Comparing Histone Deacetylase Inhibitor Responses in Genetically Engineered Mouse Lung Cancer Models and a Window of Opportunity Trial in Patients with Lung Cancer

Tian Ma1, Fabrizio Galimberti1, Cherie P. Erkmen2, Vincent Memoli3,5, Fadzai Chinyengetere1, Lorenzo Sempere4, Jan H. Beumer6,7, Bean N. Anyang6, William Nugent2, David Johnstone2, Gregory J. Tsongalis3, Jonathan M. Kurie6, Hua Li1, James DiRenzo1,5, Yongli Guo1, Sarah J. Freemantle1, Konstantin H. Dragnev4,5, and Ethan Dmitrovsky1,4,5

Abstract
Histone deacetylase inhibitor (HDACi; vorinostat) responses were studied in murine and human lung cancer cell lines and genetically engineered mouse lung cancer models. Findings were compared with a window of opportunity trial in aerodigestive tract cancers. In human (HOP62, H522, and H23) and murine transgenic (ED-1, ED-2, LKR-13, and 393P, driven, respectively, by cyclin E, degradation-resistant cyclin E, KRAS, or KRAS/p53) lung cancer cell lines, vorinostat reduced growth, cyclin D1, and cyclin E levels, but induced p27, histone acetylation, and apoptosis. Other biomarkers also changed. Findings from transgenic murine lung cancer models were integrated with those from a window of opportunity trial that measured vorinostat pharmacodynamic responses in pre- versus posttreatment tumor biopsies. Vorinostat repressed cyclin D1 and cyclin E expression in murine transgenic lung cancers and significantly reduced lung cancers in syngeneic mice. Vorinostat also reduced cyclin D1 and cyclin E expression, but increased p27 levels in post- versus pretreatment human lung cancer biopsies. Notably, necrotic and inflammatory responses appeared in posttreatment biopsies. These depended on intratumoral HDACi levels. Therefore, HDACi treatments of murine genetically engineered lung cancer models exert similar responses (growth inhibition and changes in gene expression) as observed in lung cancer cell lines. Moreover, enhanced pharmacodynamic responses occurred in the window of opportunity trial, providing additional markers of response that can be evaluated in subsequent HDACi trials. Thus, combining murine and human HDACi trials is a strategy to translate preclinical HDACi treatment outcomes into the clinic. This study uncovered clinically tractable mechanisms to engage in future HDACi trials. Mol Cancer Ther; 12(8); 1545–55. ©2013 AACR.
Prior work was built upon by studying the histone deacetylase inhibitor (HDACi) vorinostat (suberoylanilide hydroxamic acid, SAHA or Zolinza). Vorinostat inhibits class I, II, and IV, but not class III histone deacetylases (HDAC), as reviewed (22–25). HDACs remove acetyl groups from histones, leading to chromatin condensation and transcriptional repression (22–25). HDAC knockdown causes check point arrest that inhibits cancer cell growth (26). Vorinostat targets the HDAC catalytic domain; this reduces growth of diverse cancer cell lines (including lung cancer cells) by cell-cycle arrest via changes in expression of growth regulatory proteins (22–25, 27, 28).

Based on these preclinical HDACi activities, vorinostat clinical trials were conducted in advanced non–small cell lung cancer (NSCLC). Antitumor activity was modest for vorinostat treatment alone in relapsed NSCLCs (29, 30). Activity increased when vorinostat was combined with chemotherapy (31, 32). These findings implied that vorinostat effects in preclinical lung cancer models might not be recapitulated in tumors of patients with vorinostat–treated lung cancer. This study sought to address this gap in knowledge of vorinostat antineoplastic actions.

This was accomplished by determining vorinostat pharmacodynamics in human lung cancer cell lines and in murine transgenic lung cancer cell lines engineered with genetic changes present in clinical lung cancers. Vorinostat antineoplastic responses were also studied in genetically engineered mouse models that mimicked features of human lung cancer. Findings were compared with those found in a window of opportunity trial in patients with previously untreated lung and esophageal cancer. Both pre- and postvorinostat treatment tumor biopsies were obtained; serum and intratumoral vorinostat levels were measured. These findings establish the value of translating results from vorinostat treatment of lung cancer cell lines into genetically engineered mouse lung cancer models. Comparing responses to vorinostat treatment outcomes in patients with lung cancer can uncover clinically tractable pharmacodynamic mechanisms. These pathways could be engaged in future HDACi trials.

Materials and Methods

Cell culture treatments

Murine lung cancer cell lines from lung cancers of wild-type (ED-1) or proteasome degradation–resistant cyclin E (ED-2) transgenic mice exist (17, 18). The 393P lung cancer cell line was from KrasLA1/WT; p53R172H/C14 and the LKR-13 cell line was from KrasLA2/WT transgenic mice, respectively (33, 34). Murine (ED-1, ED-2, 393P, and LKR-13) and human (HOP62, H522, and H23) lung cancer cell lines were cultured in RPMI-1640 media with 10% FBS and 1% human (HOP62, H522, and H23) lung cancer cell lines were previously authenticated (33, 34). Murine (ED-1, ED-2, 393P, and LKR-13) and human (HOP62, H522, and H23) lung cancer cell lines were previously authenticated (14, 18). All lines were tested negative for mycoplasma and were passaged less than 6 months after receipt or resuscitation. Vorinostat (Supplementary Fig. S1) was obtained from Dr. Jason Sparkowski (Merck).

Logarithmically–growing lung cancer cells were seeded at optimized densities in 6-well tissue culture plates. Proliferation and apoptosis assays were conducted in triplicate and replicated at least 3 times. Cells were treated 24 hours later with vorinostat at varying concentrations versus vehicle (dimethyl sulfoxide, DMSO) controls and assayed at different times using the CellTiter-Glo Kit (Promega), as before (35). Apoptosis assays were conducted with the Annexin V:FITC Kit (AbD Serotec) in triplicate with independent replicates, as before (35). Drug washout experiments were conducted.

Immunoblot analyses

Immunoblot assays were conducted as in prior work (16, 35). These assays were with antibodies recognizing cyclin D1 (M-20, Santa Cruz Biotechnology), cyclin E (M-20, Santa Cruz Biotechnology), p27 (C-19, Santa Cruz Biotechnology), anti-acetyl-histone H3 (Millipore), or actin (C-11, Santa Cruz Biotechnology). Other antibodies recognized acetylated alpha-tubulin (6-11B-1, Sigma), alpha-tubulin (DM1A, Millipore), Akt (9272, Cell Signaling Technology), phospho-Akt (Ser473, D9E, Cell Signaling Technology), Bax (N-20, Santa Cruz Biotechnology), Bcl-2 (D55G8, Cell Signaling Technology; N-19, Santa Cruz Biotechnology), Bim (C34C5, Cell Signaling Technology), E-cadherin (24E10, Cell Signaling Technology), Chk1 (G-4, Santa Cruz Biotechnology), c-Raf (9422, Cell Signaling Technology), Mcl-1 (D53A5, Cell Signaling Technology), p21 (12D1, Cell Signaling Technology; F-5, Santa Cruz Biotechnology), or phospho-histone H2AX (Ser139, 2577, Cell Signaling Technology).

Transgenic experiments

Nine-month-old female transgenic mice expressing wild-type human cyclin E and that developed neoplastic lung lesions were injected (intraperitoneally) with vorinostat (100 mg/kg) or with an equal volume of vehicle (DMSO) daily for 5 days before being euthanized using an Institutional Animal Care and Use Committee (IACUC)–approved protocol. Lung tissues were harvested, formalin fixed, paraffin embedded, processed, and sectioned for histopathology and hematoxylin and eosin (H&E) staining, or immunostaining as before (14, 16). Quantification was by a pathologist (V. Memoli) unaware of treatment arm identities, as previously described (14, 16). Three mice were used in each study arm.

In vivo tumorigenicity

Eight-week-old female FVB mice were each tail vein injected with $8 \times 10^5$ early passage ED-1 cells suspended in phosphate-buffered saline (PBS) with 10% mouse serum (Invitrogen). Lung tumors formed by 14 days (18–20) when mice were each injected with vorinostat (200 mg/kg ip, 13 mice) dissolved in vehicle (10% DMSO, 45% PEG400, and 45% sterile water) or with an equal...
volume of vehicle (12 mice) for 14 consecutive days. Mice were euthanized using an IACUC-approved protocol. Lung tissues were harvested and processed with lung tumors scored by a pathologist (H. Li) blinded as to treatment arm identities.

Patients

Patients with clinical stage I, II, and IIIA NSCLC or stage I esophageal cancer and a pretreatment biopsy having at least 5 unstained slides were eligible. Inclusion criteria were adequate hepatic (hepatic transaminases ≤ 2.0 and total bilirubin ≤ 1.5 times the top limit of normal) and renal (serum creatinine < 1.5 times the top limit of normal and creatinine clearance ≥ 60 mL/min) functions. Exclusion criteria were prior chemotherapy or radiotherapy, age less than 18, serious uncontrolled comorbidities, active infection, inability to give informed consent because of altered mental status or cognitive impairment, prior use of HDACis, hypersensitivity to vorinostat or capsule components, concurrent use of other antineoplastics (approved or investigational) within 30 days of this study, or use of valproic acid unless its use was not as an antiepileptic. Those on therapeutic dosing for hypertension, diabetes, hyperlipidemia (except with statins), or other conditions were also excluded.

Inclusion and exclusion criteria were assessed within 14 days after resection and on the day of biopsy/surgery. The dose on the day of biopsy/surgery was taken under medical supervision and its time recorded. Serum samples were obtained before vorinostat treatment and at the time of biopsy. Serum was isolated by centrifugation at 0°C and at 1500 x g for 10 minutes before storage at −70°C. Biopsied tumors were immediately snap-frozen in liquid nitrogen and stored at −70°C until analysis. Tumor tissue was homogenized in 3 parts PBS (1:3, g/v). Vorinostat was quantitated using an established FDA-validated LC-MS/MS assay (37). Tumor homogenates were processed as serum and quantitated against a serum calibration curve. Analysis of quality control samples prepared in control human lung tumor homogenate proved that tumor homogenate could be processed as serum. Vorinostat was accurately quantitated against a serum calibration curve.

Tissue analyses

One portion of a tumor was harvested at resection, formalin fixed, and processed for histopathology and immunostaining, as before (14, 16). Another portion was immediately snap-frozen in liquid nitrogen for vorinostat pharmacodynamics and pharmacokinetics. Immunostaining for cyclin D1, cyclin E, Ki-67, caspase, p27, p21, and other markers was conducted by a pathologist (V. Memoli), who used optimized methods (15, 16, 19). The scoring system used for immunostaining and inflammatory infiltrates was previously described (14). Fluorescence-based multiplex assays (38) were performed in each tumor and comprised T cells with CD8 (Leica), macrophages with CD68 (Leica), B cells with CD20 (Leica), or myeloid cells with myeloperoxidase (MPO, Thermo Fisher Scientific).

EGFR and KRAS mutational analyses

Genomic DNA was isolated from paraffin-embedded sections from study cases and independently analyzed for EGFR and KRAS mutations using optimized methods (14).

Statistical analyses

Two-tailed t tests were used for statistical analyses. The Welch modified 2-sample t test was also used for statistical analyses of the mouse tumor experiments.

Results

Vorinostat effects in transgenic lung cancer cells

Vorinostat effects on proliferation, apoptosis, histone acetylation, and cell-cycle regulatory proteins were studied because prior work implicated these and other changes as important for HDACi responses (22–25). Genetically defined murine transgenic lung cancer cell lines were studied because they recapitulated features of human lung cancers (17, 18, 33, 34). These lines deregulated cyclin E (ED-1 and ED-2), or had KRAS (LKR-13) or KRAS and p53 (393P) mutations. Figure 1A and 1B established dose- and time-dependent effects on growth and apoptosis in ED-1 and ED-2 cells. Growth inhibition was reversible after drug washouts (Fig. 1C). Acetylation and
p27 levels increased, but cyclin D1 and cyclin E expression decreased in ED-1 cells (Fig. 1D), a representative murine lung cancer line. Vorinostat activities were also examined in LKR-13 and 393P murine transgenic lung cancer cell lines in Fig. 2. These exhibited similar, but less prominent vorinostat treatment effects than did ED-1 or ED-2 cells.

**Vorinostat effects in human lung cancer cell lines**

Vorinostat effects were investigated in HOP62, H522, and H23 human lung cancer cell lines. Similar to findings in murine lung cancer cells, vorinostat significantly reduced growth of each of these lines in a dose- and time-dependent manner (Fig. 3A). Vorinostat-mediated changes in apoptosis were confirmed in HOP62, a representative human lung cancer line (Fig 3B). Vorinostat growth inhibition was reversed by drug washout (Fig. 3C). Vorinostat exhibited dose-dependent effects on acetylation and cell-cycle regulatory proteins (Fig. 3D). Therefore, vorinostat exhibited substantial antineoplastic effects in murine and human lung cancer cells at clinically achievable concentrations (31). Other biomarkers of HDACi response were compared in murine and human lung cancer cells lines in Supplementary Fig. S2. These studies revealed that specific Bcl-2 family members and other species were regulated by vorinostat treatments of these lung cancer cells. These findings were extended by showing that c-Raf and phospho-AKT levels declined, but p21, acetylated tubulin, and expression of the epithelial–mesenchymal transition (EMT) marker E-cadherin increased after vorinostat treatments (Supplementary Fig. S2).

**Vorinostat effects in murine transgenic models**

To explore in vivo vorinostat treatment effects, studies were independently conducted using murine syngeneic transplantable and transgenic cyclin E-driven lung cancer
models. Two weeks after tail vein injections of ED-1 cells, FVB mice were treated with vorinostat (200 mg/kg) or an equal volume of vehicle as a control by daily intraperitoneal injections for 14 days. Lung cancers were scored as before (19). Figure 4A and B revealed that vorinostat significantly reduced the number of lung cancers in mice versus controls.

To evaluate pharmacodynamic effects, transgenic cyclin E mice that spontaneously developed lung cancer were injected with vorinostat (100 mg/kg/ip) or an equal volume of vehicle as a control by daily intraperitoneal injections for 14 days. Lung cancers were scored as before (19). Figure 4A and B revealed that vorinostat significantly reduced the number of lung cancers in mice versus controls.

Patient characteristics and vorinostat clinical responses

Fifteen patients with early-stage lung or esophageal cancer were enrolled between July 23, 2009 and August 23, 2011 onto a vorinostat window of opportunity trial. Ten received 2 or more days of treatment and had sufficient paired pre- versus posttreatment tumors for histopathologic, pharmacodynamic, and pharmacokinetic studies. Clinical, histopathologic, pharmacodynamic, and pharmacokinetic findings appear in Table 1. Median age was 67 years with 4 women (40%) and 6 men (60%). One (10%) patient had squamous cell cancer (SCC) of the lung, 6 (60%) had lung (ADC) or esophageal (Eso-ADC) adenocarcinomas, and 3 (30%) had adenocarcinomas with bronchioloalveolar (BAC) features. Five (50%) patients were former or never smokers; 5 (50%) were current smokers.

All cases had wild-type epidermal growth factor receptor (EGFR, data not shown) sequences; 4 had KRAS codon

Figure 2. Vorinostat effects in murine 393P (Kras<sup>LA1+/+; p53<sup>R172H</sup><sup>/+</sup>) and LKR-13 (Kras<sup>LA2/WT</sup>) transgenic lung cancer cell lines. A, vorinostat inhibited growth in a dose- and time-dependent manner and increased apoptosis in 393P cells. Effects were antagonized by vorinostat washout. B, similar proliferation, apoptosis (data not shown), and washout effects occurred in LKR-13 cells. Vorinostat treatments decreased cyclin D1 and cyclin E proteins, but increased p27 and acetyl-histone H3 protein levels in 393P (left, Q) and LKR-13 (right, Q) cells. Respective signal intensities are quantified on the right). * and **, significant changes, <i>P</i> < 0.05 and <i>P</i> < 0.01, respectively.
12 mutations in their lung cancers (Table 1). Seven cases (70%) reduced Ki-67 expression in post- versus pretreatment tumors. Cyclin E and/or cyclin D1 immunohistochemical expression decreased in 4 (40%) posttreatment tumors. Caspase levels were at the limits of detection. Induction of p27 occurred in a representative post- versus pretreatment lung cancer (Fig. 5A). Increased p21 tumor expression was also observed after vorinostat treatment (Supplementary Fig. S3). There were insufficient biopsies to analyze HDACi biomarkers shown in Supplementary Fig. S2.

Intriguingly, 8 (80%) of posttreatment, but not pretreatment tumors exhibited substantial necrosis and/or acute or chronic inflammation (Table 1). Multiplex immunohistochemical assays were used to identify cells expressing CD8, CD68, CD20, or MPO markers that respectively detected cytotoxic T cells, macrophages, B cells, or neutrophils. CD68+ and MPO+ cells with increased numbers of CD20+ and CD8+ cells appeared in posttreatment tumors (Fig. 5B), consistent with inflammatory responses. These responses were correlated with the presence of KRAS mutations in these tumors (Table 1). Esophageal cancer cases were eligible for this trial to assess vorinostat treatment effects beyond lung cancers. All 4 NSCLC cases with KRAS mutations had necrosis, and acute or chronic inflammation in posttreatment tumors. These posttreatment tumors had a decline in Ki-67 expression, 2 had a decrease in cyclin E, and 1 case had a decrease in cyclin D1 expression. Thus, responses were independent of KRAS mutations in tumors (Table 1).

**Vorinostat pharmacokinetics**

Vorinostat levels in resected tumors were quantitated in evaluable patients. Pharmacokinetic analyses confirmed serum and tumor concentrations above the detection limits of the assay in 8 (80%) and 7 (70%) patients.
respectively. One other patient took vorinostat for only 3 days with surgery carried out 4 days later; the other patient took the study drug at 25% of the dose (100 mg) for 7 days, indicating that reduced dose or duration of treatment decreased vorinostat concentrations. There was interindividual variation in serum vorinostat concentrations [range, 7.3–192.4 ng/mL; coefficient of variation (CV) 95%] and intratumoral levels (range, 15.0–80.3 ng/g; CV 59%). The highest intratumoral drug level (34,024 ng/g) was in the esophageal cancer case, likely from contaminating vorinostat in the mucosa. Lung tumor concentrations detected were similar to those observed in patient-matched serum. This is consistent with the reported volume of distribution of approximately 60 to 100 L/m², close to 1 L/kg (39). The relationship between vorinostat pharmacodynamics and intratumoral vorinostat measurements is presented in Table 1. Changes in multiple biomarkers were observed across the range of tumor vorinostat levels (range, 15.0–34024 ng/g).

Discussion

The study compared responses with the HDACi vorinostat in genetically engineered mouse models that mimic clinical lung cancers with results from a window of opportunity trial in patients with lung cancer. Figures 1 and 2 and Supplementary Fig. S2 revealed that murine transgenic lung cancer cell lines responded to this HDACi with dose- and time-dependent growth inhibition, increased apoptosis and histone acetylation and changes in expressed cell-cycle regulators: p27, cyclin D1, cyclin E, and other vorinostat biomarkers. Human lung cancer cell lines exhibited similar effects (Fig. 3 and Supplementary Fig. S2). Notably, vorinostat reduced lung cancer formation in a murine syngeneic transplantation model and decreased cyclin D1 and cyclin E expression in transgenic lung cancers (Fig. 4). Thus, prior work (40) was extended by showing that vorinostat induced similar changes in expressed cell-cycle regulatory proteins in vitro and in vivo. Vorinostat chemopreventive effects in murine carcinogen-induced lung tumors (41) were confirmed and extended here using a transplantable lung cancer model (Fig. 4).

Clinical implications of this work were explored in a vorinostat window of opportunity trial. Pharmacodynamic responses in Fig. 5 and Table 1 included changes in expressed cell-cycle regulators and reduced proliferation of human tumors despite the presence of KRAS mutations. Treatment of KRAS harboring lung cancer cases is an unmet clinical need (14). Intriguingly, vorinostat induced inflammation and necrosis in posttreatment...
tumors. Combining an agent that engages necrotic or inflammatory responses might augment clinical activity of vorinostat or another HDACi.

Differences between murine and human lung cancer responses to this HDACi could relate to the timing of posttreatment biopsies. For example, increased p27 levels and apoptosis were early indicators of vorinostat responses in cultured lung cancer cells (Figs. 1–3). Yet, the posttreatment biopsies in Table 1 were obtained at later time points. The presence of necrosis in the post-treatment human tumors implied that a wave of apoptosis and inflammation preceded the onset of necrosis. These findings indicate the value of integrating results from genetically engineered mouse models with those from a mechanistic clinical trial. This coclinical trial approach extends prior work (42, 43).

### Table 1. Clinical, pharmacodynamic, pharmacokinetic, and histopathologic responses of evaluable cases accrued to the vorinostat window of opportunity trial

<table>
<thead>
<tr>
<th>Pt</th>
<th>Age/Sex/Smoker</th>
<th>Histology</th>
<th>Treatment (days)</th>
<th>Serum ng/mL Last dose (minutes)</th>
<th>Tumor ng/mg Ki-67 %decrease</th>
<th>Cyclin E/D1 %decrease</th>
<th>K-ras</th>
<th>Necrosis increase</th>
<th>Acute/Chronic Inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59/F/Current</td>
<td>ADC</td>
<td>7</td>
<td>99.2</td>
<td>279</td>
<td>80.3</td>
<td>75</td>
<td>50/0</td>
<td>2+/3+</td>
</tr>
<tr>
<td>2</td>
<td>65/F/Former</td>
<td>ADC</td>
<td>7</td>
<td>28.0</td>
<td>366</td>
<td>42.8</td>
<td>67</td>
<td>73/0</td>
<td>1+/2+</td>
</tr>
<tr>
<td>3</td>
<td>64/F/Current</td>
<td>ADC</td>
<td>7</td>
<td>42.0</td>
<td>417</td>
<td>15.0</td>
<td>88</td>
<td>33/50</td>
<td>2+/2+/1+</td>
</tr>
<tr>
<td>4</td>
<td>71/M/Never</td>
<td>ADC/BAC</td>
<td>2</td>
<td>&lt;LLQ</td>
<td>5 days</td>
<td>&lt;LLQ</td>
<td>NA</td>
<td>NA</td>
<td>0/2+/3+</td>
</tr>
<tr>
<td>5</td>
<td>73/F/Former</td>
<td>ADC/BAC</td>
<td>7</td>
<td>36.0</td>
<td>295</td>
<td>46.9</td>
<td>0</td>
<td>ND/0</td>
<td>0/1+</td>
</tr>
<tr>
<td>6</td>
<td>66/M/Current</td>
<td>SCC</td>
<td>3</td>
<td>&lt;LLQ</td>
<td>4 days</td>
<td>&lt;LLQ</td>
<td>ND</td>
<td>ND/0</td>
<td>3+/3+/0</td>
</tr>
<tr>
<td>7</td>
<td>64/M/Current*</td>
<td>Eso-ADC</td>
<td>8</td>
<td>50.5</td>
<td>500</td>
<td>34024*</td>
<td>67</td>
<td>87/0</td>
<td>0/0</td>
</tr>
<tr>
<td>8</td>
<td>69/M/Former</td>
<td>ADC</td>
<td>7b</td>
<td>7.3</td>
<td>178</td>
<td>&lt;LLQ</td>
<td>42</td>
<td>0/1+d</td>
<td>0/0</td>
</tr>
<tr>
<td>9</td>
<td>70/M/Former</td>
<td>ADC/BAC</td>
<td>9</td>
<td>192.4</td>
<td>415</td>
<td>17.0</td>
<td>60</td>
<td>NA/0</td>
<td>0/0</td>
</tr>
<tr>
<td>10</td>
<td>69/M/Current</td>
<td>ADC</td>
<td>7</td>
<td>39.1</td>
<td>330</td>
<td>42.4</td>
<td>88</td>
<td>0/0</td>
<td>1+</td>
</tr>
</tbody>
</table>

NOTE: Pharmacodynamic changes were post-versus pretreatment. The lowest level of quantitation (LLQ) was 3 ng/mL for serum and 12 ng/mg for cancers.

Abbreviations: Pt, patient; M, male; F, female; ND, not detected; NA, no cancer in the specimen; MUT, KRAS codon 12 mutation; WT, wild-type KRAS codon 12; ADC, adenocarcinoma; SCC, squamous cell carcinoma; BAC, bronchioloalveolar carcinoma.

*Esophageal cancer (Eso-ADC).

b100 mg oral vorinostat daily treatment.

cReplicate analysis confirmed this level.

dIncreased levels.

Figure 5. Pharmacodynamic effects in lung cancers treated in the vorinostat window of opportunity clinical trial. A, histopathologic and immunohistochemical profiles for Ki-67, cyclin D1, cyclin E, and p27 (arrow) in pre-versus postvorinostat treatment from a representative lung cancer case (patient 3, >200). Necrosis (arrow) was prominent in a representative posttreatment lung cancer (case 1, >100). B, postvorinostat treatment, lung cancers exhibited marked inflammatory responses. Representative cases were used to detect immune cell subset-specific markers and H&E-stained lung tumors after vorinostat treatment. Composite images and representative fields are displayed.
Both serum and intratumoral vorinostat concentrations were measured in this clinical trial. This made possible comparisons of intratumoral pharmacodynamics and pharmacokinetics. Comparable drug concentrations were needed in in vitro models and in clinical tumors to achieve pharmacodynamic responses. Intratumoral drug levels depended on the vorinostat dose and on the timing of the posttreatment biopsy relative to the last vorinostat dosage. An esophageal cancer case had the highest vorinostat level. This was likely from the mucosal presence of vorinostat after an oral dosage. Tumor-specific expression or activity of drug transporters might also contribute to changes in vorinostat levels (44). Future work should consider these possibilities and whether clinical toxicity or resistance (22–24, 44) is reduced by the HDACi dose schedule.

Vorinostat was administered to patients with lung cancer as a single agent or as a combination regimen (29–32). Clinical activity against lung cancers increased when vorinostat was combined with chemotherapy (31, 32). This prior work indicated a need to determine vorinostat pharmacodynamics in clinical lung cancers. This was addressed in this study by determining vorinostat intratumoral pharmacodynamics and pharmacokinetics in treated human lung cancers. Vorinostat exerted substantial antineoplastic effects in these cancers, including previously unrecognized ones: induced inflammation and necrosis. There is also a need to understand the basis for differences between the current and prior (29–32) vorinostat lung cancer trials.

Several explanations could account for these differences. Clinical vorinostat responses might differ in chemotherapy-resistant versus untreated cancer cases as accrued to the window of opportunity trial. Vorinostat resistance could develop after prolonged treatments (22, 24, 44). Vorinostat can induce expression of drug resistance-associated ABC transporters in tumors (44, 45). Also, p21 is involved in drug resistance and vorinostat can augment p21 expression (46, 47). After p21 knockdown, cancer cells become sensitive to vorinostat treatment (47).

Interestingly, p21 immunohistochemical expression increased in a representative postvorinostat versus pretreatment lung cancer (Supplementary Fig. S3). This tumor might exhibit vorinostat resistance. Consistent with this is the finding that temsirolimus decreased p21 expression (without affecting cyclin D1 expression); this enhanced vorinostat antineoplastic effects in mantle cell lymphoma (47). Future cancer trials should consider using combination regimens and schedules that limit onset of vorinostat resistance. Proof of concept could be established using genetically engineered lung cancer models described here with or without xenograft lung cancer models. Given the observed pharmacodynamic effects on cyclin D1 and cyclin E levels in murine and human lung cancers, combination vorinostat regimens are appealing that would repress these cyclins and blunt increased p21 expression. There is also a rationale for a regimen that combines an HDACi with a DNA damaging agent (48).

In summary, substantial antineoplastic effects were observed for the HDACi vorinostat in murine and human lung cancer cell lines as well as in murine transgenic and transplantation lung cancer models. Findings were translated into a vorinostat window of opportunity clinical trial where induced p21 and p27 and reduced G1 cyclin expression occurred in lung cancers independent of the presence of KRAS mutations. These antitumor responses were accompanied by inflammatory and necrotic changes in human tumors.

Whether vorinostat antineoplastic activities against human tumors reported here will translate into a survival advantage is the subject of future work. Vorinostat alone may not suffice in treating lung or esophageal cancers. Yet, combining vorinostat with surgery or radiation therapy and agents that reduce cyclin D1 or blunt p21 expression could augment vorinostat therapeutic activity. Intermittent vorinostat treatments might reduce its toxicity or resistance. Genetically engineered lung cancer models described here are useful to design future vorinostat trials.

Disclosure of Potential Conflicts of Interest
J.H. Beumer has employment (other than primary affiliation; e.g., consulting) with Infinity and Salix; has commercial research grants from Bristol Myers Squibb, Infinity, Spectrum, and Millennium; and has immediate family members with employment with GlaxoSmithKline. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions
Conception and design: T. Ma, F. Galimberti, K.H. Dragnev, E. Dmitrovsky
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): T. Ma, F. Galimberti, C.P. Erikmen, V. Memoli, F. Chinyeregetere, J.H. Beumer, W. Nugent, D. Johnstone, G.J. Tsongalis, K.H. Dragnev, E. Dmitrovsky
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): T. Ma, F. Galimberti, V. Memoli, J.H. Beumer, H. Li, K.H. Dragnev, E. Dmitrovsky
Writing, review, and/or revision of the manuscript: T. Ma, V. Memoli, L. Sempere, J.H. Beumer, G.J. Tsongalis, S.J. Freemantle, K.H. Dragnev, E. Dmitrovsky
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): T. Ma, L. Sempere, B.N. Anyang, Y. Guo, K.H. Dragnev, E. Dmitrovsky
Study supervision: J. DiRenzo, K.H. Dragnev, E. Dmitrovsky
Other: Provided cell lines required for execution of these experiments, J. Kurie

Acknowledgments
The authors thank Dr. Jason Sparkowski (Merck) for providing us vorinostat. The authors also thank Dr. Eugene Demidenko, Department of Community and Family Medicine at Geisel School of Medicine at Dartmouth for biostatistical consultation and Drs. Alan Eastman, Alexey Danilov, Yolanda Sanchez, and Michael Sporn (Geisel School of Medicine) for providing several antibodies.

Grant Support
This study was supported by NIH and National Cancer Institute (NCI) grants R01-CA087546 (to E. Dmitrovsky and S.J. Freemantle), R01-CA111422 (to E. Dmitrovsky and S.J. Freemantle), and U01-CA099168 (to J.H. Beumer); by a Samuel Waxman Cancer Research Foundation award (to E. Dmitrovsky); by a grant from Merck (to K.H. Dragnev, E. Dmitrovsky); and by an American Cancer Society Clinical Research Professorship (E. Dmitrovsky) provided by a generous gift from the FM Kirby Foundation. This project used the UPCI Clinical Pharmacology Analytical...
References


destabilizes transcription factor PML/RARa and inhibits the growth of acute promyelocytic leukemia. Cancer Res 2010;70:975–85.


Molecular Cancer Therapeutics

Comparing Histone Deacetylase Inhibitor Responses in Genetically Engineered Mouse Lung Cancer Models and a Window of Opportunity Trial in Patients with Lung Cancer

Tian Ma, Fabrizio Galimberti, Cherie P. Erkmen, et al.


Updated version
Access the most recent version of this article at: doi:10.1158/1535-7163.MCT-12-0933

Supplementary Material
Access the most recent supplemental material at: http://mct.aacrjournals.org/content/suppl/2013/05/16/1535-7163.MCT-12-0933.DC1

Cited articles
This article cites 47 articles, 28 of which you can access for free at: http://mct.aacrjournals.org/content/12/8/1545.full#ref-list-1

Citing articles
This article has been cited by 1 HighWire-hosted articles. Access the articles at: http://mct.aacrjournals.org/content/12/8/1545.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.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.