Ricolinostat, the First Selective Histone Deacetylase 6 Inhibitor, in Combination with Bortezomib and Dexamethasone for Relapsed or Refractory Multiple Myeloma

Dan T. Vogl, University of Pennsylvania
Noopur Raje, Massachusetts General Hospital
Sundar Jagannath, Mount Sinai Medical Center
Paul Richardson, Dana Farber Cancer Institute
Parameswaran Hari, Medical College of Wisconsin
Robert Orlowski, MD Anderson Cancer Center
Jeffrey G. Supko, Massachusetts General Hospital
David Tamang, Acetylon Pharmaceut Inc
Min Yang, Acetylon Pharmaceut Inc
Simon S. Jones, Acetylon Pharmaceut Inc

Only first 10 authors above; see publication for full author list.

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Ricolinostat, the first selective histone deacetylase 6 inhibitor, in combination with bortezomib and dexamethasone for relapsed or refractory multiple myeloma

Dan T. Vogl1, Noopur Raje2, Sundar Jagannath3, Paul Richardson4, Parameswaran Hari5, Robert Orlowski6, Jeffrey G. Supko2, David Tamang7, Min Yang7, Simon S. Jones7, Catherine Wheeler7, Robert J. Markelewicz7, and Sagar Lonial8

1Abramson Cancer Center, University of Pennsylvania, Philadelphia
2Massachusetts General Hospital, Boston
3Mount Sinai Medical Center, New York
4Dana Farber Cancer Institute, Boston
5Medical College of Wisconsin, Milwaukee
6MD Anderson Cancer Center, Houston
7Acetylon Pharmaceuticals Inc., Boston
8Emory University, Atlanta

Abstract

Purpose—Histone deacetylase (HDAC) inhibition improves the efficacy of proteasome inhibition for multiple myeloma but adds substantial toxicity. Preclinical models suggest that the
observed synergy is due to the role of HDAC6 in mediating resistance to proteasome inhibition via the aggresome/autophagy pathway of protein degradation.

Experimental Design—We conducted a phase 1/2 trial of the HDAC6-selective inhibitor ricolinostat to define the safety, preliminary efficacy, and recommended phase 2 dose in combination with standard proteasome inhibitor therapy. Patients with relapsed or refractory multiple myeloma received oral ricolinostat on days 1–5 and 8–12 of each 21-day cycle.

Results—Single agent ricolinostat therapy resulted in neither significant toxicity nor clinical responses. Combination therapy with bortezomib and dexamethasone was well tolerated during dose escalation but led to dose-limiting diarrhea in an expansion cohort at a ricolinostat dose of 160 mg twice daily. Combination therapy at a ricolinostat dose of 160 mg daily in a second expansion cohort was well tolerated, with less severe hematologic, gastrointestinal, and constitutional toxicities compared with published data on non-selective HDAC inhibitors. The overall response rate in combination with daily ricolinostat at ≥160 mg was 37%. The response rate to combination therapy among bortezomib-refractory patients was 14%. Samples taken during therapy showed dose-dependent increases of acetylated tubulin in peripheral blood lymphocytes.

Conclusions—At the recommended phase 2 dose of ricolinostat of 160 mg daily, the combination with bortezomib and dexamethasone is safe, well tolerated, and active, suggesting that selective inhibition of HDAC6 is a promising approach to multiple myeloma therapy.

Keywords
multiple myeloma; HDAC6; aggresome; tubulin; ricolinostat

Introduction

Multiple myeloma is an incurable plasma cell malignancy with a unique biology characterized by high levels of protein synthesis and consequent endoplasmic reticulum (ER) stress and activation of the unfolded protein response (UPR). Plasma cell differentiation and survival depend on UPR activation, which results in upregulation of protein degradation by the 26S proteasome. The introduction of proteasome inhibitors into the multiple myeloma therapeutic armamentarium has led to a dramatic improvement in clinical outcomes (1–5). However, despite these advances, multiple myeloma cells inevitably develop resistance to proteasome inhibition leading to disease progression.

The aggresome/autophagy pathway is a regulated degradative process for cellular proteins (6) that is activated in response to accumulation of cytosolic polyubiquitinated proteins in the setting of proteasome inhibition, serving as an alternative route for protein degradation (7) and thereby contributing to therapeutic resistance to proteasome inhibitor therapy. Histone deacetylase 6 (HDAC6) is a cytosolic microtubule-associated deacetylase that mediates trafficking of ubiquitinated misfolded proteins to the aggresome/autophagy pathway (8). Selective inhibition of HDAC6 increases α-tubulin acetylation and accumulation of ubiquitinated proteins in multiple myeloma cells, with synergistic cytotoxicity in combination with bortezomib (9). Clinical trials with non-selective HDAC inhibitors in combination with bortezomib and dexamethasone have shown improved outcomes, but also substantially increased toxicity (10, 11). The unique role of HDAC6 in
the aggresome/autophagy pathway raises the possibility that selective inhibition of HDAC6 may yield improved efficacy and reduced toxicity when combined with proteasome inhibition.

Ricolinostat (ACY-1215) is an orally available selective HDAC6 inhibitor, with preclinical data showing anti-myeloma efficacy in combination with proteasome inhibitors, mediated by inhibition of autophagic protein degradation and increased ER stress. (12, 13). We therefore conducted a first-in-human dose escalation study of ricolinostat as a single agent and then in combination with bortezomib and dexamethasone in patients with relapsed or refractory multiple myeloma. We aimed to define the dose-limiting toxicities (DLTs) and maximum tolerated dose (MTD), pharmacokinetics and pharmacodynamics of ricolinostat alone and in combination with bortezomib and dexamethasone and to define the response rate and toxicity profile of the combination regimen.

**Methods**

**Study Design**

This study was designed as a 3-part, phase 1/2, single-arm, multicenter, open-label study in patients with relapsed or refractory multiple myeloma. Parts 1 and 2 of the study employed a sequential group dose-escalation design of ricolinostat as monotherapy (Part 1) and in combination with bortezomib and dexamethasone (Part 2), with planned enrollment of up to 20 patients in an expansion cohort at the MTD. Part 3 was intended to be a Simon optimal 2-stage phase 2 trial at the MTD; however, based on the preliminary results of the part 2 expansion cohort, we did not proceed with a formal phase 2 cohort and instead enrolled an additional expansion cohort to explore a daily dose of ricolinostat.

**Population**

Patients were eligible for enrollment if they had multiple myeloma that was relapsed (progressed after the most recent therapy) or refractory (progressed on or within 60 days after completion of the most recent therapy) after at least 2 prior lines of therapy. Patients had to have received a proteasome inhibitor, an immunomodulatory drug, and an autologous stem cell transplant as part of their prior therapy, unless they were considered not to be a candidate for these therapies by their treating physician. At enrollment, patients had to have measurable disease parameters according to the International Myeloma Working Group (IMWG) Criteria (14). Patients were at least 18 years old and had a Karnofsky Performance Status of ≥70, adequate bone marrow reserve (absolute neutrophil count ≥1.0×10^9/L and platelet count ≥5×10^9/L [≥50×10^9/L in patients in whom ≥50% of bone marrow nucleated cells were plasma cells], calculated creatinine clearance ≥30 mL/min, adequate hepatic function (serum bilirubin <2.0 mg/dL, serum alanine transaminase (ALT) and aspartate transaminase (AST) <3 times the upper limit of normal [ULN]), and corrected serum calcium ≤ULN. Patients could not have received radiotherapy or systemic anticancer therapy within 2 weeks of starting therapy, autologous stem cell transplant within 12 weeks, or any allogeneic stem cell transplant or HDAC inhibitor. Patients were excluded if they had grade 2 or higher neuropathy, an active systemic infection, other active malignancies, known human immunodeficiency virus, active hepatitis B virus, or active hepatitis C virus infection.
Patients were also excluded if they had New York Heart Association Class 3 or 4 congestive heart failure, unstable angina, cardiac arrhythmia, QTcF >480 msec, myocardial infarction or stroke within 6 months, or severe hypertension, diabetes mellitus, or chronic obstructive pulmonary disease. All patients provided written informed consent prior to study participation.

Study treatment

Ricolinostat was administered as an oral liquid formulation on days 1–5 and 8–12 of each 21-day cycle. Ricolinostat was initially prepared as a 20 mg/mL solution; mid-way during the study a 12 mg/mL solution was introduced with improved stability at room temperature. Prespecified ricolinostat doses during single-agent dose escalation (part 1) were 40, 80, 160, 240, and 360 mg daily in successive cohorts. In the combination cohorts (part 2), patients received bortezomib immediately after ricolinostat on days 1, 4, 8, and 11, with dexamethasone 20 mg administered orally 30 minutes after ricolinostat on days 1, 2, 4, 5, 8, 9, 11, and 12. During dose escalation, bortezomib was administered intravenously; when data became available regarding subcutaneous administration of bortezomib, we permitted switching to subcutaneous dosing after completion of the first 3-week cycle. In cohort 4 (dose expansion at ricolinostat 160 mg daily), patients could start therapy with either intravenous or subcutaneous bortezomib administration, at the investigator’s discretion. Prespecified dose levels for the combination therapy dose escalation cohorts are shown in Table 1, with ricolinostat doses ranging from 40 mg daily (qd) to 160 mg twice daily (bid) and bortezomib administered at 1.0 mg/m² in the first combined dose cohort and the standard 1.3 mg/m² subsequently.

Endpoints

The primary endpoints in the dose escalation cohorts were identification of dose limiting toxicities (DLTs) and the maximum tolerated dose (MTD). Dose escalation occurred using a standard 3+3 design, with enrollment of 3 patients to each dose cohort, enrollment of a second group of 3 patients if 1 of the first 3 experienced an DLT, and enrollment to the next dose level permitted if fewer than 1 of 3 or 2 of 6 patients at a given dose level experienced a DLT. Toxicities were assessed using the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0. DLT was defined as one of the following events occurring during the first cycle of therapy and considered to be related to ricolinostat: grade 4 neutropenia lasting >5 days; febrile neutropenia; grade 4 nausea, vomiting, or diarrhea; grade 3 nausea or vomiting persisting >72 hours; grade 3 diarrhea persisting >48 hours; or any other ≥grade 3 non-hematologic toxicity, with the exception of hyperglycemia in diabetic patients. Thrombocytopenia was considered a DLT for patients receiving single-agent ricolinostat if it was grade 3 with grade ≥2 bleeding or grade 4, and for patients receiving combination therapy if it was grade 4 on two separate occasions unresponsive to transfusion support. The MTD was defined as the dose immediately below the dose level at which ≥2 of up to 6 patients experienced a DLT.

Responses were assessed according to standard IMWG criteria (15). The overall response rate (ORR) was defined as the sum of the rates of stringent complete response (sCR), complete response (CR), very good partial response (VGPR), and partial response (PR). The
clinical benefit rate was defined as the sum of the ORR and the rate of minimal response (MR). Response assessments (serum and urine) were conducted on day 15 of each cycle, with a bone marrow aspirate and biopsy required to confirm complete response.

Patients receiving bortezomib completed a neurotoxicity-directed questionnaire from the Functional Assessment of Cancer/Gynecology Oncology Group (FACT/GOG) survey of neurotoxicity (16, 17).

**Pharmacokinetic Studies**

Serial blood samples for pharmacokinetic assessments were collected during the first cycle of therapy. In Part 1, samples were collected before and at 0.25, 0.5, 1, 2, 4 and 24 hours after the first dose of ricolinostat, on days 4 and 8 before, 0.25 and 1 hour after the morning ricolinostat dose, on day 11 before and at 0.25, 0.5, 1, 2 and 4 hours after ricolinostat, and on day 15. In Part 2, samples were collected at the same time intervals after the administration of ricolinostat and bortezomib with additional samples obtained 6 hours after dosing on days 1 and 11. Blood (6 mL) was drawn from a peripheral vein into plastic tubes containing freeze-dried sodium heparin, mixed by inversion, and placed over ice until centrifuged (1300 g, 10 min, 4°C). Plasma was removed and stored at −80°C until assayed. The concentrations of ricolinostat and bortezomib were concurrently determined using an analytical method involving high performance liquid chromatography with tandem mass spectrometric detection. The assay was validated and applied to the analysis of study samples as recommended by the FDA Guidance for Industry: Bioanalytical Method Validation, May 2001 (http://www.fda.gov.cder/guidance/index.htm). The lower limit of quantitation for both compounds was 0.50 ng/mL. Pharmacokinetic parameters were estimated by analysis of the ricolinostat and bortezomib plasma concentration-time curves for individual patients by standard noncompartmental methods using WinNonlin Professional 5.0 software (Pharsight Corp., Cary, NC) and are reported as the geometric mean ± SD at each dose level.

**Pharmacodynamic Studies**

Blood samples for pharmacodynamic assessment of acetylated tubulin and acetylated histone levels were collected at baseline and (in part 1) 1 and 4 hours after the first ricolinostat dose or (in part 2) 0.25, 0.5, 1, 2, 4, 6, and 24 hours after the first bortezomib dose. Blood samples were cryopreserved in equal volume of freezing solution (10% dimethyl sulfoxide in PBS v/v) and stored at −80°C. Upon thaw, samples were fixed with 3.8% formaldehyde (2:1 formaldehyde/sample v/v) and stained with primary antibodies for anti-tubulin (Sigma-Aldrich, #T7451) and anti-histone H2BK5 (Cell Signaling Technologies, #2574) or isotype controls (Rabbit IgG polyclonal Control antibody, Cell Signaling Technologies, #2729; Mouse IgG2b, clone MOPC-141, Sigma-Aldrich, #M5534). Samples were labeled with secondary DyLight488 antibodies (DyLight 488 Goat anti-Mu-IgG, KPL #072-03-18-06; DyLight 488 Goat anti-Rb-IgG, KPL #072-03-15-16). The target population was labeled with anti-CD3 (PE-Cy5 Mouse anti-human CD3, clone UCHT1, eBioscience # 15-0038). Data analysis was performed by gating on the CD3+ cells in FlowJo software (Treestar, Inc., Ashland, OR) and applying an MFI statistic. Fold change of tubulin or histone acetylation was calculated by subtracting the control IgG MFI from the sample MFI and dividing the MFI of treated groups by the MFI of the control. (Fold change =
Horizontal bars represent arithmetic means. Error bars represent the standard error of the mean.

**Statistical Analysis**

The size of the dose escalation cohort was based on the conventional 3 + 3 dose-escalation design for phase 1 trials; consequently, a formal sample size estimation was not performed. All patients who received at least one dose of ricolinostat were included in the toxicity analysis. Patients who discontinued the study before the first response evaluation at the beginning of cycle 2 were considered unevaluable for response. Data were summarized using descriptive statistics. Time-to-event analysis, including progression free survival, was estimated using the Kaplan-Meier method. The MFI of pharmacodynamic samples used to calculate the fold change were assessed for statistical difference by comparing the pre-dose signal to the 1 hr post-dose signal of a given group using a paired, two tail T-test. Groups where $p \leq 0.05$ are indicated with an “*”. Groups where $p > 0.05$ are indicated by “ns” (not significant). Statistical analyses were performed using SAS version 9.3 (SAS Institute, Inc, Cary, NC) and R version 3.2.3 (R Foundation, Vienna, Austria).

**Results**

**Population**

We enrolled 15 patients to 5 single-agent ricolinostat cohorts between August 2011 and September 2012 and 57 patients to 9 combination dose cohorts between May 2012 and September 2015. Patient characteristics in the single-agent ricolinostat cohorts (Table 2) included a median age of 70 years and median number of prior regimens of 4 (range 2–11). Patient characteristics in the combination dose cohorts (Table 2) included a median age of 65 years and median number of prior regimens of 5 (range 2–12). All patients had received prior bortezomib, and 33% in the single-agent ricolinostat cohorts and 63% in the combination dose cohorts were refractory to bortezomib, respectively.

**Single-agent ricolinostat**

**Dose limiting toxicities and adverse events**—We did not observe any dose limiting toxicities during single-agent dose escalation and therefore did not identify a maximum tolerated dose. We did not explore doses higher than 360 mg qd because pharmacokinetic analysis showed evidence of a plateau in exposure at dose levels ≥160 mg, suggestive of saturable absorption of the drug (see below).

The most common adverse events observed during therapy (many attributed to disease or intercurrent illness) were renal insufficiency (33%), fatigue (27%), anemia (20%), and diarrhea (20%). Diarrhea occurred only at ricolinostat doses ≥60 mg qd. The only grade 3 or 4 adverse events assessed by the investigator as possibly related to ricolinostat were seen at doses ≥60 mg qd and were all hematologic abnormalities, including anemia (at 160 mg qd) and neutropenia and leukopenia (at 360 mg qd). Two patients experienced a serious adverse event (SAE), including Grade 5 (fatal) cardiac arrest at 40 mg qd (which occurred 27 days after study treatment was discontinued for disease progression) and an exacerbation...
of chronic pulmonary disease at 160 mg qd; neither was considered to be related to ricolinostat. No patient discontinued single-agent ricolinostat because of a treatment emergent adverse event. Serial triplicate electrocardiograms showed no evidence of QT interval prolongation.

**Response**—Of the 15 patients treated with single-agent ricolinostat, 6 had stable disease for a median of 11 weeks (range 5 – 30), and no patients had a minor or partial response (Figure 1).

**Combination dosing with ricolinostat, bortezomib, and dexamethasone**

**Dose limiting toxicities and adverse events**—The first combination cohort was expanded to six patients due to an asymptomatic increase in amylase. No other dose-limiting toxicities were observed during dose escalation. Toxicities observed during dose escalation (Table 3) were primarily low grade gastrointestinal toxicities, cytopenias, and fatigue. Based on the combined pharmacokinetic and pharmacodynamic data from the monotherapy and combination dose escalation cohorts, we enrolled an expansion cohort at the top dose of ricolinostat (160 mg bid).

Of the 21 patients treated on the 160 mg bid expansion cohort, 29% were hospitalized for reasons potentially related to diarrhea and dehydration. Because of a concern that this represented excess toxicity with twice daily dosing, we enrolled a second expansion cohort at a lower dose of ricolinostat (160 mg qd) to better understand the relationship between dose and both toxicity and efficacy. Among the 24 patients who received ricolinostat 160 mg bid (3 patients treated in the dose escalation cohort and 21 patients in the dose expansion cohort), the most common treatment emergent adverse events were thrombocytopenia (71%), diarrhea (67%), anemia (42%), fatigue (42%), nausea (38%), hypokalemia (33%), vomiting (29%), peripheral neuropathy (29%), hyperglycemia (25%), and renal insufficiency (21%) (Table 3). Because of similar pharmacokinetic exposure at doses of 240 mg daily and 160 mg daily (see below), we analyzed patients treated at these two doses as a single cohort.

Of the 20 patients receiving once daily ricolinostat at a dose of 160 mg or greater (including 3 patients each treated at 160 mg qd and 240 mg qd during dose escalation and an additional 14 patients treated at 160 mg qd during dose expansion), the most common treatment emergent adverse events were thrombocytopenia (40%), anemia (35%), diarrhea (30%), hypertension (25%), fatigue (25%), hyperglycemia (25%), renal insufficiency (25%), nausea (25%), hypophosphatemia (20%), and hyponatremia (20%) (Table 3). Similar to the single-agent cohort, we did not observe any significant prolongation of the QT interval with combination dosing.

Because the toxicity of bortezomib is greater with intravenous dosing than with subcutaneous dosing (18), we analyzed toxicity rates by route of bortezomib administration. Of the 20 subjects treated with doses of ricolinostat ≥160 mg qd in combination with bortezomib and dexamethasone, 10 received at least one full cycle of intravenous bortezomib (8 subsequently switched to subcutaneous dosing), and 9 started and continued with subcutaneous dosing. The toxicity profiles in these two groups were not significantly different (data not shown).
Responses—Of the 57 patients treated with ricolinostat, bortezomib, and dexamethasone, with a median follow-up of 5 months (range 1–22), the overall response rate (partial response or better) was 29%, and the clinical benefit rate (minor response or better) was 39% (Figure 1). Of the 20 patients receiving ricolinostat at 160 or 240 mg qd, with median follow-up of 6 months (range 1–14), the overall response rate was 37% and the clinical benefit rate was 53%. Of the 24 patients receiving ricolinostat 160 mg bid, with median follow-up of 5 months (range 1–22), the overall response rate was 25% and the clinical benefit rate was 38%.

Of the 35 bortezomib-refractory patients treated with combined ricolinostat, bortezomib, and dexamethasone, the overall response rate was 14% among all combination doses, 17% with ricolinostat 160 or 240 mg qd in combination with bortezomib and dexamethasone, and 20% with ricolinostat 160 mg bid in combination with bortezomib and dexamethasone (Figure 1).

Pharmacokinetics

Ricolinostat was rapidly absorbed following oral administration, with the maximum observed concentration in plasma (C_{max}) occurring at a median time of 1.0 hour (range, 0.25–2.0 hours) for both the initial dose and the dose given on day 11. As shown in Fig 2A, the mean C_{max} of ricolinostat increased proportionately as the dose was escalated from 40 to 160 mg with no significant increase at doses greater than 160 mg when given alone or in combination with bortezomib, suggestive of saturable drug absorption. The concentration of ricolinostat in plasma remained above the 0.50 ng/mL lower limit of quantitation of the analytical method for 24 hours after taking the initial 160 mg dose in 18 of 20 patients. Overall mean (± SD) values of the pharmacokinetic parameters for the initial 160 mg daily dose of ricolinostat for cohort 4′ were as follows (n=14): C_{max}, 610 ± 163 ng/mL; apparent biological half-life, 3.26 ± 0.46 hours; apparent oral clearance, 111 ± 25 L/h; apparent oral total body volume of distribution, 521 ± 166 L. Drug accumulation upon repeated qd (1.10 ± 0.20) or bid (1.01 ± 0.26) dosing was negligible. In addition, the mean C_{max} on day 11 for bid administration (524 ± 150 ng/mL, n=10) was not significantly different from that for qd dosing (648 ± 200 ng/mL, n=11). These findings suggest that the concurrent administration of bortezomib does not have a clinically relevant effect on the plasma pharmacokinetics of ricolinostat on days 1 or 11 (Figure 2A).

The pharmacokinetics of bortezomib were not altered by ricolinostat coadministration (Figure 2B). The observed C_{max} of bortezomib occurred in the first plasma sample collected 15 minutes after i.v. injection of the drug, with few exceptions, for the doses given on days 1 and 11. The mean C_{max} of bortezomib given in combination with varying doses of ricolinostat was comparable in each cohort of patients evaluated. The mean C_{max} of bortezomib was consistently greater for the dose given on day 11 as compared to day 1. In particular, for the patients in cohort 4′, the mean C_{max} was 24 ± 11 ng/mL (n=14) for the day 1 dose and 40 ± 15 ng/mL (n=11) for the day 11 dose. This behavior is consistent with the previously documented reduction in the clearance of bortezomib upon repeated dosing for this administration schedule (19, 20). The sampling schedule did not allow bortezomib pharmacokinetic parameters, such as the terminal phase half-life and total body clearance, to be estimated from the plasma concentration-time profiles. Nevertheless, there is no evidence
to suggest that the concurrent administration of ricolinostat affects the pharmacokinetics of bortezomib.

Pharmacodynamics

To assess the effects of ricolinostat on HDAC activity, levels of acetylated tubulin (a substrate of HDAC6) and acetylated histones (a substrate of class I HDACs) in CD3⁺ lymphocytes from peripheral blood were measured before and 1 hour after the first dose of ricolinostat. In patients treated with single agent ricolinostat, we observed an increase in acetylated tubulin (Figure 3A) in samples from patients receiving 160 mg of ricolinostat and an upward trend at the 240 mg dose level. Histone acetylation in the 160 mg dosing group was unchanged (Figure 3B), but a qualitative increase was observed at the 240 mg dose level.

In patients treated with ricolinostat, bortezomib, and dexamethasone, at ricolinostat doses of either 40, 80 or 160 mg, levels of acetylated tubulin (Figure 4A) increased one hour after the first dose. Acetylated histone (Figure 4B) was increased one hour after the first dose only at the 160 mg dose level. A ricolinostat dose of 160 mg induced similar levels of acetylated tubulin and acetylated histone as the 80 mg dose. In a subset of 14 patients receiving 160 mg of ricolinostat, we obtained both ricolinostat levels in plasma and levels of acetylated tubulin and acetylated histone at 1, 2, 4, 6 and 24 hours after the first ricolinostat dose. Levels of both acetylated tubulin and acetylated histone peaked at 1–2 hours, coinciding with the peak plasma concentration of ricolinostat at 1 hour post-dose (Figure 4C). Taken together, the single agent pharmacodynamic results suggest that ricolinostat can selectively inhibit HDAC6, and the combination therapy pharmacodynamics show a time-dependent on-target pharmacodynamic effect at the recommended phase 2 dose of 160 mg daily in combination with bortezomib and dexamethasone.

Conclusion

We have shown that the combination of ricolinostat, a selective inhibitor of HDAC6, with bortezomib and dexamethasone is safe, well tolerated, and active as an anti-myeloma regimen. Twice daily ricolinostat dosing in combination with bortezomib and dexamethasone was associated with gastrointestinal toxicity, that while not formally dose limiting is clinically relevant. However a dose of 160 mg qd on days 1–5 and 8–12 of each 21 day cycle is well tolerated and pharmacodynamically active, produces clinical responses, and is therefore the recommended phase 2 dose in combination with bortezomib and dexamethasone. Our observed responses in patients with bortezomib-refractory myeloma validate HDAC6 as a therapeutic target and suggest that combined inhibition of the proteasomal and aggresome/autophagy protein degradation pathways is a useful approach to multiple myeloma therapy. These results support the further development of ricolinostat as an anti-myeloma agent.

Our trial was initiated prior to the approval of panobinostat for the treatment of relapsed/refractory multiple myeloma. Panobinostat, a pan-HDAC inhibitor, was approved in combination with bortezomib and dexamethasone based on the results of a randomized, placebo-controlled study that demonstrated superior responses and progression-free survival.
compared to bortezomib and dexamethasone alone (11). Panobinostat is also associated with significantly increased toxicity in this combination, with levels of diarrhea (25% vs 7% grade 3/4), thrombocytopenia (35% vs 12% grade 4), and fatigue (24% vs 12% grade 3/4) that limit its clinical utility, as well as an increase in cardiac events, including arrhythmias and ischemia. The risks of severe diarrhea and cardiac events has led to a black-box warning on the FDA-approved prescribing information. The increased toxicity of panobinostat is similar to the effect of vorinostat, another pan-HDAC inhibitor, in combination with bortezomib and dexamethasone (16% vs 8% grade 3/4 diarrhea, 22% vs 8% grade 4 thrombocytopenia, and 17% vs 5% grade 3/4 fatigue), though the clinical benefits of vorinostat were much more marginal (10). Our investigation of ricolinostat is predicated on the theory that the efficacy of HDAC inhibition in combination with proteasome inhibitors is due to inhibition of HDAC6-mediated trafficking of polyubiquitinated proteins to the aggresome/autophagy pathway, while the adverse effects seen with other HDAC inhibitors are due to adverse alterations in gene expression due to inhibition of class I HDACs. Ricolinostat does retain a lower level of class I HDAC inhibition compared to pan-HDAC inhibitors, but our data support the concept that ricolinostat’s reduced inhibition of class I HDACs results in an improved therapeutic window. In subjects treated with once daily ricolinostat at or above the recommended phase 2 dose of 160 mg, our observed toxicity profile (5% grade 3/4 diarrhea, 20% grade 3/4 thrombocytopenia, and 5% grade 3/4 fatigue) compares favorably to the published toxicity rates of panobinostat and vorinostat listed above. The pharmacokinetic behavior of ricolinostat is characterized by rapid but saturable absorption after oral administration and a relatively short terminal phase half-life, resulting in negligible drug accumulation upon repeated dosing either qd or bid. The pharmacokinetics of bortezomib in our trial was consistent with previously reported findings of the drug in adult cancer patients (19, 20). There was no evidence to suggest that the pharmacokinetics of ricolinostat or bortezomib were affected by their coadministration.

Our results validate the preclinical models and mechanistic rationale for combining a selective inhibitor of HDAC6 with proteasome inhibition for myeloma therapy. HDAC6 is unique within the HDAC family in that its substrate is acetylated tubulin in the cytoskeleton rather than acetylated histones in the nucleus. When proteasomal degradation of misfolded and unneeded proteins is inhibited, those proteins are shuttled along the cytoskeleton in an HDAC6-dependent manner to perinuclear aggresomes, which subsequently fuse with degradative autophagic lysosomes (6, 7). In contrast, nuclear HDACs modulate gene expression through control of histone acetylation, and while inhibition of nuclear HDACs is an effective therapeutic strategy in other malignancies, HDAC inhibition has no single agent activity in multiple myeloma, and the role of nuclear HDACs in mediating myeloma resistance to proteasome inhibition is unclear. We therefore chose to investigate a selective inhibitor of HDAC6, though ricolinostat does retain some inhibitory activity against nuclear HDACs (21). As noted above, we observed less toxicity than described in previous reports of nonselective HDAC inhibitors in the same therapeutic combination, suggesting that inhibition of nuclear HDACs is responsible for much of the toxicity of nonselective HDAC inhibitors in this setting. We also observed responses in patients with bortezomib-refractory myeloma, suggesting that HDAC6 inhibition can overcome chemoresistance to proteasome inhibitor therapy and validating in a clinical setting the mechanistic rationale for this
combination. Our pharmacodynamic data show that when given as a single agent at clinically relevant doses, ricolinostat induces a greater change in acetylated tubulin levels (the putative on-target effect) than in acetylated histone levels, though the difference in these effects is not as clear in samples from patients receiving combination therapy. While it is possible that a more selective HDAC6 inhibitor would have an even better therapeutic index, it is also possible that retaining a smaller effect on nuclear histone acetylation is important to the clinical efficacy of ricolinostat.

Our data support the combination of HDAC6 inhibition with proteasome inhibition for multiple myeloma therapy, but other therapeutic combinations appear to have efficacy as well. Ongoing trials are exploring the combination of ricolinostat with lenalidomide and dexamethasone (NCT01583283) or with pomalidomide and dexamethasone (NCT01997840 and NCT02189343). The demonstration of clinical responses from combination ricolinostat with lenalidomide and dexamethasone in patients with multiple myeloma previously refractory to lenalidomide therapy suggests synergistic efficacy (22). The cellular mechanism underlying this synergy has not been fully elucidated, but the retention of a low and tolerable level of class 1 HDAC inhibition appears relevant (23, 24). One aspect limiting further clinical development of ricolinostat is the challenge in deriving a solid dose formulation and the observed exposure plateau. ACY-241, an HDAC6-selective inhibitor that is structurally very similar to ricolinostat, has been developed in tablet form and does not exhibit the exposure plateau observed with ricolinostat. ACY-241 is in phase 1 trials in combination with pomalidomide and dexamethasone for the treatment in myeloma (NCT02400242) (25), as well as in combination with taxanes or immune checkpoint inhibitors for the treatment of solid tumors.

Our demonstration of the therapeutic utility of combined selective HDAC6 and proteasome inhibition validates preclinical models that showed the specific role of HDAC6 in mediating autophagic protein degradation as a mechanism of resistance to proteasome inhibitor therapy. Our results also show the value of developing isoform-selective HDAC targeted therapies based on preclinical models, an approach to drug development that promises improved efficacy and toxicity. Combining selective and better tolerated HDAC inhibitors with proteasome inhibitors and immunomodulatory drugs offers the possibility of improving outcomes in multiple myeloma.

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TRANSLATIONAL RELEVANCE

Ricolinostat is the first isoform selective histone deacetylase inhibitor in human clinical trials, an approach to drug development that utilizes rational targeting to achieve an improved therapeutic index. Our data show that the combination of HDAC6-selective inhibition using ricolinostat with the proteasome inhibitor bortezomib overcomes bortezomib resistance in relapsed multiple myeloma, with a favorable safety profile that offers potential advantages compared to non-selective histone deacetylase inhibition. Our findings validate preclinical data showing that HDAC6-mediated trafficking of ubiquitinated proteins to the aggresome/autophagy pathway is a relevant alternative mechanism of protein degradation for cells exposed to proteasome inhibition and therefore a mechanism of therapeutic resistance to proteasome inhibition. Incorporation of HDAC6-selective inhibition into the anti-myeloma armamentarium therefore offers the prospect of improved myeloma outcomes.
Figure 1. Treatment responses

(A) Responses for the entire cohort of patients treated with (from left to right) single agent ricolinostat, ricolinostat with bortezomib (Bz) and dexamethasone (dex), ricolinostat ≥160 mg qd with Bz/dex, and ricolinostat 160 mg bid with Bz/dex. (B) Responses among patients with bortezomib-refractory myeloma treated with (from left to right) ricolinostat with Bz/dex, ricolinostat ≥160 mg qd with Bz/dex, or ricolinostat 160 mg bid with Bz/dex. Numbers indicate the percentage of patients in each category of response. VGPR, very good partial response; PR, partial response; MR, minimal response; SD, stable disease; PD, progressive disease; ORR, overall response rate; CBR, clinical benefit rate.
Figure 2.
Pharmacokinetics of ricolinostat and bortezomib. (A) Observed maximum concentration of ricolinostat in plasma at each dose level for patients receiving single agent ricolinostat (open markers) or combination dosing with bortezomib (closed markers). (B) Observed maximum concentration of bortezomib (given at a dose of 1.0 mg/m² in cohort 1 and 1.3 mg/m² in all other cohorts) in combination with ricolinostat (doses outlined in table 1). Markers represent data for individual patients and the horizontal bars the geometric mean of the data for each group. Circles denote levels on treatment day 1 and squares day 11.
Figure 3.
Pharmacodynamics for ricolinostat monotherapy. Peripheral blood from patients receiving dose-escalating ricolinostat monotherapy was collected and the CD3+ lymphocytes were assessed for acetylated tubulin (A) and acetylated histones (B) at 1 hour after dosing. Horizontal lines represent the arithmetic mean.
Figure 4. Pharmacodynamics of ricolinostat combination therapy. (A,B) Changes compared to baseline in levels of acetylated tubulin (A) and acetylated histones (B) in peripheral blood lymphocytes taken 1 hour after the first ricolinostat dose from patients receiving ricolinostat in combination with bortezomib and dexamethasone. Solid circles indicate patients receiving ricolinostat qd where open circles indicate bid dosing. Horizontal lines represent the arithmetic mean. (C) Changes over 24 hours after the first ricolinostat dose in peripheral blood lymphocyte levels of acetylated tubulin (red line, left axis), acetylated histones (blue line, left axis), and in plasma ricolinostat levels (black dashed line, right axis). Error bars represent the standard error of the mean.
Table 1

Phase 1b Dose escalation schema.

<table>
<thead>
<tr>
<th>Phase 1b Cohorts</th>
<th>N</th>
<th>Ricolinostat Days 1–5, 8–12</th>
<th>Bortezomib Cycles 1–5: Days 1, 4, 8, 11 Cycles 6+: Days 1, 8</th>
<th>Dexamethasone Cycles 1–5: Days 1, 2, 4, 5, 8, 11 Cycles 6+: Days 1, 2, 8, 9</th>
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<tbody>
<tr>
<td>Cohort 1</td>
<td>7</td>
<td>40 mg QD</td>
<td>1.0 mg/m²</td>
<td>20 mg</td>
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<tr>
<td>Cohort 2</td>
<td>3</td>
<td>40 mg QD</td>
<td>1.3 mg/m²</td>
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<tr>
<td>Cohort 3</td>
<td>3</td>
<td>80 mg QD</td>
<td>1.3 mg/m²</td>
<td>20 mg</td>
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<tr>
<td>Cohort 4</td>
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<td>160 mg QD</td>
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<td>20 mg</td>
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<tr>
<td>Cohort 6’ expansion</td>
<td>21</td>
<td>160 mg BID</td>
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<td>20 mg</td>
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<tr>
<td>Cohort 4’ expansion</td>
<td>14</td>
<td>160 mg QD</td>
<td>1.3 mg/m²</td>
<td>20 mg</td>
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Table 2

Patient Demographics

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<tr>
<th>Characteristic</th>
<th>Single-agent ricolinostat</th>
<th>Ricolinostat with bortezomib and dexamethasone</th>
<th>Combination therapy with ≥ 160 mg QD ricolinostat dosing (Cohorts 4, 4′, 5)</th>
<th>Combination therapy with 160 mg BID ricolinostat dosing (Cohorts 6, 6′)</th>
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<tr>
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<td>N=15</td>
<td>N=57</td>
<td>N=20</td>
<td>N=24</td>
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<td>Age, years</td>
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<td>65 (47–84)</td>
<td>65 (47–83)</td>
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<td>14 (25)</td>
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<td>8 (33)</td>
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<td>15 (26)</td>
<td>5 (25)</td>
<td>6 (25)</td>
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<td>10 (50)</td>
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### Table 3

Treatment emergent adverse events (in order of frequency of grade 3/4 events in patients receiving combined therapy with ricolinostat, bortezomib, and dexamethasone).

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>Single agent ricolinostat N=15 (%)</th>
<th>Ricolinostat with bortezomib and dexamethasone N=57 (%)</th>
<th>Ricolinostat ≥160 mg QD with bortezomib and dexamethasone (Cohorts 4, 4′, 5) N=20 (%)</th>
<th>Ricolinostat 160 mg BID with bortezomib and dexamethasone (Cohorts 6, 6′) N=24 (%)</th>
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<tbody>
<tr>
<td></td>
<td>All grades</td>
<td>Grade 3/4</td>
<td>All grades</td>
<td>Grade 3/4</td>
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<td>11 (19)</td>
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