

## Evaluation of the Pharmacodynamic Effects of MGCD0103 from Preclinical Models to Human Using a Novel HDAC Enzyme Assay

Claire Bonfils,<sup>1</sup> Ann Kalita,<sup>1</sup> Marja Dubay,<sup>1</sup> Lillian L. Siu,<sup>2</sup> Michael A. Carducci,<sup>3</sup> Gregory Reid,<sup>1</sup> Robert E. Martell,<sup>1</sup> Jeffrey M. Besterman,<sup>1</sup> and Zuomei Li<sup>1</sup>

**Abstract Purpose:** The pharmacodynamic properties of MGCD0103, an isotype-selective inhibitor of histone deacetylase (HDAC), were evaluated in preclinical models and patients with a novel whole-cell HDAC enzyme assay.

**Experimental Design:** Boc-Lys( $\epsilon$ -Ac)-AMC, a HDAC substrate with fluorescent readout, was found to be cell permeable and was used to monitor MGCD0103-mediated HDAC inhibition in cultured cancer cells *in vitro*, in peripheral WBC *ex vivo*, in mice *in vivo*, and in human patients.

**Results:** MGCD0103 inhibited HDAC activity in several human cancer cell lines *in vitro* and in human peripheral WBC *ex vivo* in a dose-dependent manner. Unlike suberoylanilide hydroxamic acid, the HDAC inhibitory activity of MGCD0103 was time dependent and sustained for at least 24 hours following drug removal in peripheral WBC *ex vivo*. Inhibitory activity of MGCD0103 was sustained for at least 8 hours *in vivo* in mice and 48 hours in patients with solid tumors. HDAC inhibitory activity of MGCD0103 in peripheral WBC correlated with induction of histone acetylation in blood and in implanted tumors in mice. In cancer patients, sustained pharmacodynamic effect of MGCD0103 was visualized only by dose-dependent enzyme inhibition in peripheral WBC but not by histone acetylation analysis.

**Conclusions:** This study shows that MGCD0103 has sustained pharmacodynamic effects that can be monitored both *in vitro* and *in vivo* with a cell-based HDAC enzyme assay.

Recent evidence shows that cancer is associated with identifiable epigenetic changes and dysfunctional transcriptional regulatory mechanisms, thus providing a novel therapeutic strategy (1–3). Both acetylation and deacetylation of histones play a fundamental role in the remodeling of chromatin and epigenetic regulation of gene expression. Transcriptionally active genes are associated with hyperacetylated chromatin, whereas transcriptionally silent genes are associated with hypoacetylated chromatin. Histone deacetylases (HDACs) remove the acetyl groups from the acetylated lysines in histones and function as transcriptional corepressors (4–6). HDACs are grouped in four classes based on the structure of their accessory domains. Class I, II, and IV HDACs are Zn<sup>2+</sup>-

dependent enzymes, whereas class III enzymes are defined by their dependency on NAD<sup>+</sup> and are referred to as sirtuins. Class I HDACs include HDAC 1 to 3, and 8; class II HDACs include HDAC 4 to 7, 9, and 10. HDAC 11 belongs to class IV but shares features of both class I and II enzymes.

Dysregulation of HDACs and aberrant chromatin acetylation and deacetylation have been implicated in the pathogenesis of cancer. Consequently, inhibition of HDAC activity has been explored as a therapeutic strategy in cancer (4–8). The anticancer activity of HDAC inhibitors is thought to occur through a decrease in transcriptional repression, resulting in inhibition of proliferation, induction of apoptosis, and/or terminal cell differentiation (9, 10). In preclinical studies, several structurally diverse HDAC inhibitors have been found to have potent antitumor activities and tumor specificity. Among these inhibitors are the hydroxamic acids suberoylanilide hydroxamic acid (SAHA)/vorinostat (Zolinza; refs. 11–13), NVP-LAQ-824 (14), LBH589 (15), and PXD101/belinostat (16, 17); benzamides MS-275 (18–20) and MGCD0103 (21–23); and natural products such as depsipeptide/romidepsin (24). SAHA was the first HDAC inhibitor approved and is indicated for treatment of advanced refractory cutaneous T-cell lymphoma (12).

Unlike SAHA, MGCD0103 is a nonhydroxamate isotype-selective HDAC inhibitor that targets HDAC isotypes 1 to 3 and 11 (25). Preclinical studies showed that MGCD0103 has significant *in vivo* antitumor activity with low toxicity. Induction of histone acetylation in tumors by MGCD0103 has been observed to correlate with antitumor activity in mouse models with human tumor xenografts (23).

**Authors' Affiliations:** <sup>1</sup>MethylGene, Inc., Montreal, Quebec, Canada; <sup>2</sup>Princess Margaret Hospital, Toronto, Ontario, Canada; and <sup>3</sup>Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland  
Received 9/24/07; revised 2/6/08; accepted 2/8/08.

**Grant support:** MethylGene, Inc., Taiho Pharmaceuticals (Japan), and Pharmion Corporation.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

**Note:** Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

**Requests for reprints:** Zuomei Li, MethylGene, Inc., 7220 Frederick-Banting, Montreal, Quebec, Canada H4S 2A1. Phone: 514-337-3333, ext. 245; Fax: 514-337-0550; E-mail: [liz@methylgene.com](mailto:liz@methylgene.com).

© 2008 American Association for Cancer Research.

doi:10.1158/1078-0432.CCR-07-4427

As efforts in the development of HDAC inhibitors for therapeutic treatment progress, the need for assays to determine the pharmacodynamic effects of HDAC inhibitors is emerging. In past clinical trials of SAHA and MS-275, pharmacodynamic effects in patients were monitored by analyzing induction of histone acetylation in peripheral WBC by immunoblotting, ELISA, or fluorescence-activated cell sorting (11, 18) or in tumor tissues by immunocytochemistry (18, 20). However, for many of these assays, the sensitivity or limitation of clinical materials has created constraints. In addition, current enzyme assays relying on purified enzymes or lysates do not necessarily reflect *in situ* activity, because they are likely to disrupt normal protein-protein interactions. Our aim was to develop a HDAC assay using a cell-permeable small-molecule substrate in intact cells from human patients. Here, we present our findings that Boc-Lys( $\epsilon$ -Ac)-AMC, a substrate used previously *in vitro* for HDAC enzyme assay (26), is cell permeable and is suitable to monitor the inhibitory activity of MGCD0103 in several preclinical models, *in vitro* and *in vivo*. This assay was then applied to monitoring pharmacodynamic effects of MGCD0103 in a clinical phase I study on patients with advanced solid tumors.

## Materials and Methods

**Materials.** MGCD0103, *N*-(2-amino-phenyl)-4-[(4-(pyridin-3-yl)pyrimidin-2-ylamino)-methyl]-benzamide dihydrobromide, was designed and synthesized at MethylGene (21). The free base form of MGCD0103 was used for all *in vitro* assays and mouse *in vivo* analyses. For clinical trials, MGCD0103 was used as the dihydrobromide salt. Compound A is an inactive analogue of MGCD0103 missing an amino group and was also designed and synthesized at MethylGene. All other comparator HDAC inhibitors used were synthesized at MethylGene. Boc-Lys( $\epsilon$ -Ac)-AMC was purchased from Bachem. Fluor-de-Lys Developer was purchased from BioMol.

**Cell culture.** Cancer cell lines HCT116 (human colon cancer), Jurkat (human acute T-cell leukemia), T24 (human bladder cancer), MCF-7 (human breast adenocarcinoma), PANC-1 (human pancreas carcinoma), HeLa (human cervix carcinoma), A549 (human non-small cell lung cancer), 293T (human embryonic kidney), DU145 (human prostate cancer), and SW48 (human colorectal adenocarcinoma) were purchased from the American Type Culture Collection. HMEC (human mammary epithelial cells) were purchased from BioWhittaker. All cell lines were cultured following vendor instructions.

**Peripheral WBC isolation.** Whole blood was collected in sodium heparin tubes (BD Biosciences) and shipped at room temperature within 24 h. Buffy-coat cells were separated from erythrocytes by centrifugation at 1,850 rpm for 5 min and further purified with EL lysis buffer (Qiagen) following the manufacturer's instructions.

**Whole-cell HDAC enzyme assay.** Boc-Lys( $\epsilon$ -Ac)-AMC (Bachem) has been described previously as a small-molecule substrate for HDAC enzymes *in vitro* (27). In the present study, it was used with intact cells as sources of HDAC enzymes. Cells were seeded in 50  $\mu$ L in 96-well culture plates (Corning) at a density that has been found previously to be in the linear range of the assay (for HCT116,  $1 \times 10^5$  cells per well were seeded; for DU145, A549, 293T, T24, Jurkat, MCF-7, PANC-1, and HeLa,  $5 \times 10^4$  cells per well; for isolated buffy-coat cells,  $3 \times 10^5$ – $8 \times 10^5$  cells per well). The reaction was initiated by adding 1  $\mu$ L of 15 mmol/L Boc-Lys( $\epsilon$ -Ac)-AMC (Bachem; stock solution in DMSO) to reach a final concentration of 0.3 mmol/L. Cells were incubated with the substrate for 1 h at 37°C with 5% CO<sub>2</sub>. The reaction was then stopped by adding 50  $\mu$ L buffer [25 mmol/L Tris-HCl (pH 8.0), 137 mmol/L NaCl, 2.7 mmol/L KCl, 1 mmol/L MgCl<sub>2</sub>] containing 1  $\mu$ mol/L trichostatin A (BioMol), 1:60 diluted Fluor-de-Lys Developer,

and 1% NP-40 (Sigma-Aldrich Canada). Trichostatin A in the stop mixture prevented any further deacetylation, whereas Fluor-de-Lys Developer released a measurable fluorescent moiety from the deacetylated product, and NP-40 lysed the cells to allow intracellular deacetylated product to be accessible to the developer. The reaction was allowed to develop for  $\geq 15$  min at 37°C, and the fluorescent signal was detected by fluorometer (GeminiXS; Molecular Devices) at excitation 360 nm, emission 470 nm, and cutoff 435 nm. A standard curve of Boc-Lys-AMC (Bachem) allowed the conversion of fluorescent signal into picomoles of deacetylated product.

**Detection of histone acetylation.** Isolated peripheral WBC or frozen tumor pieces were lysed in the presence of 5 mmol/L sodium butyrate and histones were acid extracted as described in ref. 28. Protein concentrations were determined using Bradford protein assay reagent (Bio-Rad), and 10  $\mu$ g from each sample were resolved by electrophoresis on a 4% to 20% SDS-polyacrylamide gel. Proteins were transferred to a polyvinylidene difluoride membrane, probed with anti-H3Ac antibody (Upstate; 1:3,000 dilution) and either anti-pan-histone (Chemicon; 1:2,000 dilution) or anti-H4 antibody (Upstate; 1:2,000 dilution), and then revealed with horseradish peroxidase-conjugated anti-rabbit antibody (Sigma; 1:5,000 dilution) and ECL<sup>+</sup> chemiluminescent detection reagents (Amersham Biosciences). The signal was scanned and quantified on Storm860 imaging system (Amersham Biosciences).

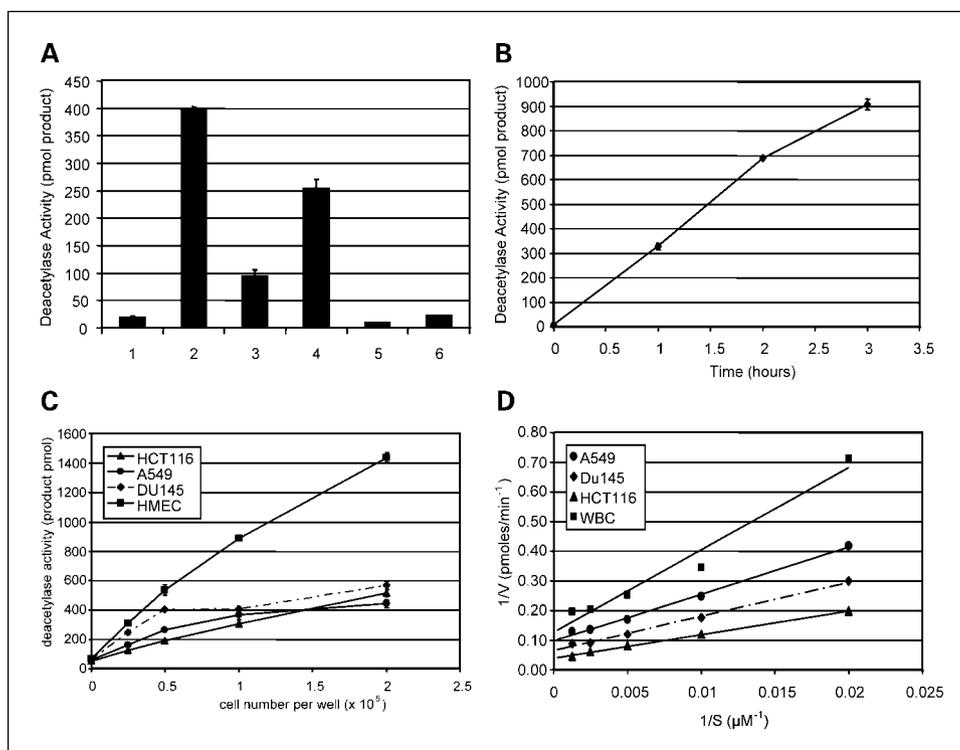
**In vivo studies.** Female CD-1 normal mice (Charles River Laboratories) or 8- to 10-week-old CD-1 nude mice (Charles River Laboratories) bearing either human colon SW48 tumors or HCT116 tumors were used. MGCD0103 or its inactive analogue (compound A) with an amino group deletion were dissolved in PBS acidified with 0.1 N HCl and animals were dosed once by oral gavage (200  $\mu$ L) using either vehicle or compounds. At the end of each experiment, blood and tumors were collected from animals. For HDAC activity, the blood was stored overnight before processing to mimic shipment conditions of clinical samples. To evaluate histone acetylation by immunoblot analysis, tumors and blood were harvested and immediately flash-frozen.

**Pharmacodynamic analyses in cancer patients with advanced solid tumors.** An open-label, nonrandomized, dose-escalation, multicenter phase I study was done in patients with advanced solid malignancies. The trial was conducted at the Kimmel Cancer Center at Johns Hopkins and Princess Margaret Hospital. The study was approved by the local institutional ethics committee of each institution, and patients provided informed consent for their participation. MGCD0103 was administered in 3-week cycles consisting of an oral dose ranging from 12.5 to 56 mg/m<sup>2</sup>/d given three times weekly for 2 weeks followed by a 7-day rest period. This cycle was repeated every 3 weeks. Pharmacodynamic samples were collected before onset of treatment, as well as at 4 h, 24 h, day 3 (48 h after the first dose), and day 8 (72 h after the third dose) for evaluation of total HDAC enzyme activity in peripheral WBC.

**Statistical analysis.** *In vivo* xenograft data were subjected to ANOVA followed by Dunnett's test comparing each treated group with the vehicle control, with a level of significance of 0.01. The data on inhibition of HDAC activity in clinical samples were subjected to ANOVA followed by a Student's-Newman-Keuls' procedure for pairwise comparisons with a level of significance of 0.05. In addition, a linear trend test, which evaluates whether the response is dose dependent, was applied. To circumvent the influence of an outlier in the day 3 time-point data set, the data were also ranked and analyzed with ranked linear trend test and ranked ANOVA followed by ranked Student's-Newman-Keuls' procedure.

## Results

**Boc-Lys( $\epsilon$ -Ac)-AMC is a cell-permeable HDAC substrate.** In the absence of cells (Fig. 1A, column 1), the substrate Boc-Lys( $\epsilon$ -Ac)-AMC was not converted to product and did not generate

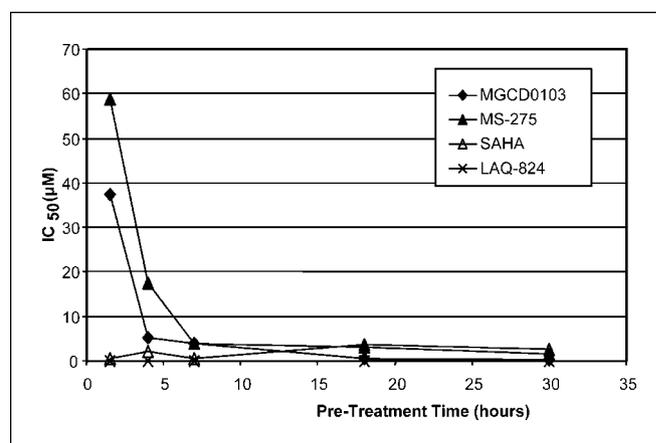


**Fig. 1.** Validation of whole-cell HDAC assay. *A*, 1, no cell control: all reagents were included in the wells, except for cells; 2, total activity: 293T cells ( $5 \times 10^4$  per well) were incubated with the substrate, then were lysed and the product was converted with Fluor-de-Lys Developer (BioMol) into a measurable fluorescent compound; 3, cell fraction: 293T cells were incubated with the substrate, but before lysis the supernatant was replaced with fresh medium; 4, supernatant: 293T cells were incubated with the substrate as in 2, but before stopping the reaction the supernatant was collected, cleared from potentially floating cells by centrifugation, and transferred to empty wells; 5, no substrate control: all the components of the reaction were added, except for Boc-Lys( $\epsilon$ -Ac)-AMC; 6, conditioned medium: 293T cells were incubated in medium without substrate, then the medium was centrifuged to remove any cells, and Boc-Lys( $\epsilon$ -Ac)-AMC was added to the clear, conditioned medium. *B*, assay linearity as a function of time. 293T cells were incubated with Boc-Lys( $\epsilon$ -Ac)-AMC for various amounts of time before the reaction was stopped. Bars, SE. *C*, assay linearity as a function of cell number. Cell lines (HCT116 colon carcinoma, A549 lung carcinoma, DU145 prostate carcinoma, and HMEC human mammary epithelial cells) were seeded in 96-well plates at the indicated cell densities and incubated with Boc-Lys( $\epsilon$ -Ac)-AMC for 1 hour before the reaction was stopped. *D*, determination of  $K_m$  in cultured cell lines and peripheral WBC. HDAC activity was measured with various amounts of Boc-Lys( $\epsilon$ -Ac)-AMC for a determined amount of time. The velocity  $V$  (pmol/min) was plotted as a function of substrate concentration  $S$  ( $\mu\text{mol/L}$ ) in a double reciprocal graph.

fluorescence. Likewise, when no substrate was added, the cells alone generated very low background fluorescence (Fig. 1A, column 5). Measurements of cell-associated and cell-free product showed that Boc-Lys( $\epsilon$ -Ac)-AMC is cell permeable (Fig. 1A, column 2) and that the deacetylated product Boc-Lys-AMC also diffuses out of the cells (Fig. 1A, columns 3 and 4). There was no extracellular HDAC activity, because conditioned medium, incubated previously with cells (Fig. 1A, column 6), did not generate more fluorescence than background (Fig. 1A, column 1).

**Characteristics and kinetics of whole-cell HDAC enzyme assay in human cancer cell lines in vitro.** The assay was time dependent and linear over 2 hours in 293T cells (Fig. 1B). It was also cell number dependent and linear up to  $5 \times 10^4$  cells per well (for 1 hour of reaction time) for several cell lines examined (HCT116, A549, DU145, and HMEC; Fig. 1C). To calculate  $V_{\max}$  and  $K_m$  for several cell lines, HDAC activity was measured as a function of time and substrate concentration (Fig. 1D).  $V_{\max}$  values (expressed in pmol converted product/min) were  $9.47 \pm 0.37$  for A549,  $14.06 \pm 0.70$  for DU145,  $28.56 \pm 1.19$  for HCT116, and  $6.16 \pm 0.38$  for peripheral WBC.  $K_m$  values ( $\mu\text{mol/L}$ ) were  $130 \pm 16$  for A549,  $140 \pm 21$  for DU145,  $255 \pm 26$  for HCT116, and  $122 \pm 23$  for WBC. Based on these results, the substrate concentration for subsequent assays was set at  $300 \mu\text{mol/L}$ .

**Inhibitory activity of MGCD0103 and other HDAC inhibitors in human cancer cell lines in vitro.** The whole-cell HDAC activity assay was used to evaluate the potency of HDAC inhibitors. Unlike hydroxamate-based compounds such as



**Fig. 2.** Time dependency of benzamide-based HDAC inhibitors. HDAC activity was assayed in HCT116 with doses of MGCD0103, MS-275, SAHA, or NVP-LAQ-824 in serial dilutions ranging from 0.006 to  $60 \mu\text{mol/L}$ .  $\text{IC}_{50}$ , defined as the concentration ( $\mu\text{mol/L}$ ) of compound inhibiting 50% of initial HDAC activity, was determined and plotted as a function of time of pretreatment (hours).

**Table 1.** IC<sub>50</sub> values of compounds for HDAC inhibition in cancer cell lines, mean ± SE (n)

	IC <sub>50</sub> (μmol/L)			
	HCT116	Jurkat	T24	MCF-7
MGCD0103	0.45* ± 0.05 (11)	0.11 ± 0.02 (5)	0.22 ± 0.04 (6)	0.21 ± 0.01 (5)
MS-275	2.50 ± 0.26 (5)	0.26 ± 0.01 (5)	0.27 ± 0.04 (5)	0.36 ± 0.01 (5)
SAHA	2.98* ± 0.18 (4)	0.46 ± 0.06 (5)	0.43 ± 0.07 (5)	0.63 ± 0.11 (5)
Compound A	>50 (3)	>50 (4)	>50 (4)	>50 (4)

NOTE: Cells were seeded on day 0 at  $1 \times 10^5$  (HCT116) or  $5 \times 10^4$  (all other lines) per well in a 96-well culture plate and assayed for HDAC activity on day 2 following an 18-hour treatment with various doses of MGCD0103, MS-275, SAHA, or compound A. The numbers represent the concentration (μmol/L) inhibiting 50% of the total HDAC enzyme activity. NA, not available.

\*These values were presented in Fournel et al.<sup>4</sup> and are used here for the sake of comparison.

SAHA or NVP-LAQ-824, MGCD0103 and MS-275 show increasing potency over time. Their IC<sub>50</sub> values were time dependent, shifting with time over a 100-fold range, and only reaching full potency after 16 hours of incubation with cells at 37°C (Fig. 2). Consequently, the drug exposure time was set at 16 to 18 hours before adding Boc-Lys(ε-Ac)-AMC in further activity assays.

Alamar Blue (BioSource, Invitrogen), an indicator of mitochondrial metabolism, did not reveal any cell cytotoxicity within the exposure time of the assay (data not shown). In addition, other cytotoxic, non-HDAC inhibitors, such as paclitaxel, did not inhibit HDAC whole-cell activity in this assay (data not shown).

To ensure that the observed decrease in substrate conversion on treatment with HDAC inhibitors was not due to HDAC down-regulation or depletion, we detected the levels of several major HDAC isotypes by immunoblot analysis (Supplementary Fig. S1) and found that, after 16 hours of pretreatment with either MGCD0103 or NVP-LAQ-824 (at a concentration ~ 10 times their respective IC<sub>50</sub> values), the levels of HDAC 1 to 3, 6, and 8 were unaffected. HDAC7 was down-regulated by both MGCD0103 and NVP-LAQ-824 as reported previously for other HDAC inhibitors (29). This HDAC7 down-regulation, however, was not correlated with HDAC inhibition measured by the whole-cell assay (Supplementary Fig. S2). For example, at 7 hours of pretreatment, 5 μmol/L MGCD0103 (~ 10 times the IC<sub>50</sub>) could inhibit HDAC activity without down-regulating HDAC7. Moreover, NVP-LAQ-824 could very efficiently inhibit activity with no time dependency even at time points where HDAC7 levels were not yet affected (≤3 hours pretreatment). Therefore, the measured decrease in substrate deacetylation on treatment with HDAC inhibitors is probably not much influenced by down-regulation of HDAC gene expression. Rather, it is believed to mostly reflect direct inhibition of HDAC activity.

The inhibitory activities of MGCD0103, MS-275, and SAHA were compared using a panel of cell lines (Table 1). Although the measured IC<sub>50</sub> varied between cell lines, MGCD0103 was always more potent than the comparator molecules in all cases examined. MGCD0103 was more than 7-fold more potent than SAHA in PANC-1 pancreatic cancer cells and more than 6-fold

in HCT116 colon cancer. Compound A showed no inhibition in any cell line up to 50 μmol/L.

**Profile and persistence of MGCD0103 inhibitory activity in human peripheral WBC ex vivo.** The whole-cell HDAC enzyme activity assay was also shown to behave in a linear fashion as a function of time and cell number in healthy human peripheral WBC *ex vivo* (Fig. 3A). MGCD0103 induced significant inhibition of HDAC activity in a dose-dependent manner in human peripheral WBC treated *ex vivo* for 18 hours (Fig. 3B), and the calculated IC<sub>50</sub> ± SE of MGCD0103 was  $0.35 \pm 0.08$  μmol/L ( $n = 16$ ). Inhibition with MGCD0103 reached a plateau at 1.5 μmol/L, which corresponds approximately to a 70% reduction of total HDAC activity. Some enzyme activity remained uninhibited even at very high doses of the compound (up to 50 μmol/L). Nonselective hydroxamate-based inhibitors such as SAHA and NVP-LAQ-824, on the other hand, were able to inhibit the cellular pool of HDAC activity almost completely, with an IC<sub>50</sub> of 0.9 μmol/L for SAHA and 0.05 μmol/L for NVP-LAQ-824.

The HDAC inhibitory activity of MGCD0103 was long-lasting in human peripheral WBC treated *ex vivo* with MGCD0103, and whole-cell HDAC inhibition was sustained even 24 hours after removal of the compound (Fig. 3C). In contrast, SAHA showed very rapid reversal of HDAC inhibition on drug removal, as enzyme activity was fully restored as soon as the compound was removed.

**In vivo pharmacodynamic effects in tumor and peripheral WBC following oral dosing of MGCD0103 in mice.** With the aim of using the whole-cell HDAC activity assay as a pharmacodynamic endpoint during clinical trials, it was necessary to validate the use of blood as a surrogate tissue for tumors. Mice ( $n = 5$  per group) were administered MGCD0103 (free base) orally at 90 mg/kg, a dose that has antitumor efficacy in all xenograft models we have tested previously (23). At several time points, blood was collected and stored overnight to mimic shipment conditions of clinical samples. As shown in Fig. 4A, the HDAC inhibitory activity was time dependent, with maximal inhibition (40% of initial level) rapidly achieved within the first hour following administration. Inhibition was sustained for ~8 hours, and HDAC activity returned to baseline levels by 24 hours. Inhibition was highly significant, with  $P < 0.01$  as determined by post-ANOVA Dunnett's test, for all time points up to and including 8 hours.

The observed *in vivo* HDAC inhibition was associated with an increase in histone acetylation in SW48 tumors implanted in

<sup>4</sup> M. Fournel, C. Bonfils, Y. Hou, et al. MGCD0103, a novel isotype-selective histone deacetylase inhibitor, has broad-spectrum antitumor activity *in vitro* and *in vivo*, *Mol Cancer Ther* 2008;7:759–68.

**Table 1.** IC<sub>50</sub> values of compounds for HDAC inhibition in cancer cell lines, mean ± SE (n) (Cont'd)

IC <sub>50</sub> (μmol/L)			
PANC-1	HeLa	A549	293T
0.09 ± 0.03 (3)	0.08 ± 0.01 (4)	0.38 ± 0.04 (9)	0.45 ± 0.02 (57)
0.45 ± 0.04 (3)	0.34 ± 0.03 (4)	0.54 ± 0.16 (4)	2.65 ± 1.06 (5)
0.69 ± 0.32 (3)	0.39 ± 0.03 (4)	0.53 ± 0.06 (4)	2.59 ± 0.52 (5)
>50 (2)	>50 (4)	>50 (3)	NA

nude mice. As shown in Fig. 4B and C, MGCD0103 induced histone H3 hyperacetylation in SW48 tumors as early as 4 hours following acute administration of MGCD0103 free base (90 mg/kg). Acetylation increased over time, with a 4.3-fold peak at 16 hours. After 48 hours, the levels of H3 acetylation in tumors returned to baseline values. In peripheral WBC of the same mice, H3 acetylation levels, normalized with total H4 histone levels, paralleled those of SW48 tumors, although to a lesser degree with a 2.6-fold peak after 16 hours, with values returning to baseline levels within 48 hours (Fig. 4B and C). H3 hyperacetylation in both tumor cells and peripheral WBC correlated with inhibition of HDAC activity (Fig. 4A). In this model, H3 acetylation in peripheral WBC appears to be a valid surrogate for MGCD0103 activity in the tumor.

H3 acetylation was also examined in HCT116 tumors implanted in nude mice (Fig. 4D). In this model, MGCD0103 induced a very modest but highly significant ( $P < 0.01$ , post-ANOVA Dunnett's test) H3 hyperacetylation. The effect reached a plateau 8 hours post-treatment and lasted for at least 48 hours.

**Baseline whole-cell HDAC activity levels in human peripheral WBC from volunteers.** We determined the natural variability in HDAC activity levels in peripheral WBC from eight healthy volunteers at two different time points, 2 months apart. Figure 5A shows that the baseline HDAC activity levels in WBC, as determined by this whole-cell HDAC activity assay, are constant across individuals (with a coefficient of variation of 6.6% for the first time-point and 8.2% for the second time-point) and across time points (with coefficients of variation of 1.0-11.3%). Moreover, we tested various shipment-mimicking conditions with blood from healthy donors. Blood was collected in heparin-coated regular or BD-Vacutainer-CPT tubes and stored at 4°C or ambient temperature for 0, 24, or 48 hours after collection before isolation of peripheral WBC. No significant difference in baseline HDAC activity was observed among these conditions (data not shown).

**Pharmacodynamic effects of MGCD0103 in peripheral WBC from patients with solid tumors in phase I trial.** The whole-cell HDAC enzyme assay was used to monitor the inhibitory activity of MGCD0103 in peripheral WBC from subjects with solid tumors in a phase I clinical trial. The overall clinical outcome of MGCD0103 in this trial and pharmacodynamic effects of MGCD0103 at 24 hours were reported in a separate publication (30). A closer examination of the dose- and time-dependent pharmacodynamic effects at later time-points is presented here.

To determine the dynamic range of the HDAC enzyme assay, we measured the inhibitory activity of MGCD0103 in four groups of subjects treated orally at doses of 27, 36, 45, or 56 mg/m<sup>2</sup> MGCD0103. As shown in Fig. 5B and C, MGCD0103-mediated enzyme inhibition was dose dependent and sustained

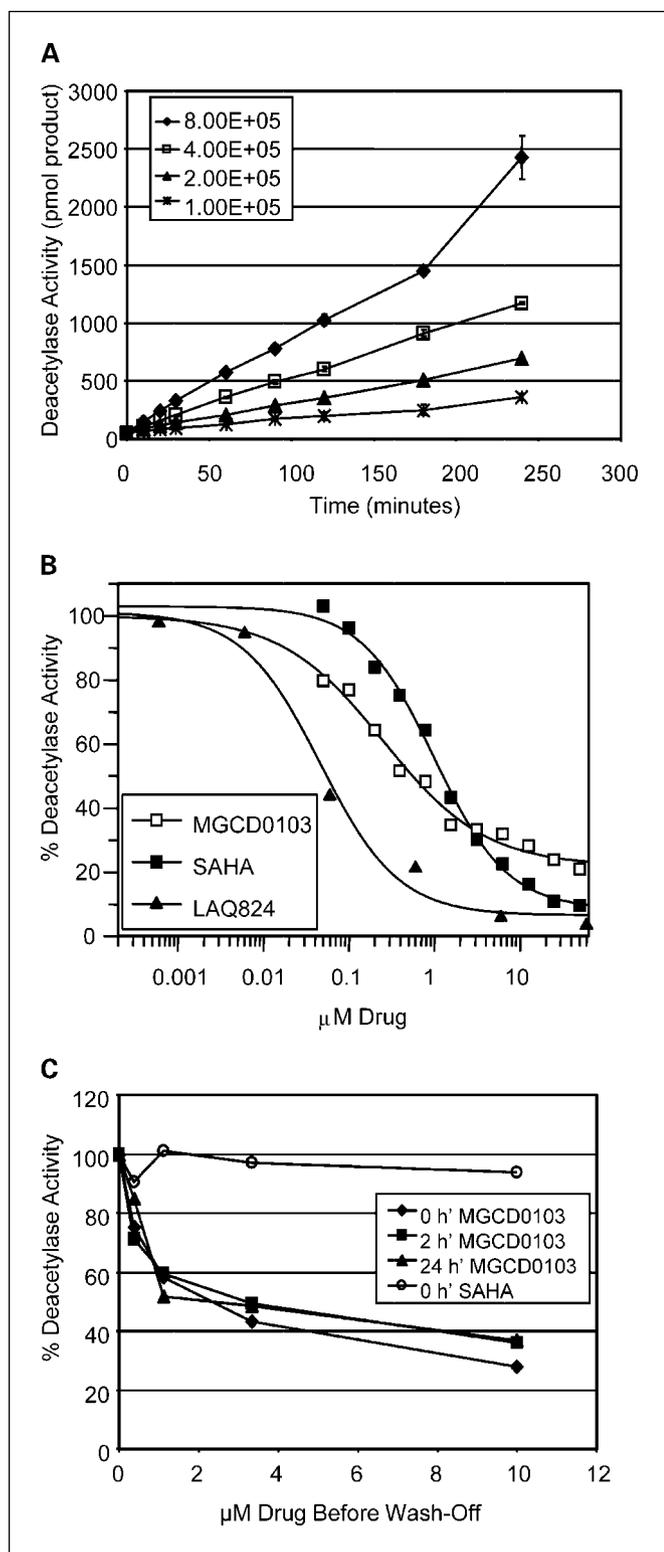
for at least 48 hours after the first administration (day 3, Fig. 5B) and up to 72 hours after the third administration (day 8, Fig. 5C). At the highest dose (56 mg/m<sup>2</sup>), HDAC inhibition was significantly higher than at 27 and 36 mg/m<sup>2</sup> in both day 3 and day 8 time points, as determined by the Student's-Newman-Keuls' procedure following ANOVA, with a significance level of 0.05. However, one of the samples from the day 3 data set treated at 27 mg/m<sup>2</sup> met all the criteria of an outlier, explaining why the average in this group was so different from the median (Fig. 5B). It was therefore justifiable to perform a nonparametric ranked analysis. When the ranked Student's-Newman-Keuls' test was applied to the day 3 data set, it appeared that HDAC inhibition at 56 mg/m<sup>2</sup> was also significantly higher than at 45 mg/m<sup>2</sup> ( $P < 0.05$ ). The ranked analysis did not change conclusions for the day 8 time point. Moreover, whether the data were analyzed raw or ranked, the linear trend test showed that the dose dependency was highly significant ( $P < 0.01$ ) at both time points.

## Discussion

The whole-cell HDAC enzyme assay allows the assessment of HDAC activity in a context in which interactions of HDAC enzymes with associated proteins remain intact. This is important as the complexes involving HDACs differ in various cell lines and tissues. Indeed, the differences in IC<sub>50</sub> values reported in Table 1 reflect this disparity. In contrast, *in vitro* measurements rely on the purification of specific HDAC isotypes, which may create artificial conditions and alter normal protein-protein interactions. Therefore, assessing HDAC activity in a native, whole-cell context may be more physiologically relevant.

The cell-based activity assay was also informative regarding inhibitor selectivity. Only a portion of total cellular HDAC activity could be abrogated by MGCD0103, whereas SAHA and NVP-LAQ-824 almost completely inactivated the cellular enzyme pool at high concentrations. Obviously, the cellular HDAC activity measured in this assay is defined by the isotypes that can use the substrate. Boc-Lys(ε-Ac)-AMC is a pan-HDAC substrate, although class I enzymes use it with a greater turnover rate than class II or III isotypes.<sup>5</sup> Class III isotypes (sirtuins) are not inhibited by NVP-LAQ-824, SAHA (31), or trichostatin A; yet those inhibitors almost completely abrogate the measured activity, so it is unlikely that the deacetylase activity measured in this assay comes from sirtuins. Therefore, the isotypes contributing to the total measured activity are class I and II enzymes, with a preference for class I isotypes. Unlike

<sup>5</sup> A-H. Lu and J. Rahil, unpublished data.



**Fig. 3.** Whole-cell HDAC assay in human peripheral WBC *ex vivo*. HDAC enzyme activity was monitored with Boc-Lys( $\epsilon$ -Ac)-AMC in human peripheral WBC (from buffy coat) isolated from volunteer blood. **A**, time dependency. **B**, human peripheral WBC were treated with a range of doses of either SAHA, MGCD0103, or NVP-LAQ-824 for 16 hours and then assayed for HDAC activity with Boc-Lys( $\epsilon$ -Ac)-AMC. **C**, human peripheral WBC were treated with a range of doses of either SAHA or MGCD0103 for 16 hours before compounds were removed from the medium by washing cells with PBS. Cells were then resuspended in fresh medium at time 0 hour and assayed at indicated time points postwash for whole-cell HDAC activity with Boc-Lys( $\epsilon$ -Ac)-AMC.

NVP-LAQ-824 or SAHA, MGCD0103 specifically inhibits only a subset of the measured HDAC isotypes. The uninhibited enzymes account for  $\sim 35\%$  of the total measured cellular HDAC activity at  $1.5 \mu\text{mol/L}$  in peripheral WBC. These results are consistent with the finding that MGCD0103 *in vitro* primarily inhibits only human HDAC 1 and 2 and less potently HDAC 3 and 11 (25, 32).<sup>4</sup>

Interestingly, the  $\text{IC}_{50}$  values of MGCD0103 and other benzamide-based inhibitors, such as MS-275, shift with time over a 100-fold range. Closer examination of their biochemical properties revealed that benzamide-based inhibitors have the characteristics of a slow-tight inhibitor, which will be described elsewhere.<sup>6</sup> Consequently, one might argue that the inevitable delay between blood collection and time of assay, due to shipment, might amplify the apparent MGCD0103 inhibitory activity. However, the shift in  $\text{IC}_{50}$  is dramatic only in the first 8 hours of exposure in cultured cell lines; therefore, clinical samples at later time-points are not likely to be meaningfully affected by this factor. Otherwise, we found no significant differences in the basal level of HDAC activity between samples from healthy donors, whether they were fresh or stored for 24 or 48 hours or taken at several time points. Thus, the delay due to shipment does not appear to affect the measurement of HDAC activity per se, and changes in HDAC activity levels during clinical trials are believed to be accounted for by the treatment rather than by natural fluctuations.

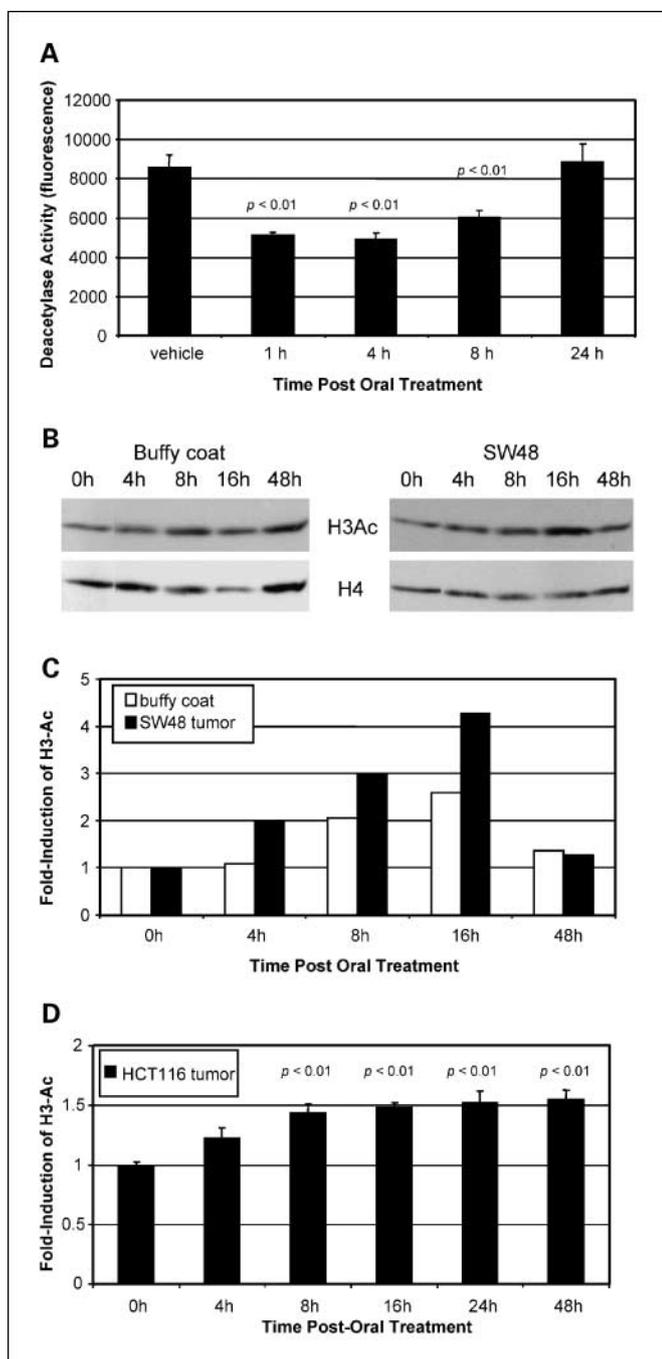
As a corollary of the slow-tight kinetic properties of MGCD0103, the off-binding rate is very slow and can account for the prolonged inhibitory effect of MGCD0103 observed in *ex vivo* treated cells after the compound had been removed (Fig. 3C). One implication of these results is that patients may receive fewer doses of MGCD0103 while the pharmacodynamic effects are maintained. Another implication is that detection of HDAC inhibition in isolated peripheral WBC from patients would not be compromised by unavoidable washing steps during the isolation protocol. The clinical pharmacodynamic data are, therefore, assumed to be relevant to the actual inhibitory activity of MGCD0103.

The utility of blood as a surrogate tissue for solid tumors was validated in nude mice bearing implanted tumors (Fig. 4). At a dose shown previously to have efficacy against implanted tumors (90 mg/kg; ref. 23), we observed reduction in peripheral WBC HDAC activity in a time-dependent manner, concomitant with induction of histone acetylation both in peripheral WBC and in implanted tumors. These data indicate that MGCD0103 was biologically active as expected. Moreover, in mice,  $t_{1/2}$  for MGCD0103 was found to be  $\sim 0.7$  hour,<sup>7</sup> whereas HDAC inhibition was sustained for at least 8 hours and histone acetylation peaked at 16 hours in SW48 tumors and lasted for at least 48 hours in HCT116 tumors. Therefore, in mice, the pharmacodynamic effect of MGCD0103, as monitored by HDAC inhibition and histone acetylation, outlasted detectable plasma exposures.

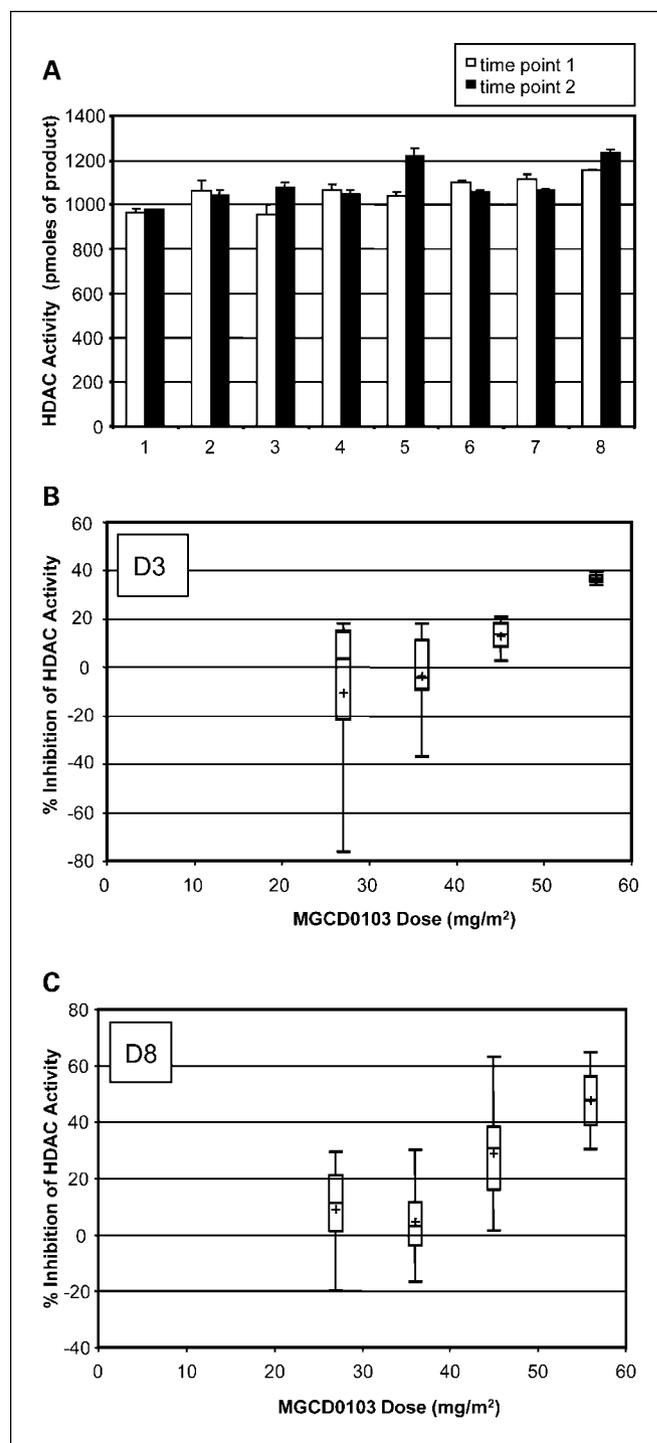
Pharmacokinetic variables for MGCD0103 in plasma from treated patients have been analyzed (32–34). Correlation with HDAC inhibition has been observed and was described in detail elsewhere (30). Briefly, in patients receiving  $27 \text{ mg/m}^2$

<sup>6</sup> A-H Lu, J. Rahil, et al., unpublished data.

<sup>7</sup> J.Wang et al., unpublished data.



**Fig. 4.** HDAC activity in mice *in vivo*. **A**, CD-1 mice ( $n = 5$  per group) were given oral MGCD0103 free base (90 mg/kg). At indicated time points, blood was collected and peripheral WBC were isolated from individual mice and assayed for HDAC activity using  $4 \times 10^5$  cells per well. Columns, averages for each group of mice; bars, SE.  $P < 0.01$ , Dunnett's test following ANOVA. **B** and **C**, CD-1 nude mice ( $n = 3$  per group) with SW48 colon cancer xenografts ( $\sim 1,000 \text{ mm}^3$ ) were given a single oral administration of MGCD0103 free base (90 mg/kg) and then were sacrificed after specified amount of time. Both blood and tumors were collected and peripheral WBC were isolated from blood. Histones were acid extracted from individual animals and pooled for immunoblot analysis (10  $\mu\text{g}/\text{lane}$ ) using antibodies against acetylated H3 histones and total H4 histones (**B**). Signals were quantified by densitometry and normalized by dividing the H3Ac signal with total H4 to correct for uneven loading (**C**). **D**, CD-1 nude mice ( $n = 5$  per group) with HCT116 colon cancer xenografts were treated with a single oral dose of MGCD0103 free base (90 mg/kg). At specified times, mice were sacrificed and tumors were collected. Histones were acid extracted and analyzed by immunoblot (10  $\mu\text{g}/\text{lane}$ ) using antibodies against acetylated H3 histones and pan-histones. Signals were quantified and normalized by dividing the H3Ac signal with the signal for pan-histones.



**Fig. 5.** HDAC activity in WBC from healthy volunteers and cancer patients *in vivo*. **A**, basal HDAC activity levels were measured in peripheral WBC ( $8 \times 10^5$  cells per well) isolated from eight different volunteers, at two time-points 2 mo apart, using the whole-cell HDAC activity assay. **B** and **C**, phase I trial subjects with solid tumors were treated three times a week with MGCD0103 at 27, 36, 45, or 56 mg/m<sup>2</sup>. At specified time points, HDAC activity was evaluated in peripheral WBC ( $8 \times 10^5$  cells per well) from all evaluable subjects. The results were expressed relative to the 0-hour time point (before onset of treatment) and graphed as box plots. The boxes are delimited by the lower and upper quartiles, whereas the middle bar represents the median. The whiskers reach to the smallest and the largest data points of each set. The cross represents the average. **B**, day 3 time point, collected 48 h after onset of treatment and before the second dose, at 27 mg/m<sup>2</sup> ( $n = 6$ ), at 36 mg/m<sup>2</sup> ( $n = 6$ ), at 45 mg/m<sup>2</sup> ( $n = 9$ ), and at 56 mg/m<sup>2</sup> ( $n = 2$ ). **C**, day 8 time point, collected 72 h after the third dosing, at 27 mg/m<sup>2</sup> ( $n = 6$ ), at 36 mg/m<sup>2</sup> ( $n = 4$ ), at 45 mg/m<sup>2</sup> ( $n = 11$ ), and at 56 mg/m<sup>2</sup> ( $n = 2$ ).

dose,  $C_{\max} \pm SD$  was  $71.6 \pm 50.4$  ng/mL, which translates to  $0.18 \pm 0.12$   $\mu\text{mol/L}$ . In patients receiving  $56 \text{ mg/m}^2$  dose,  $C_{\max} \pm SD$  was  $172.0 \pm 28.3$  ng/mL, which corresponds to  $0.43 \pm 0.07$   $\mu\text{mol/L}$ .  $t_{1/2}$  was  $\sim 10$  hours and  $T_{\max}$  was  $\sim 1$  hour regardless of the dose. The magnitude of HDAC inhibition was, not surprisingly, rather limited in the  $27 \text{ mg/m}^2$  dose group because the  $C_{\max}$  was well below the  $IC_{50}$  measured in *ex vivo* treated peripheral WBC. In the  $56 \text{ mg/m}^2$  dose group, however,  $C_{\max}$  was above the  $IC_{50}$  as determined in *ex vivo* assay ( $0.35 \mu\text{mol/L}$ ). In this dose group, a significant reduction of HDAC enzyme inhibition was seen at 24 hours (30) and 48 hours after the first administration as well as 72 hours after the third administration. The whole-cell HDAC enzyme assay appears to be applicable to determine the pharmacodynamic effect of MGCD0103 at least in peripheral WBC from patients with solid tumors. In leukemia patients, whose peripheral WBC contain leukemia blasts, we need to consider that apoptotic cells get rapidly cleared from the blood (35). If MGCD0103-mediated HDAC inhibition increased apoptosis of cancer cells, as has been suggested elsewhere,<sup>5</sup> the detected HDAC inhibition in peripheral WBC from leukemia patients would likely be underestimated.

Interestingly, HDAC enzyme inhibition was sustained in MGCD0103-treated cancer patients for up to 48 to 72 hours (Fig. 5B and C), although plasma concentrations of the drug were decreased to below the limits of detection at these time points. This finding is further supported by a similar observation in both the *ex vivo* assay with peripheral WBC from human volunteers and *in vivo* in mice. This suggests that a less frequent dosing schedule may be desired to reach the best therapeutic index. Indeed, the observed half-life of MGCD0103, combined with the sustained pharmacodynamic, allow for dosing schedules of two to three times per week (32, 36).

Histone acetylation was measured in samples from MGCD0103-treated solid tumor patients (30). A modest but

significant induction of histone acetylation ( $\sim 1.8$ -fold), correlating with HDAC inhibition, was observed transiently 24 hours after the first administration in patients who received the highest dose of MGCD0103 (30). However, no significant histone acetylation was found in peripheral WBC from subjects treated with MGCD0103 at the other dose levels or time points.<sup>8</sup> This lack of sensitivity for the histone acetylation assay in human patients may be due to the constraints of the clinical trial setup and the fact that the samples were evaluated after overnight shipment rather than fresh. In contrast, the whole-cell HDAC activity assay revealed inhibitory activity at all time points and the dynamic range was wider than for the acetylation assay under the current shipment conditions.

In conclusion, the data presented here confirm the HDAC inhibitory activity and ability of MGCD0103 to induce histone acetylation in mice and show that the MGCD0103-mediated HDAC inhibition can be monitored by a novel whole-cell HDAC enzyme assay in patients. This enzyme assay is more sensitive than histone acetylation assays and provides rapid results while using few peripheral WBC from patients. Thus, it could prove to be a useful pharmacodynamic assay in future clinical studies of MGCD0103 and other HDAC inhibitors.

### Disclosure of Potential Conflicts of Interest

C.B., A.K., M.D., G.R., R.E.M., J.M.B., and Z.L. are employed by MethylGene, whose potential product was studied in the present work. M.A.C. is a consultant of MethylGene.

### Acknowledgments

We thank our MethylGene colleagues Theresa Yan, Jianhong Liu, Marielle Fournel, Dr. A. Robert MacLeod, H el ene Ste-Croix, Laura Pearce, Tracy-Ann Patterson, Dr. Christiane Maroun, Dr. Jubrail Rahil, Dr. James Wang, Dr. Lori Martell, and Eugene Kovtun for assistance and discussions; our colleagues from Pharmion and Taiho Pharmaceuticals, especially Dr. Eric Laille for discussion of pharmacokinetic variables of MGCD0103, Dr. Richard McNally for helping with the statistical analysis, and Dr. Carla Heise for discussion of experimental results; and the clinical teams at Sidney Kimmel Cancer Center at John Hopkins University, Princess Margaret Hospital, University of Toronto, and MethylGene as well as all patients and their families for participation in the clinical trial.

<sup>8</sup> Unpublished data.

### References

- Gore SD, Baylin S, Sugar E, et al. Combined DNA methyltransferase and histone deacetylase inhibition in the treatment of myeloid neoplasms. *Cancer Res* 2006;66:6361–9.
- Rasheed WK, Johnstone RW, Prince HM. Histone deacetylase inhibitors in cancer therapy. *Expert Opin Investig Drugs* 2007;16:659–78.
- Santini V, Gozzini A, Ferrari G. Histone deacetylase inhibitors: molecular and biological activity as a premise to clinical application. *Curr Drug Metab* 2007;8:383–93.
- Bolden JE, Peart MJ, Johnstone RW. Anticancer activities of histone deacetylase inhibitors. *Nat Rev Drug Discov* 2006;5:769–84.
- Johnstone RW. Histone-deacetylase inhibitors: novel drugs for the treatment of cancer. *Nat Rev Drug Discov* 2002;1:287–99.
- Acharya MR, Sparreboom A, Venitz J, Figg WD. Rational development of histone deacetylase inhibitors as anticancer agents: a review. *Mol Pharmacol* 2005;68:917–32.
- Monneret C. Histone deacetylase inhibitors for epigenetic therapy of cancer. *Anticancer Drugs* 2007;18:363–70.
- Garcia-Manero G, Issa JP. Histone deacetylase inhibitors: a review of their clinical status as antineoplastic agents. *Cancer Invest* 2005;23:635–42.
- Marks P, Rifkin RA, Richon VM, Breslow R, Miller T, Kelly WK. Histone deacetylases and cancer: causes and therapies. *Nat Rev Cancer* 2001;1:194–202.
- Gallinari P, Di Marco S, Jones P, Pallaoro M, Steinkuhler C. HDACs, histone deacetylation and gene transcription: from molecular biology to cancer therapeutics. *Cell Res* 2007;17:195–211.
- Kelly WK, O'Connor OA, Krug LM, et al. Phase I study of an oral histone deacetylase inhibitor, suberoylanilide hydroxamic acid, in patients with advanced cancer. *J Clin Oncol* 2005;23:3923–31.
- Marks PA. Discovery and development of SAHA as an anticancer agent. *Oncogene* 2007;26:1351–6.
- Duvic M, Vu J. Vorinostat: a new oral histone deacetylase inhibitor approved for cutaneous T-cell lymphoma. *Expert Opin Investig Drugs* 2007;16:1111–20.
- Kato Y, Salumbides BC, Wang XF, et al. Antitumor effect of the histone deacetylase inhibitor LAQ824 in combination with 13-*cis*-retinoic acid in human malignant melanoma. *Mol Cancer Ther* 2007;6:70–81.
- Giles F, Fischer T, Cortes J, et al. A phase I study of intravenous LBH589, a novel cinnamic hydroxamic acid analogue histone deacetylase inhibitor, in patients with refractory hematologic malignancies. *Clin Cancer Res* 2006;12:4628–35.
- Qian X, LaRochelle WJ, Ara G, et al. Activity of PXD101, a histone deacetylase inhibitor, in preclinical ovarian cancer studies. *Mol Cancer Ther* 2006;5:2086–95.
- Plumb JA, Finn PW, Williams RJ, et al. Pharmacodynamic response and inhibition of growth of human tumor xenografts by the novel histone deacetylase inhibitor PXD101. *Mol Cancer Ther* 2003;2:721–8.
- Gojo I, Jiemjit A, Trepel JB, et al. Phase 1 and pharmacologic study of MS-275, a histone deacetylase inhibitor, in adults with refractory and relapsed acute leukemias. *Blood* 2007;109:2781–90.
- Hess-Stumpff H, Bracker TU, Henderson D, Polit O. MS-275, a potent orally available inhibitor of histone deacetylases—the development of an anticancer agent. *Int J Biochem Cell Biol* 2007;39:1388–405.
- Ryan QC, Headlee D, Acharya M, et al. Phase I and pharmacokinetic study of MS-275, a histone deacetylase inhibitor, in patients with advanced and refractory solid tumors or lymphoma. *J Clin Oncol* 2005;23:3912–22.

21. Moradei O, Leit S, Zhou N, et al. Substituted *N*-(2-aminophenyl)-benzamides, (*E*)-*N*-(2-aminophenyl)-acrylamides and their analogues: novel classes of histone deacetylase inhibitors. *Bioorg Med Chem Lett* 2006;16:4048–52.
22. Bonfils C, Kalita A, Liu J, Besterman JM, Li Z. Development of whole cell HDAC enzyme assay to analyze inhibitory activity of MGCD0103 *in vitro* and *in vivo*. 96th Annual Meeting of the AACR. *Proc Am Assoc Cancer Res* 2005;46:A606.
23. Li Z, Zhou N, Fournel M, et al. Antitumor activities of MGCD0103, a novel isotype-selective histone deacetylase inhibitor. 16th EORTC-NCI-AACR Symp Mol Targets Cancer Ther. *Eur J Cancer Suppl* 2004;2:A83.
24. Byrd JC, Marcucci G, Parthun MR, et al. A phase 1 and pharmacodynamic study of depsipeptide (FK228) in chronic lymphocytic leukemia and acute myeloid leukemia. *Blood* 2005;105:959–67.
25. Vaisburg A. Discovery and development of MGCD0103—an orally active HDAC inhibitor in human clinical trials. XIXth International Symposium on Medicinal Chemistry. *Int Symp Med Chem* 2006;31(Suppl. A): Abs L57.
26. Heltweg B, Dequiedt F, Verdin E, Jung M. Nonisotopic substrate for assaying both human zinc and NAD<sup>+</sup>-dependent histone deacetylases. *Anal Biochem* 2003;319:42–8.
27. Heltweg B, Jung M. A microplate reader-based nonisotopic histone deacetylase activity assay. *Anal Biochem* 2002;302:175–83.
28. Zweidler A. Resolution of histones by polyacrylamide gel electrophoresis in presence of nonionic detergents. *Methods Cell Biol* 1978;17:223–33.
29. Dokmanovic M, Perez G, Xu W, et al. Histone deacetylase inhibitors selectively suppress expression of HDAC7. *Mol Cancer Ther* 2007;6:2525–34.
30. Siu LL, Pili R, Duran I, et al. A phase I study of MGCD0103 given as a three-weekly oral dose in patients with advanced solid tumors. *J Clin Oncol* 2003;26:1940–7.
31. Xu WS, Parmigiani RB, Marks PA. Histone deacetylase inhibitors: molecular mechanisms of action. *Oncogene* 2007;26:5541–52.
32. Siu LL, Carducci M, Patterson T, et al. Phase I study of isotype-selective histone deacetylase (HDAC) inhibitor MGCD0103 given as a three-times weekly oral dose in patients (pts) with advanced solid tumors. 18th EORTC-NCI-AACR Cancer Symposium. *Eur J Cancer* 2006;412:A300.
33. Carducci M, Siu LL, Sullivan R, et al. Phase I study of isotype-selective histone deacetylase (HDAC) inhibitor MGCD0103 given as three-times weekly oral dose in patients (pts) with advanced solid tumors. 2006 ASCO Annual Meeting. *Proc Am Soc Clin Oncol* 2006;25:A3007.
34. Siu LL, Carducci M, Pearce L, et al. Phase I study of isotype-selective histone deacetylase (HDAC) inhibitor MGCD0103 given as three-times weekly oral dose in patients (pts) with advanced solid tumors. AACR-NCI-EORTC Intl Conf Mol Targ Cancer Ther. *Clin Cancer Res* 2005;11:C77.
35. Savill J, Fadok V. Corpse clearance defines the meaning of cell death. *Nature* 2000;407:784–8.
36. Garcia-Manero G, Yang AS, Giles F, et al. Phase I/II study of the oral isotype-selective histone deacetylase (HDAC) inhibitor MGCD0103 in combination with azacitidine in patients (pts) with high-risk myelodysplastic syndrome (MDS) or acute myelogenous leukemia (AML). 48th American Society of Hematology. *Blood* 2006;108:A1954.

# Clinical Cancer Research

## Evaluation of the Pharmacodynamic Effects of MGCD0103 from Preclinical Models to Human Using a Novel HDAC Enzyme Assay

Claire Bonfils, Ann Kalita, Marja Dubay, et al.

*Clin Cancer Res* 2008;14:3441-3449.

**Updated version** Access the most recent version of this article at:  
<http://clincancerres.aacrjournals.org/content/14/11/3441>

**Cited articles** This article cites 35 articles, 11 of which you can access for free at:  
<http://clincancerres.aacrjournals.org/content/14/11/3441.full#ref-list-1>

**Citing articles** This article has been cited by 6 HighWire-hosted articles. Access the articles at:  
<http://clincancerres.aacrjournals.org/content/14/11/3441.full#related-urls>

**E-mail alerts** [Sign up to receive free email-alerts](#) related to this article or journal.

**Reprints and Subscriptions** To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at [pubs@aacr.org](mailto:pubs@aacr.org).

**Permissions** To request permission to re-use all or part of this article, use this link  
<http://clincancerres.aacrjournals.org/content/14/11/3441>.  
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.