MS-27-275, an Inhibitor of Histone Deacetylase, Has Marked \textit{in Vitro} and \textit{in Vivo} Antitumor Activity against Pediatric Solid Tumors

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\textbf{ABSTRACT}

The antitumor efficacy of the synthetic benzamide derivative MS-27-275 (MS-275), an inhibitor of histone deacetylation [T. Suzuki et al., J. Med. Chem., 42: 3001–3003, 1999], was evaluated in a series of pediatric solid tumor cell lines, including neuroblastoma, rhabdomyosarcoma, Ewing’s sarcoma (EWS), retinoblastoma, medulloblastoma, undifferentiated sarcoma (US), osteosarcoma, and malignant rhabdoid tumors. Treatment with MS-275 results in an increase in acetylation of histones within 4 h of drug exposure. The cell lines were treated with various concentrations of MS-275 for 3 days and incubated with [3 H]thymidine for 20 h before cell harvest. MS-275 inhibited [3 H]thymidine uptake in a dose-dependent manner in all tumor cell lines examined. The IC₅₀ ranged from 50 nM in the D283 medulloblastoma cell line to 1.3 μM in the US. A common feature of MS-275 treatment of pediatric tumor cell lines was induction of p21mRNA. However, the effects on cell cycle were diverse because in some cases MS-275 induced an increase in G₁ or G₂, whereas in others, there was an induction of apoptosis. In EWS, the EWS/Fli chimeric transcription factor created by the t(11;22) suppresses transforming growth factor (TGF) RII mRNA and restore TGF–RII signaling. Using xenograft orthotopic models of US, EWS, and neuroblastoma, we find that the growth of established tumors is inhibited with treatment of MS-275.

\textbf{INTRODUCTION}

The genesis of a number of pediatric solid tumors is influenced by alterations in transcription factors. These include amplification of N-myc in NB⁴ (1), deletions of the RB gene in retinoblastoma (2) and the WT1 gene in Wilms’ tumor (3), and the generation of chimeric transcription factors EWS/FLI in the EWS family of tumors (4) and PAX/Forkhead in alveolar RH (5). Tumor-associated alterations in transcription factor pools may lead to misregulation of genes important in normal growth and development (6). Chimeric transcription factors may cause transcriptional regulation of growth regulatory target genes by the aberrant recruitment of transcriptional corepressors and their associated HDAC activity (7–9). In human acute promyelocytic leukemia, chimeric transcription factors involving retinoic acid receptor (PML-RARα and PLZF-RARα) have been found to repress transcription of target genes such as the RARβ gene. Transcriptional corepressor complexes contain HDAC activity and transcriptional coactivator complexes contain histone acetyltransferase activity. Recent studies indicate that HDAC inhibitors such as trichostatin A or NaBu are able to relieve the transcriptional repression caused by the chimeric transcription factors PML-RARα and PLZF-RARα (8, 9). HDAC inhibitors alone or in combination with retinoids have been shown to induce leukemia remission and prolonged survival in an animal model of acute promyelocytic leukemia without apparent side effects (10).

Acetylation and deacetylation of histones alter higher order chromatin structure by influencing histone interaction with DNA (11, 12). Transcription factors may also be acetylated (13), and the acetylated status of these proteins may influence their interaction with DNA, as well as their ability to interact with other transcriptional coregulatory proteins. For example, acetylation of p53 enhances its sequence-specific DNA binding activity (14, 15). Deacetylated histones are associated with cell growth, whereas hyperacetylated histones (16, 17) are associated with cell growth arrest, differentiation, and/or apoptosis. Finally, tubulin can be acetylated, and this may affect the dynamic equilibrium between soluble and polymerized tubulin and the structure of microtubules (18).

HDAC inhibitors differ with regards to their antitumor activity, toxicity, and stability (17, 19). High concentrations of NaBu are required to induce tumor cell apoptosis or differentiation (20). Additionally, NaBu has a short half-life that limits its effectiveness as a therapeutic agent. Hybrid polar compounds such as M-carboxyoxynonacid bishydroxamide have been shown to induce apoptosis in NB cells \textit{in vitro}, although the mechanism has not been fully elucidated (21). Saito et al. (22) recently evaluated the efficacy of MS-275, a synthetic benzene derivative that inhibits HDAC. In a number of adult tumor cell lines, MS-275 inhibited tumor cell growth with an IC₅₀ in the submicromolar range. The inhibition of cell growth was accompanied by a cell cycle arrest and an induction of the cell cycle inhibitor p21. MS-275 administered p.o. inhibited the growth of established adult tumor lines s.c. implanted in nude mice with minimal toxicities.

To date, there have been few studies (21, 23) using HDAC inhibitors in pediatric solid tumors, although these tumors are marked by alterations in the expression and structure of transcription factors. In this study, we assessed the antitumor activity of MS-275 against several pediatric solid tumor cell lines \textit{in vitro} and against selected \textit{in vivo} orthotopic xenograft murine models of pediatric solid tumors. We found that MS-275 inhibited \textit{in vitro} cell growth in all cell lines tested with an IC₅₀ ranging from 50 nM to 1.3 μM. In NB, EWS, and US orthotopic murine xenograft models, the oral administration of MS-275 inhibited tumor growth with few side effects.

\textbf{MATERIALS AND METHODS}

\textbf{Cell Culture.} The pediatric tumor cell lines used in this study are listed on Table 1. All cell lines were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM glutamine, 50 units/ml penicillin, and 50μg/ml streptomycin at 37°C with 5% CO₂ as described previously (26). N-(2-Aminophenyl)-4-[N-(pyridin-3-yl-methoxy carbonyl) aminomethyl] benzamide (MS-275) was kindly provided by Nihon Schering and was made as a 200 μM stock solution in 100% DMSO.
DNA Synthesis, Cell Cycle, and TUNEL Analyses. Five to 10,000 cells from several pediatric solid tumor cell lines (Table 1) were seeded into 96 flat-bottomed well plates, and six replicate wells were incubated with different concentrations of MS-275 for 72 h. Twenty-four h before termination of culture, cells were incubated with 1 μCi of [3H]thymidine, cultured for an additional 16 h, and harvested with Packard FilterMate harvester (Packard Instrument Company, Meriden, CT). [3H]Thymidine was assessed in a Packard Top Count-NXT Scintillation counter (Packard Instrument Company). Two to four dose response curves were established for each cell line, and the variability among experiments on the same cell line was <10%. For the cell cycle, cells were mechanically detached at the indicated time after drug treatment, washed twice in ice-cold PBS, and processed for cell cycle analysis as described previously (24). TUNEL analyses were using the ApoDirect kit as per manufacturer’s recommendation (BD-PharMingen, Mountain View, CA).

Ac-H3 Analysis. For acetylated histone assessment by Western analysis, cells were trypsinized, washed twice with PBS, resuspended in lysis buffer [0.02 M Tris (pH 7.4), 0.2 M Triton X-100, and 0.02% 2-mercaptoethanol] with 2 ng/ml aprotinin, and solubilized by sonication. Protein extracts (10 μg) were subjected to SDS-PAGE and transferred to polyvinylidene difluoride membranes. Membranes were probed with Ac-H3 (Upstate Biotechnology, Lake Placid, NY). Glyceraldehyde-3-phosphate dehydrogenase (American Research Products, Belmont, MA) was used as a control. Cytosins of trypsinized cells were fixed in 95% ethanol/5% acetic acid and blocked in 8% BSA in PBS. After an overnight incubation with anti-Ac-H3 cells, cells were stained with horse anti-rabbit FITC-conjugated secondary antibody (Vector Laboratories, Eugene, OR). Slides were scanned using a CompuCyte Laser Scanning Cytometer equipped with WinCyte software (Cambridge, MA).

RNA Preparation and Northern Blot Analysis. Control and MS-275-treated cells were detached mechanically from the 150-cm² plate, washed twice with ice-cold PBS, and processed for RNA extraction with the RNAeasy kit (Qiagen, Inc., Chatsworth, CA) according to the manufacturer’s instructions and analyzed by Northern blot analysis as described previously (24). Probes were labeled with [32P]dCTP using RediPrime II (Amersham Pharmacia Biotech) according to manufacturers’ recommendations.

In Vivo Animal Model. The US (2 million) cells and the EWS cell line (TC71; 2 million cells) were injected orthotopically into the gastrocnemious muscle of SCID-beige mice. The NB cell line (KCNR; 2 million cells) was injected into the periaxial fat pad of SCID-beige mice as described previously (28). A post test-Bonferroni’s multiple comparison test was used to assess the differences in the US and TC71 experiment, and an unpaired t test was used to assess the differences in the KCNR experiment.

RESULTS

MS-275 Inhibits DNA Synthesis. The characteristics of the pediatric tumor cell lines used in this study are detailed in Table 1. Treatment of several adult tumor cell lines with MS-275 results in a dose-dependent decrease in cell viability (22). To assess whether MS-275 alters DNA synthesis in pediatric tumor cell lines, cells were seeded into a 96-well flat-bottomed plate and cultured with various concentrations of MS-275 for 72 h. MS-275 inhibited [3H]thymidine uptake in a dose-dependent manner in all tumor cell lines examined (a representative of two to four experiments from each cell line is plotted in Fig. 1). The MS-275 IC₅₀s ranged from 50 nM for medulloblastoma cell line D283 to 1.3 μM for an US cell line (Table 1). MS-275 inhibits DNA synthesis in the osteosarcoma cell line HOS and a retinoblastoma cell line SK, which lacks RB. The RH cell line RD retains sensitivity to MS-275, although it contains a mutation in exon 2 of p53 and shows alterations in DNA damage responses (26, 29). All of the tumor cell lines were more sensitive to the growth inhibiting effects of MS-275 than NIH3T3, an immortalized rodent fibroblast cell line (Table 1).

Morphological examination by light microscopy revealed that all of the pediatric tumor cell lines treated with MS-275 had a dose-dependent decrease in cell number and extensive change in morphology to rounded, denser, and more refractive cells at 48 h. When the NB cell line KCNR, the RH cell line Rh30, and the EWS cell line TC-106 rounded, denser, and more refractive cells at 48 h. When the NB cell line KCNR, the RH cell line Rh30, and the EWS cell line TC-106 rounded, denser, and more refractive cells at 48 h. When the NB cell line KCNR, the RH cell line Rh30, and the EWS cell line TC-106 rounded, denser, and more refractive cells at 48 h.
an increase in cells with a sub-G₁ content of DNA. At 48 h, KCNR, TC106, and US had an increase in the percentage of cells with a sub-G₁ content of DNA and decreases in the other phases of the cell cycle. U20S showed a transient accumulation of cells in G₂-M at 24 h followed by an increase in cells in modest increase in cells in G₁ at 48 h.

Typically, a sub-G₁ content of DNA is indicative of apoptosis. To assess another parameter of apoptosis, a TUNEL assay was performed to assess DNA fragmentation. KCNR and TC106 cells were cultured with 1/1000 M MS-275 for 48 h and DNA fragmentation was assessed as detailed in "Materials and Methods." There was a 7-fold increase in TdT-labeled FITC-dUTP-labeled DNA in KCNR (Fig. 3, left panel), whereas in TC106, there was a 3-fold increase in FITC-dUTP-labeled DNA (Fig. 3, left panel). These data indicate that in the KCNR and TC106 cell lines, MS-275 induces apoptosis. It is likely that in the other cell lines in which MS-275 induces an increase in cells with a sub-G₁ content of DNA, this is attributable to the induction of apoptosis.

**MS-275 Increases Acetylation of Histone H3.** Treatment with MS-275 was shown to increase acetylated histones in adult tumor cell lines (22). To assess the effects of MS-275 on acetylation of histones in pediatric tumor cell lines, cell lines were treated with different concentrations of MS-275 for 4 and 18 h, and protein lysates were evaluated for Ac-H3 by Western analysis. In Fig. 4A, we have shown the Western analysis of three cell lines with different sensitivities to MS-275. At 4 h, the increases in Ac-H3 occur at 10–100 nM in TC32 cell line (IC₅₀ = 100 nM), 100–500 nM for SK-N-AS (IC₅₀ = 660 nM), and at 500–1000 nM in TC71 (IC₅₀ = 1000 nM; Fig. 4A). However, by 18 h maximum, Ac-H3 is detected even at low doses of MS-275. KCNR and the US cells were treated with 1/100 M MS-275, and intact cells were evaluated for acetylation of histone H3 after 4 and 18 h.
Ethanol-fixed cells were incubated with anti-Ac-H3. There is increase FITC-Ac-H3 staining in KCNR treated with 1 μM MS-275 compared with untreated cells. Fluorescence-activated cell sorting analysis of KCNR cells indicated that the relative fluorescent intensity as assessed by mean channel number (MC#) was 8.10 in control cells. After 4 h, the values increased to 9.52 and by 18 h had reached 26.51, indicating that there was a 3.3-fold increase in the relative fluorescent intensity for Ac-H3 that occurred in the majority of cells (Fig. 4B). However, in the less MS-275 sensitive US cell line, the relative fluorescent intensity was 3.5, unchanged at 4 h, and only slightly increased to 4.43 at 18 h, indicating that there was not a dramatic increase in Ac-H3 under these conditions.

**MS-275 Induced Changes in Gene Expression in Pediatric Tumors.** Other HDAC inhibitors (30) as well as MS-275 (22) have been shown to induce p21. We evaluated induction of p21 in selected pediatric tumor cell lines and found that in six of seven cell lines, there was an increase in p21 that occurred from 4–18 h after treatment with 1 μM MS-275 (Fig. 5A). Induction of p21 occurred regardless of the p53 status. Rh30 has an Arg to Ser mutation in codon 280 of p53 and shows a marked induction of p21 within hours of treatment with MS-275. The US cell line, which was the least sensitive to MS-275, did not show an increase in or induction of p21 under these conditions, but p21 was induced using 3 μM MS-275 (data not shown).

Previously, Hahm et al. (31) had shown that the chimeric transcription factor EWS/FLI suppresses TGF-βRII expression in EWS. Because HDAC inhibitors have been shown to relieve transcriptional repression (8, 9), we hypothesized that MS-275 may relieve the transcriptional repression of the TGF-βRII gene in EWS cell lines. Three EWS cell lines were treated with 1 μM MS-275, and cells were harvested and RNA extracted at various times from 4 to 48 h after treatment. Expression of TGF-βRII increased within 18 h of treatment (Fig. 5B). Quantitative PCR indicated that the expression of the EWS/FLI did not significantly change after MS-275 treatment (data not shown). This indicates that despite the presence of the chimeric transcription factor that represses TGF-βRII expression, MS-275 treatment of cells can lead to an increase in TGF-βRII expression. Most EWS cell lines constitutively express one of the TGF-β isoforms.

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>G&lt;sub&gt;1&lt;/sub&gt;</th>
<th>S</th>
<th>G&lt;sub&gt;2&lt;/sub&gt;-M</th>
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<td>24&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>56.2</td>
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<td>4.1</td>
<td>2.5</td>
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</tr>
<tr>
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<td>13.0</td>
<td>28.9</td>
</tr>
<tr>
<td>KCNR</td>
<td>2.6</td>
<td>3.0</td>
<td>26.2</td>
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<sup>a</sup> 1 μM MS-2775.
<sup>b</sup> Hours after treatment.
<sup>c</sup> Percentage of events.

**Fig. 2.** Morphological assessment of effects MS-275 on selected cell lines. The morphology of the KCNR neuroblastoma cell line (left panel), the Rh30 RH (middle panel), and the TC106 EWS cell line (right panel) at initiation of treatment (time = 0; top panels) and 48 h (bottom panels) after treatment with 1 μM MS-275.

**Fig. 3.** TUNEL labeling of KCNR and TC106. After cells were treated with 1 μM MS-275 for 24 h, they were labeled with FITC-dUTP using TdT processed as described in “Materials and Methods.” Samples labeled with FITC-dUTP in the absence of TdT served as controls. M1 represents the background FITC-dUTP labeling, and M2 represents TdT-dependent FITC labeling.
constitutively (32). To assess whether the TGF-β pathway was active after MS-275 treatment, the expression of a downstream target of the TGF-β signaling path, PAI, was assessed. The pattern of PAI expression indicated that there was an early increase that occurred within 4 h of MS-275 treatment and before the detection of an increase in expression of TGF-βRII. However, a second increase in PAI expression could be detected that was coincident or subsequent to the MS-275-induced increase in TGF-βRII expression. These data are consistent with the hypothesis that the TGF-β signaling path becomes active in these cells after MS-275 treatment.

Patients with NB tumors that contain amplification and overexpression of N-myc have a poor prognosis (33). We evaluated the expression of N-myc in NB cells treated with 1 μM MS-275 for various times. Within 4 h of MS-275 treatment, there was a decrease in N-myc levels, and expression was barely detected at 48 h (Fig. 5C). Similar results have been detected in an additional 4 of 4 N-myc-amplified NB cell lines examined (data not shown). In contrast, c-myc levels were increased in the EWS cells after treatment with MS-275. Thus, there is differential regulation of members of the myc gene family after treatment with MS-275.

Antitumor Effect of MS-275 in Tumor-bearing SCID-Beige Mice. MS-275 has been shown to be effective in inhibiting the in vivo growth of most of the adult tumor cell lines grown s.c. in nude mice (22). To evaluate effects of MS-275 on the in vivo growth of the US cell line, which was most resistant to the effects of MS-275 in vitro, 2 × 10⁶ US cells were injected into the gastrocnemiumus muscle and allowed to grow until the tumor reached 0.3 cm³. At that time (~5 days), the animals received various doses of MS-275 ranging from 8.25 to 24.5 mg/kg based on doses in the murine study of adult tumor cell lines that did not have significant toxicity. Both control and MS-275 tumors grew during the course of therapy. However, at the termination of therapy (4 weeks), the volume of the tumors in the MS-275 animals was decreased compared with controls. There was a ~60% decrease in tumor volume in the animals treated with the maximal dose (24.5 mg/kg/day every 5 days) of MS-275 (Fig. 6A). Using a Bonferroni’s multiple comparison test, (34) only the US tumor size in the 24.5 mg/kg was significantly different (P < 0.001) from the size of the US tumors in the untreated group.

The EWS cell line TC71 (2 × 10⁶ cells) was placed into the gastrocnemious muscle of SCID-beige mice. When tumors reached 0.6 cm³, mice received placebo or MS-275 at 12.25 or 24.5 mg/kg/day for 5 days by oral gavage. There was a dose-dependent decrease in tumor volume in MS-275-treated mice (Fig. 6B). The mean tumor volume in mice that received 24.5 mg/kg MS-275 was relatively...
unchanged from initiation of therapy. Using a Bonferroni’s multiple comparison test, both the TC71 tumor size in the 24.5 mg/kg and the 12.5 mg/kg group were significantly different ($P < 0.001$) from the size of the TC71 tumors in the untreated group. One mouse in the control group was lost because of a gavage accident.

The NB cell line KCNR (2 $\times$ 10$^6$ cells) was injected orthotopically to the adrenal gland of 18 mice. After 1 week, 2 animals were sacrificed, and small periadrenal masses could be visualized (28). Remaining animals were randomly placed into two groups, and 24.5 mg/kg MS-275 (12 mice) or diluent (10 mice) were administered by oral gavage five times/week. After 1 month, when the tumor volume in the placebo cohort had reached $\sim 1.5$ cm$^3$ (as determined by external palpation), the animals were sacrificed, and tumors were excised, measured, and weighed. In the placebo group, all animals had tumors, and the tumor volume ranged from 1 to 7 cm$^3$ with a mean tumor volume of 3.5 cm$^3$. In contrast, only 50% of animals had a visually detectable tumor in the MS-275 group, and the volume of these tumors ranged from 0.6 to 2.7 cm$^3$, which was significantly different from the controls ($P = 0.0099$ using an unpaired $t$ test, Fig. 6C). NB tumors and xenografts growth in orthotopic sites in SCID-beige mice are highly vascular; KCNR tumors in the placebo group are also well vascularized (Fig. 7A). In the MS-275-treated group, there was a marked decrease in vascularization (Fig. 7B). Consistent with in vitro studies, there was a decrease in N-myc mRNA and an increase in $p21^{waf/cip1}$ mRNA in tumors from mice treated with MS-275 compared with control (inset panel on Fig. 7).

**DISCUSSION**

Alterations in transcription factors are a common feature in pediatric solid tumors, and it is thought that these altered transcription factors affect target genes that regulate growth, differentiation, and survival pathways and contribute to a tumorigenic genotype. Transcription factors serve as the basis for the recruitment of higher ordered complexes that affect basic chromatin structure to activate or repress gene transcription. In this study, we have shown that the HDAC inhibitor MS-275 has antitumor activity against a broad panel of pediatric solid tumors. As was seen in studies examining the effects of MS-275 on adult tumor cell lines in vitro (22) and breast cancer cells (35), we found that DNA synthesis in all of the pediatric solid tumor cell lines evaluated was markedly inhibited by MS-275. The IC$_{50}$ of these tumor cell lines ranged from 50 to 1300 nM. We found that within 4 h of treatment, there was a dose-dependent increase in Ac-H3 in pediatric tumor cell lines that paralleled the sensitivity of cells to the drug (Fig. 4A). However, as in the study on adult tumor cell lines, we found that concentrations of MS-275 significantly less than the IC$_{50}$ were able to induce increases in Ac-H3 after 24 h.

In contrast to adult studies in which MS-275 induced a G$_1$ arrest in all cell lines examined, the effects of MS-275 on cell growth and induction of apoptosis were varied among different pediatric tumor cell lines evaluated. In some cell lines, apoptosis was induced, whereas in others, cell cycle arrest occurred in either the G$_1$ or G$_2$ phase of the cell cycle. The variability in response of the cell lines to the drug may reflect the nature of the genetic alterations and the signal transduction pathways altered in the different cell lines. As has been shown for a number of HDAC inhibitors, $p21^{waf/cip1}$ was induced in most of the pediatric solid tumor cell lines evaluated irrespective of the p53 status of the cells. This suggests that HDAC inhibitors as a class may induce cell death irrespective of the status of p53.

Although we cannot exclude that there is a common mechanism by which MS-275 affects the growth and survival of different tumor cell lines, it is equally possible that the effect of MS-275 on the different pediatric solid tumor cell lines may be dependent on the nature of the genetic alteration in the different tumor cell lines. We evaluated changes in TGF-βRII mRNA, which is known to be transcriptionally repressed by the EWS/FLI transcription factor in EWS and the N-myc gene whose amplification is associated with a poor prognosis in NB tumors. Studies on MS-275 indicate that it induces the expression of TGF-βRII mRNA and restores TGF-β signaling in breast cancer cell lines (35). We have found that in EWS in which the chimeric transcription factor EWS/FLI transcriptionally represses the TGF-βRII gene (31), MS-275 is able to stimulate increases in TGF-βRII mRNA despite the presence of the EWS/FLI transcriptional repressor. Our finding that there is an increase in PAI mRNA, a downstream target of the TGF-β signal transduction pathway coincident or subsequent to the MS-275-induced increase in TGF-βRII mRNA, is consistent with the restoration of TGF-β signaling in EWS. Previous studies indicate that restoration of the TGF-β pathway in EWS cell lines inhibits their growth in animal models (31). Additional studies will be needed to assess whether the effects of MS-275 on the growth of TC71 cells in vivo are...
dependent on the induction of TGF-βRII and restoration of the TGF-β signal transduction path.

Previous studies using the HDAC inhibitor CHBA have indicated induction of p21 in NB cell lines and inhibition of NB tumor growth in vitro and in vivo (21, 23). Our results using MS-275 are similar. Typically, the use of HDAC inhibitors has been found to increase gene expression, whereas repression of gene expression by HDAC inhibitors is not common. In N-myc-amplified NB cells, we found that MS-275 induced a decrease in N-myc mRNA expression (Fig. 5 and data not shown), and similar findings were found in tumors taken from animals treated with MS-275 (Fig. 7). Decreases in N-myc are known to affect the survival and growth of NB cells (36, 37). Although this may affect the biology of N-myc-expressing tumor cell lines, we also found that MS-275 was able to inhibit the growth of a non-N-myc-amplified cell line, SK-N-AS. Thus the mechanism by which MS-275 affects NB cell growth and survival may not require N-myc down-regulation or may be distinct in different NB cell types. However, both KCNR and AS contain 1pLOH and increases in 17q, common alterations or may be distinct in different NB cell types. However, both KCNR and AS contain 1pLOH and increases in 17q, common although uncharacterized genetic lesions in NB, and it is possible that MS-275 may complement these genetic alterations to alter NB cell growth.

In our animal studies, we found that the growth of established tumors in vivo was markedly affected by treatment with MS-275. Toxicities were minimal with ruffled fur being the consistent alteration noted in all of the experiments. Initial studies indicated that 24.5 mg/kg every 5–7 days by oral gavage was the most effective dose noted in all of the experiments. Initial studies indicated that 24.5 mg/kg every 5–7 days by oral gavage was the most effective dose noted in all of the experiments. Initial studies indicated that 24.5 mg/kg every 5–7 days by oral gavage was the most effective dose noted in all of the experiments. Initial studies indicated that 24.5 mg/kg every 5–7 days by oral gavage was the most effective dose noted in all of the experiments. Initial studies indicated that 24.5 mg/kg every 5–7 days by oral gavage was the most effective dose noted in all of the experiments.

In this report we have evaluated the in vitro and in vivo effects of the HDAC inhibitor MS-275 in a number of pediatric solid tumor cell lines. Future studies on the changes in gene expression induced by MS-275 has the potential to reveal genes with expressions that are affected by the altered transcription factors in these different pediatric solid tumors. As in vitro studies indicated that MS-275 induced apoptosis in many cases, histological and molecular genetic analysis of the xenograft tumors will enable us to determine whether induction of apoptosis and/or inhibition of angiogenesis affects tumor growth in vivo. Currently, MS-275 is in Phase I clinical trials in adults and these studies indicate that it should be considered for the treatment of pediatric malignancies as well.

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