5), and oxamflatin; (c) cyclic tetrapeptides containing a 2-amino-8-oxo-9,10-epoxy-decanoyl moiety, trapoxin A (6); (d) cyclic peptides not containing the 2-amino-8-oxo-9,10-epoxy-decanoyl moiety, depsipeptide and apicidin (7); and (e) benzamides, including MS-275 (8).

MS-275, 3-pyridylmethyl-N-[4-[[2-aminophenyl]carbamoyl]benzyl]carbamate, is a HDAC inhibitor that differs from other clinically tested HDAC inhibitors in its chemical structure and some of its targets. Treatment of human leukemia cell lines with MS-275 results in potent antiproliferative activity, with the induction of p21(CIP1/WAF1)-mediated growth arrest and expression of differentiation markers (CD11b) at lower concentrations (9). At higher concentrations of MS-275, there is a marked induction of reactive oxygen species, mitochondrial damage, caspase activation, and apoptosis (9). Treatment of sensitive tumor cell lines with MS-275 induces gelsolin, a maturation marker, and produces a change in the cell cycle distribution with a decrease in S phase and an accumulation of cells G1 (8). The in vivo therapeutic efficacy of MS-275 has been shown in a variety of human tumor xenograft models (8).

Preclinical pharmacology studies indicated a half-life (T1/2) of approximately 1 h after oral administration with good oral bioavailability. Pharmacokinetic profiles are similar in rats,
mice, and dogs (10). Tumor growth suppression in xenografts requires frequent exposure to MS-275 over 3 to 4 weeks (11). Intermittent administration schedules and shorter lengths of exposure are less efficacious in animal models. These properties seem to be concordant with a mechanism of action that requires persistent drug exposure to maintain altered gene expression patterns.

In light of these data and the short half-life noted in preclinical pharmacokinetic studies, the initial design of the trial reported herein was to administer daily doses of MS-275 (12). However, this daily schedule was poorly tolerated, and initial pharmacokinetic data from this trial indicated a significantly longer than predicted plasma half-life than predicted, of approximately 30 to 50 h. Consequently, the trial design was altered, and an administration schedule of once every other week was pursued (12). This was well tolerated and pharmacokinetic data suggested that MS-275 could be safely administered on a weekly schedule providing more continuous exposure. The administration of MS-275 was changed to a weekly schedule (once a week for 4 weeks, with 2 weeks off) and patients treated on this final treatment program are reported herein.

The objectives of this trial were to determine the dose-limiting toxicities (DLT), maximum tolerated dose (MTD), and pharmacokinetics of oral MS-275 in humans. MS-275 was administered with food on a once weekly schedule for 4 weeks with 2 weeks off, for an overall cycle length of 6 weeks. We also evaluated the effect of MS-275 on the activity of HDAC by measuring the level of acetylation of proteins in peripheral blood mononuclear cells (PBMC) before and after MS-275 treatment.

### Eligibility criteria

Patients were eligible for this study if they had pathologically confirmed metastatic or unresectable malignancy for which there were no acceptable standard therapies; an Eastern Cooperative Oncology Group performance status ≤2 ( Karnofsky >50%); age >18 years; and adequate organ and marrow functions defined as follows: leukocytes ≥3,000/µL, absolute neutrophil count ≥1,500/µL, platelets ≥100,000/µL, creatinine within normal limits or measured creatinine clearance ≥60 mL/min, total bilirubin ≤1.5 × the upper limit of normal, aspartate aminotransferase and alanine aminotransferase ≤2.5 × upper limit of normal, serum albumin ≥75% of lower limit normal, normal cardiac ejection fraction, and no history of a gastrointestinal disorder that could impair drug absorption. Prior anticancer therapy must have been completed at least 4 weeks before starting study drug; toxicities were required to have recovered to eligibility levels. Patients were excluded if they had an uncontrolled intercurrent illness, brain metastases within the past 6 months, or a history of Gilbert’s syndrome as glucuronidation, which is one of the eligibility levels. Patients were excluded if they had an uncontrolled gastrointestinal disorder that could impair drug absorption.

### Study design and drug administration

This was an open-label, single-arm phase 1 study of MS-275 in patients with advanced malignancies. MS-275 was supplied by the National Cancer Institute Division of Cancer Treatment and Diagnosis under a Collaborative Research Agreement with Schering AG. MS-275 was administered orally once a week as film-coated tablets for 4 weeks followed by 2 weeks off in each 6-week cycle.

Based on the results of the initial design of this trial that evaluated MS-275 (12), the starting dose for this study was 2 mg/m². Because preclinical studies had shown enhanced drug bioavailability in animal models in the presence of food, MS-275 was administered with food in this study (10). However, the type and quantity of food was not specified. Dose escalation was done using a modified Fibonacci dose escalation scheme with three to six patients entered per dose level: dose level 1, 2 mg/m²; level 2, 4 mg/m²; level 3, 6 mg/m²; level 4, 8 mg/m²; and level 5, 10 mg/m². Concurrent accrual was allowed within the same dose level. No intrapatient dose escalation was permitted. Higher dose levels were not opened to accrual until at least one patient in a cohort was observed for the entire 6-week cycle, and all three patients in a cohort had been observed for at least 4 weeks. Adverse events were graded according to National Cancer Institute Common Toxicity Criteria version 2.0. DLT was defined as an adverse event likely related to the study drug that fulfilled one of the following criteria: (a) ≥grade 3 nonhematologic toxicity (except for nausea/vomiting without maximal symptomatic/prophylactic treatment); (b) grade 2 nonhematologic toxicity, which was intolerable to the patient, interrupted dosing courses, or failed to recover to grade 1 at the time treatment was due (except alopecia); and (c) grade 4 hematologic toxicity.

If none of three patients experienced a first-course DLT, subsequent patients were enrolled at the next higher dose level. If one of three patients experienced a first-course DLT, three additional patients were added at that dose level, for a total of six patients. If two of three or two of six patients experienced a first-course DLT, no further patients were started at that dose level, and the dose level was determined to have exceeded MTD. Three additional patients were then entered on the next lower dose level. MTD was defined as the dose at which ≤1 of 6 patients or ≤33% experience a first-course DLT (one dose level below the dose at which ≥2 of 3 to 6 patients experience DLT).

### Safety and efficacy evaluations

Complete blood count with differential and serum chemistries were obtained every week while on study. Due to concern about the cardiac toxicity observed with other agents in this class, electrocardiograms were done weekly for the first 4 weeks of cycle 1 and then weeks 1 and 4 for all subsequent cycles, as well as at off-study evaluation. Resting multiple gated acquisition scans were done at baseline, and patients were required to have a normal cardiac ejection fraction for eligibility. Multiple gated acquisition scans were repeated at the end of week 4 of therapy and every two cycles thereafter. However, because there was no evidence of cardiac toxicity in terms of decreased ejection fractions in the initial phase of this clinical trial, the protocol was amended, and resting multiple gated acquisition scans were done only at baseline (to document normal ejection fraction) and at the end of treatment on study. Electrocardiograms continued to be done as outlined above. Radiographic assessments were done every two cycles for tumor response as evaluated by the Response Evaluation Criteria in Solid Tumors criteria (13).

### Treatment modifications

Grade 1 toxicities were treated symptomatically, as clinically indicated, without interruption of MS-275 treatment. For grade 2 toxicities, the dosing of MS-275 was continued along with symptomatic treatment. If symptom control was not achieved, MS-275 was withheld until toxicity declined to ≤grade 1. In the event of persistent grade 2 toxicity that had interrupted the treatment cycle, patients were allowed to resume therapy at a lower dose level if toxicity had resolved within 2 weeks. If the toxicity resulted in a treatment interruption of longer than 2 weeks, the patient was taken off study.

For grade 3 nonhematologic toxicity and grade 3 and 4 hematologic toxicity: at dose level 1, no drug could be administered until the toxicity recovered to ≤grade 1. Reintroduction of the dose with a 50% dose reduction could be attempted at the principal investigator’s discretion.
For dose level 2 or higher, no drug could be administered until the toxicity recovered to grade 1, and then drug was reintroduced at the next lower dose level.

Grade 4 nonhematologic toxicity resulted in the patient coming off study.

Pharmacokinetic studies

Peripheral blood samples were collected in 7-mL tubes with sodium heparin used as anticoagulant before drug administration and at 0.5, 1, 2, 6, 12, 24, 48, 60, and 72 h after the first drug administration in cycle 1. Samples were immediately centrifuged at 4°C, and the plasma supernatant was divided into two aliquots, both of which were stored at -70°C until analysis. Plasma samples were analyzed using a validated assay for MS-275 that uses liquid chromatography coupled with mass spectrometry. Experimental details and validation data for this assay have been published previously (14). A 24-h urine sample was also collected from 0 to 24 h after the first dose of MS-275.

Noncompartmental pharmacokinetic analysis of data for each individual was undertaken using the software package WinNonlin version 5.0 (Pharsight Corp.). The peak plasma concentration (C_{max}) and the time to peak plasma concentration (T_{max}) are reported as observed values. The area under the plasma concentration versus time curve (AUC_{last}) was calculated using the linear trapezoidal method from time 0 (at drug administration) to the time of the last sample with measurable drug concentration for each patient (C_{last}). The AUC_{inf} value was calculated by extrapolation by dividing C_{last} (the last measurable drug concentration) by the rate constant of the terminal phase. The apparent oral clearance (Cl/F) was calculated by dividing the administered dose by AUC_{last}.

Multiple linear regression was used to assess the effect of sex on drug pharmacokinetic parameters. Noncompartmental pharmacokinetic analysis of data for each tumor type was undertaken using WinNonlin version 5.0 (Pharsight Corp.). The peak plasma concentration (C_{max}) and the time to peak plasma concentration (T_{max}) were reported as observed values. The area under the plasma concentration versus time curve (AUC_{last}) was calculated using the linear trapezoidal method from time 0 (at drug administration) to the time of the last sample with measurable drug concentration for each patient (C_{last}). The AUC_{inf} value was calculated by extrapolation by dividing C_{last} (the last measurable drug concentration) by the rate constant of the terminal phase. The apparent oral clearance (Cl/F) was calculated by dividing the administered dose by AUC_{last}.

Statistical analysis for pharmacokinetic studies

All pharmacokinetic data are presented as mean ± SD, unless otherwise noted. Multiple linear regression was used to assess the effect of dose on all pharmacokinetic variables. Normality (skewness and kurtosis) and equality of variance was assessed for each variable, grouped by cohort. For normal groups of data, equal variance was measured using the variance-ratio test. If the data were not normal, a modified Levene equal-variance test was used. The results of these tests dictated which two-sample test was applied to the data for each variable. For normal data sets with equal variance, a two-sided equal-variance t test was used. For data sets that were not normal, but of equal variance, the Mann-Whitney U test was done. For data sets that were not normal and unequal variance, the Mann-Whitney U test was used to evaluate differences in median. All statistical calculations were carried out using Number Cruncher Statistical System 2004 (NCSS, J. Hintze, Kaysville, UT). P < 0.05 was considered to be statistically significant.

Pharmacodynamic studies

Cell preparation. Peripheral blood samples were collected in 7-mL heparin tubes, and mononuclear cells were isolated by Ficoll density gradient centrifugation. Viable cell number was determined, and the cells were viably frozen in 10% DMSO in fetal bovine serum and stored at -80°C.

Multivariable flow cytometric assay. The cells were analyzed by a flow cytometric technique capable of detecting the level of protein acetylation. Methodologic details and application of this technique to the pharmacodynamic analysis of HDAC inhibitors have been described in detail (15, 16). For flow cytometric analysis, the cells were thawed and washed and viable cell number was determined by trypan blue exclusion. The cells were resuspended in fixation buffer (0.4% paraformaldehyde in PBS), incubated for 5 to 10 min at 37°C, and washed with wash buffer (PBS containing 0.1% bovine serum albumin). The fixed cells were resuspended in permeabilization buffer (0.4% Triton X-100 in wash buffer), incubated for 5 min at room temperature, and washed with wash buffer.

After fixation and permeabilization, the cells were resuspended in 100 μL of wash buffer and incubated with anti–acetylated lysine polyclonal antibody (1:100 dilution; Cell Signaling Technology) for 1 h at room temperature and washed with wash buffer. The cells were incubated with FITC-conjugated goat F(ab')2 anti-rabbit IgG (1:200 dilution; H+L; Caltag Laboratories) for 1 h at room temperature and washed with wash buffer. For multicolor experiments, antibodies against cell surface markers [allophycocyanin-Cy7–conjugated anti-CD14 antibody (monocyte; Caltag Laboratories), Cy-Chrome–conjugated anti-CD19 (B cells; BD Transduction Laboratories), and phycoerythrin–conjugated anti-CD3 (T cells; BD Transduction Laboratories)] were added simultaneously at the secondary antibody incubation step. To establish the fluorescence compensation variables, tubes were prepared in which the cells were stained separately with each fluorophore to be analyzed.

For analysis of protein acetylation versus forward-scatter or side-scatter, cells were run on a FACS Calibur (Becton Dickinson). For analysis of multicolor staining, an LSRII (Becton Dickinson) equipped with the following lasers was used: (a) a Coherent Sapphire 488 nm diode-pumped solid state laser was used for FITC-conjugated secondary antibody, phycoerythrin-conjugated anti-CD3, and phycoerythrin-Cy5 (cyochrome)–labeled anti-CD19 and (b) a JDS Uniphase helium-neon 633 nm (red) laser was used for allophycocyanin-Cy7–conjugated anti-CD14.

Positive controls were prepared by exposing healthy donor PBMC to MS-275 in vitro. For this analysis, buffy coats, provided anonymously as a byproduct of whole blood donations from paid healthy volunteer donors through an Institutional Review Board–approved protocol, were centrifuged on Ficoll-Paque Plus, and mononuclear cells were incubated with MS-275 in vitro for various times and at varying drug concentrations. Cells were then processed for protein hyperacetylation in the same manner as the patient samples.

Results

A total of 22 patients were enrolled from March 2005 to February 2006. Patient characteristics are summarized in Table 1.

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Total no. patients</th>
<th>22</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. evaluable patients</td>
<td>19</td>
</tr>
<tr>
<td>Age (y)</td>
<td>Median 56, Range 23-71</td>
</tr>
<tr>
<td>Sex</td>
<td>Male 14, Female 9</td>
</tr>
<tr>
<td>Performance status (ECOG)</td>
<td>0 4, 1 18, 2 0, Median 1</td>
</tr>
<tr>
<td>Tumor types</td>
<td>Colorectal adenocarcinoma 10, GI, other 4, Non–small cell lung cancer 2, Renal cell cancer 1, Melanoma 1, Non–Hodgkin’s lymphoma 1, Pheochromocytoma 1, Sarcoma 2, Percentage of patients with ≥4 prior chemotherapy regimens (%) 67</td>
</tr>
</tbody>
</table>

Abbreviations: ECOG, Eastern Cooperative Oncology Group; GI, gastrointestinal.
Table 1. Patients had a variety of solid tumors and lymphoid malignancies, and the majority was heavily pretreated. Nineteen were considered evaluable for toxicity (having completed at least one cycle of therapy or having stopped therapy early due to toxicities; Table 2). The three patients not evaluable for toxicity had evidence of clinical disease progression before completing one cycle of therapy, documented by imaging studies.

At dose level 1, 2 mg/m², apart from one patient developing grade 3 lymphopenia, no other significant toxicities were observed in the three patients at this dose. At the 4 mg/m² dose, one of the first three patients developed grade 3 hypophosphatemia, which was a DLT, necessitating expansion of the dose level to a total of six patients. No other DLTs were observed, and the dose was escalated to dose level 3 (6 mg/m²). No DLTs were observed in the first three patients at this dose level. As per protocol, dose was escalated to 8 mg/m², and a total of three patients enrolled at this dose level. This dose level was poorly tolerated with two of the three patients developing DLTs, and the third patient had grade 3 neutropenia and thrombocytopenia. No grade 4 events were observed. One patient developed grade 3 fatigue, hyponatremia, and grade 3 anemia following two doses of study medication, which responded to intravenous fluids and RBC transfusions. The patient also had evidence of disease progression and was taken off study. One patient with T-cell lymphoma developed grade 3 hypophosphatemia requiring oral phosphorus supplementation. He also developed grade 3 anemia requiring transfusions in the setting of clinical evidence of disease progression. Because two of the three patients at 8 mg/m² had DLTs, the dose was de-escalated and three additional patients were added to dose level 3 (6 mg/m²). One of the three patients developed a grade 2 allergic reaction to MS-275 and treatment was stopped. Because this was the only patient who developed an allergic reaction to MS-275 and because she had a history of allergic reactions to multiple food products and drugs, the decision was made to replace this patient. Thus, a total of seven patients were enrolled on dose level 3. One patient developed grade 3 hypoalbuminemia in the setting of an acute attack of gout (a baseline condition) and evidence of disease progression. This was felt to be a DLT given the known effects of MS-275 treatment on albumin levels in other patients. Thus, one of seven patients at dose level 3 (6 mg/m²) had a DLT; therefore, by definition, this was determined to be the MTD.

Hypophosphatemia, generally grade 1 or 2, did not have any clinical consequences and was easily correctable by oral supplementation. No patient required supplementation with intravenous phosphates.

Given the concern for cardiac toxicities, serial electrocardiograms and multiple gated acquisition scans (total of 53 scans) were done. No clinical cardiac toxicity was observed, and there was no significant difference in the left ventricular ejection fraction between baseline and off-study scans for the patients enrolled on this trial. No new abnormalities were detected in electrocardiograms done during and at the completion of the trial compared with baseline evaluations.

Table 2. Observed treatment-related toxicities of any grade by dose level and number of patients

<table>
<thead>
<tr>
<th>Cycle 1</th>
<th>2 mg/m², n = 3 patients</th>
<th>4 mg/m², n = 6 patients</th>
<th>6 mg/m², n = 7 patients</th>
<th>8 mg/m², n = 3 patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematologic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anemia</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2 (2)*</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>2 (1)*</td>
<td>4</td>
<td>2 (2)*</td>
<td></td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>3</td>
<td>1 (1)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>1 (1)*</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukopenia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonhematologic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoalbuminemia</td>
<td>3</td>
<td>6 (1)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypophosphatemia</td>
<td>2 (1)*</td>
<td>2</td>
<td>2 (1)*</td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>2</td>
<td>1 (1)*</td>
<td>2 (1)*</td>
<td></td>
</tr>
<tr>
<td>Hyponatremia</td>
<td>2</td>
<td>3</td>
<td>2 (1)*</td>
<td></td>
</tr>
<tr>
<td>Anorexia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GI (nausea)</td>
<td>1</td>
<td>4 (1)*</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>GI (vomiting)</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Headache</td>
<td>2</td>
<td>3</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Allergic reaction</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

*The number in parenthesis indicates the number of patients with grade 3 toxicities.
No patients had a partial or complete response to therapy on this study. One patient with rapidly progressive colorectal cancer, who received MS-275 at 4 mg/m², had evidence of disease stabilization for over 8 months. This patient had received multiple prior chemotherapy combination regimens, including the following agents: fluorouracil, leucovorin, oxaliplatin, irinotecan, bevacizumab, and cetuximab. He had extensive liver metastases with adenopathy in the retroperitoneal and porta hepatis regions. Two computed tomography scans done less than 4 weeks apart before enrollment on study showed rapidly progressive disease with the mass in the porta hepatis increasing in size from 2.5 to 3.5 cm and a liver lesion from 1.8 to 3.2 cm. While on study, metastatic lesions stabilized completely for the first 4 months with slow progression over the next 4+ months. Patient tolerated treatment well with minimal side effects.

Pharmacokinetics. Nineteen of the 22 patients receiving MS-275 had assessable pharmacokinetics. Two subjects, one each in the 2 and 4 mg/m² dose groups, did not have measurable concentrations of drug in plasma, despite analysis using an assay with a 1 ng/mL limit of quantitation. All patients who did not have measurable MS-275 concentrations were excluded from the analysis.

For several patients, a large percentage of the AUCₘᵢₓ value was derived from extrapolation, due to the low plasma concentrations of MS-275. AUCₘᵢₓ was deemed to be a more accurate measure of drug exposure for comparison between groups because it eliminated any error or additional variability due to extrapolation, although these values may underestimate total drug exposure. Figure 1 shows an averaged MS-275 plasma concentration-time profile for assessable patients in the cohort that received the MTD. The mean pharmacokinetic variables are provided in Table 3. The mean terminal half-life (t₁/₂) of MS-275 was 33.9 ± 26.2 h across all dose levels. The T_max ranged from 0.5 to 24 h. It should be noted that the actual peak plasma concentration and AUC may be slightly underestimated due to the rapid absorption of MS-275 in some patients. The first sample drawn following drug administration occurred after 30 min, thereby preventing detection of higher concentrations that may have occurred earlier. Clearance was calculated for 11 patients, as described previously. Mean clearance for these 11 patients was 72.0 ± 107.2 L/h/m². Clearance seems to decrease with increasing dose, suggesting nonlinearity. However, this may be artifactual, due to the inability to detect drug in some samples, leading to poor characterization of the terminal phase.

Multiple linear regression analysis showed a positive correlation between MS-275 dose and both peak plasma concentration and AUCₘᵢₓ (P = 0.03 and 0.0001, respectively; data not shown). Figure 2 shows the increase in AUCₘᵢₓ with dose. There was no correlation found between dose and T_max (P = 0.39) or t₁/₂ (P = 0.67) when analyzed using multiple linear regression. Significant interindividual variability was noted, with a coefficient of variation for AUCₘᵢₓ/dose of 138%.

**Pharmacodynamic analysis.** Pharmacodynamic analysis was done on PBMCs using multivariable flow cytometry to detect protein hyperacetylation in response to MS-275. This technique can readily examine multiple variables, including protein hyperacetylation versus cell lineage, as shown in Fig. 3. This four-color analysis examined acetylation in response to MS-275.

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**Table 3. The pharmacokinetics of MS-275**

<table>
<thead>
<tr>
<th>Dose (mg/m²)</th>
<th>C_max (ng/mL) Mean (SD)</th>
<th>AUCₘᵢₓ (ng*h/mL) Mean (SD)</th>
<th>CL/F* (L/h/m²) Mean (SD)</th>
<th>t₁/₂ (h) Mean (SD)</th>
<th>T_max (h) Median (range)</th>
<th>AUCₘᵢₓ/dose (ng*h/mL per mg/m²) Mean (SD)</th>
<th>C_max/dose (ng/mL per mg/m²) Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed</td>
<td>2</td>
<td>3.6 (0.4) 2</td>
<td>58.8 (34.6) 2</td>
<td>—</td>
<td>1.0 (1)</td>
<td>29.4 (17.3) 2</td>
<td>1.8 (0.2) 2</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>31.7 (30.0) 7</td>
<td>67.6 (49.7) 7</td>
<td>112.8 (152.1) 5</td>
<td>10.2 (6.4) 5</td>
<td>16.9 (12.4) 7</td>
<td>7.9 (8.3) 7</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>30.9 (30.1) 7</td>
<td>174.9 (97.4) 7</td>
<td>47.5 (44.9) 4</td>
<td>33.4 (21.8) 5</td>
<td>29.2 (16.2) 7</td>
<td>5.2 (5.0) 7</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>146.9 (190.2) 3</td>
<td>267.7 (132.0) 3</td>
<td>18.9 (3.8) 2</td>
<td>52.3 (17.6) 3</td>
<td>33.5 (16.5) 3</td>
<td>18.4 (23.8) 3</td>
</tr>
<tr>
<td>All groups combined</td>
<td></td>
<td>33.9 (26.2) 15</td>
<td>253.1 (15.2) 19</td>
<td>7.9 (10.9) 19</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Clearance was only calculated using the formula dose/AUCₘᵢₓ for subjects with <50% of the AUCₘᵢₓ an extrapolated value. If extrapolation was >50%, apparent oral clearance was not calculated or included in this summary data.

†Subjects with half-life greater than 100 h or for which drug was not measurable before the 4-h time point were considered to be outliers and were not included in the summary statistics.
in patient peripheral blood T cells, B cells, and monocytes. T cells had the most robust response to MS-275, B cells responded to a lesser extent, and monocytes had the highest basal level and the lowest response among the PBMC populations analyzed. These data show that all of the cell types analyzed by the flow technique responded to MS-275 with an increase in protein acetylation. However, the degree of basal, pretreatment acetylation differed, as did the magnitude of the response. A total of eight patients were analyzed pre-MS-275 and post-MS-275, including three at the 2 mg/m² dose and five at the 4 mg/m² dose. All of the patients showed a change in acetylation during at least one of the time points tested (Fig. 3B).

Discussion

MS-275 administered on the weekly schedule evaluated in this trial was well tolerated with hypoalbuminemia, easily correctable electrolyte abnormalities, and mild gastrointestinal and bone marrow side effects as the principal toxicities. The etiology of the hypoalbuminemia is unclear. Based on previous reports with the every 2-week schedule (12), the decrease in albumin may be related to a decline in protein production because no evidence of excessive renal or gastrointestinal loss of albumin was shown.

MS-275 offers the advantage of oral dosing, and unlike other HDAC inhibitors, no cardiac events were noted by serial electrocardiograms or multiple gated acquisition scans. Although no patient had an objective response to therapy, one patient with colorectal cancer had prolonged disease stabilization with minimal side effects.

This trial also highlights certain early drug development pitfalls because current animal models do not always accurately reflect efficacy or toxicity of an agent in humans (17–19). The initial schedule of daily dosing was poorly tolerated likely because MS-275 in humans has a t½ of 30 to 50 h. This long t½ was not predicted from preclinical pharmacology studies because the available data suggested a t½ of 1 h in animals (20). Subsequent studies with a more sensitive assay indicated a rapid distribution phase and a terminal elimination phase of t½ approximately 12 to 16 h. Because of the poor tolerability of the daily schedule in humans and the observed longer t½, the study design was altered to an intermittent schedule, initially every 2 weeks and then weekly. Both the every 2-week (12) and the weekly schedule evaluated in this trial are well tolerated. However, as the weekly schedule gives more persistent exposure to the drug, which is believed to be mechanistically important based on preclinical studies, we recommend administering MS-275 on a weekly schedule.

There was considerable interpatient variability in the pharmacokinetics of MS-275. The reason for the high degree of variability is unclear. Preliminary data (data not shown) suggests that there may be less variability when MS-275 is taken in the fasting state. However, this would need to be confirmed in a formal food effect study. Pharmacokinetic linearity could not be established, in part due to the low or undetectable concentrations of MS-275 detected in some plasma samples, resulting in poor characterization of the terminal phase. This could have potentially led to artifactual inaccuracies in the half-life and clearance variables calculated.

Pharmacodynamic analyses in previous HDAC inhibitor clinical trials have used a technique, such as Western blot analysis, which averages data from large numbers of cells, or single-variable flow cytometry to measure acetylation (15, 16). In contrast, our study used a multivariable technique. Because of the capabilities of multivariable flow cytometry, we were able to analyze the contribution of individual cell types to the hyperacetylation response. This analysis showed that within PBMCs, a surrogate commonly used for HDAC inhibitor pharmacodynamic assessments, mononuclear cells differ in the basal level of protein acetylation and in the magnitude of their response to MS-275. The data also suggest that the...

Fig. 3. Flow cytometric analysis of protein acetylation in PBMC pre-MS-275 and post-MS-275. A, three-color analysis of protein acetylation in PBMC pre-MS-275 (left) and 24 h post-MS-275 (right). CD3+ T cells (red), CD19+ B cells (blue), and CD14+ monocytes (green). B, flow cytometric analysis of protein acetylation in PBMC from patients treated at 2 and 4 mg/m². Columns, mean of the percentage change in acetylation after MS-275 treatment versus the level of acetylation pretreatment; bars, SD.
response was higher at 4 mg/m² than at 2 mg/m², but larger numbers of patient samples would be required to confirm these apparent trends. The assay used here, which can be done on as little as 0.1 mL of blood, would be particularly useful in a larger trial or, as a multivariable technique, in a HDAC inhibitor combination therapy trial (16).

In conclusion, MS-275 is well tolerated when given on a weekly schedule with food; drug exposures increase linearly with dose. Using multivariable flow cytometry, we showed an increase in protein acetylation in all subpopulations of PBMCs following treatment with MS-275.

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References
Phase I Trial of MS-275, a Histone Deacetylase Inhibitor, Administered Weekly in Refractory Solid Tumors and Lymphoid Malignancies


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