Ricolinosist (ACY-1215), a Selective Inhibitor of HDAC6, Synergizes with Immunomodulatory Drugs (IMiDs) to Induce Apoptosis of Multiple Myeloma (MM) Cells

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Introduction

Histone deacyetylase (HDAC) enzymes represent attractive therapeutic targets in multiple myeloma (MM), but unfortunately non-selective HDAC inhibitors have led to dose-limiting toxicities in patients. Ricolinosist (ACY-1215) is a new generation, orally available HDAC inhibitor that is 11-fold selective for HDAC6, and synergizes in vitro and in vivo with bortezomib in preclinical models of MM without inducing unfavorable toxicities (Sanito et al, Blood, 2012). Consistent with these findings, ongoing Phase Ib clinical trials (NCT03327351 and NCT01583283) with ricolinosist have thus far demonstrated an excellent safety and tolerability profile (Yee, et al, ASH, 2013).

The IMiD class of drugs, including lenalidomide and pomalidomide, exhibit striking anti-myeloma properties in a variety of MM models, and have demonstrated significant clinical activity in MM patients. Prior studies have shown potential clinical activity of a combination of the non-selective HDAC inhibitor vorinostat with lenalidomide and dexamethasone in myeloma patients (Siegol, et al, Blood Cancer J, 2014). However, many patients experienced significant toxicities with this regimen that limits its clinical utility. In support of our ongoing clinical development program for ricolinosist in MM, we show here that combining ricolinosist with either lenalidomide or pomalidomide leads to synergistic decreases in the viability of MM cells, as well as increased levels of apoptosis and cell cycle arrest in vitro. At the molecular level, MM cells are known to be dependent on expression of the MYC and IRF4 transcription factors. Both ACY-1215 and IMiDs as single agents reduced expression of the critical genes MYC and IRF4, which were reduced even further by combination treatment. Further, the combination of ricolinosist, lenalidomide, and dexamethasone was well tolerated in vivo in mice with no overt evidence of toxicity. By demonstrating that a selective inhibitor of HDAC6 synergizes with IMiDs while maintaining an improved safety profile, these results provided a rational basis for the clinical development of the all oral combination of ricolinosist and lenalidomide plus dexamethasone in an ongoing Phase Ib clinical trial for the treatment of MM.

HDAC Inhibitor Selectivity Profiles

Figure 1 - Isoform selectivity profiles of HDAC inhibitors currently under clinical investigation, as well as the highly selective HDAC6 inhibitor ACY-775. (A) Heat map of relative IC50 values for each compound across HDAC-9. Ricolinosist (ACY-1215) is approximately 11-fold selective for HDAC6 over HDAC1/2, and 1500-fold selective over HDAC3. SAHA + vorinostat; LBH-589 = pomalidomide; MS-275 = entinostat

Selective HDAC6 Inhibition Reduces MM Cell Growth

Figure 2 - Selective inhibition of HDAC6 by ricolinosist reduces the growth of MM cells. (A) MM1.s cells were exposed to ricolinosist (10 nM) plus pomalidomide (100 nM) for 1-7 days and cell growth was assessed by mTS. (B) Ricolinosist (+) strongly reduced cell growth compared to pomalidomide (○) and ricolinosist alone (□). (C) Induction of apoptosis was assessed in H929 cells by binding of Annexin V and cellular permeability to propidium iodide after 7 days exposure to increasing doses of ricolinosist. At doses of ricolinosist exhibiting selective inhibition of HDAC6 (≥ 2 μM), cell viability was decreased by only minimal induction of apoptosis, while at non-selective doses gross cytotoxicity was observed. (D) MM1.s cells were exposed to ricolinosist alone (□) or in combination with either pomalidomide (○) or lenalidomide (●). Significant decreases in cell growth were observed as early as 5 to 7 days following 10 nM ricolinosist plus 1 μM pomalidomide or lenalidomide.

Ricolinosist Increases Apoptosis in Combination with IMiDs

Figure 5 - Treatment of MM cells with ricolinosist plus IMiDs causes increased cellular apoptosis. H929 [5x105] (A) and MM1.s [1x106] (B) myeloma cells were exposed to drug for 3-7 days and apoptosis was assessed by measuring Annexin V binding and cellular permeability to propidium iodide. Cells were treated with DMSO, ACY-1215 (2 μM), lenalidomide (1 μM), pomalidomide (1 μM), or combinations of ACY-1215 with either IMiD, and the relative fraction of apoptotic cells was determined.

Combination Treatment Reduces MYC and IRF4 Expression

Figure 6 - The expression of MYC and IRF4 are decreased by treatment with ricolinosist and IMiDs. H929 cells were treated with DMSO, ACY-1215 (2 μM), lenalidomide (1 μM), pomalidomide (1 μM), or combinations of ACY-1215 with either IMiD, and quantitative PCR was used to assess the relative transcript levels of MYC (A) and IRF4 (B) as MM cells were previously shown to exhibit dependence on both transcription factors (Nature, 454, 2010). The decrease in MYC and IRF4 expression was confirmed at the protein level by immunoblot after 48 h of treatment (C). Consistent with enhanced induction of apoptosis, increased cleavage of PARP was seen after combination treatment (arrow). Inhibition of HDAC6 by rocolinosist was confirmed by the detection of hyperacetylation of α-tubulin, and treatment with IMiDs resulted in the degradation of IκBα.

Combination Treatment Is Well Tolerated In Vivo

Figure 7 - Treatment with ricolinosist plus lenalidomide is well tolerated in vivo. SCID-beige mice were treated with vehicle, ACY-1215 alone (100 mg/kg PO BD), lenalidomide (15 mg/kg IP QD) plus dexamethasone (5 mg/kg IP QD), or the triple combination of lenalidomide, dexamethasone, and ACY-1215. Percent body weight change was determined relative to the start of dosing, and the mean change ± SD is plotted. All treatments were dosed five days per week for 3 cycles, and all were well tolerated with no overt evidence of toxicity and complete recovery after minimal body weight loss.

Conclusions

- Treatment of MM cell lines with ricolinosist, a selective inhibitor of HDAC6, in combination with either lenalidomide or pomalidomide significantly reduces cellular viability.
- Combination treatment leads to increased cell cycle arrest and increased apoptosis that accumulates with prolonged exposure to clinically relevant doses of each agent.
- Both ricolinosist and IMiDs as single agents reduce expression of the critical genes MYC and IRF4, which are reduced even further by combination treatment. MM cells exhibit dependence on both MYC and IRF4, and thus this finding may explain the synergistic relationship between these agents. The molecular mechanism underlying this effect is currently under investigation to determine if there is a contribution from lower level of inhibition of Class I HDACs.
- Treatment with the combination of ricolinosist, lenalidomide, and dexamethasome is well tolerated in vivo, as well as in patients (Yee, et al, ASH, 2013).

Collaborations Welcome!

Acetylon welcomes collaborations to better understand HDAC biology and to expand the therapeutic utility of HDAC inhibitors for patients with unmet medical needs. If you believe your research may benefit from compounds that selectively target HDACs, then please contact us at: qualey@acetylon.com. For an electronic copy of this presentation scan the QR code below.

Conflict of Interest

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