

Jennifer Amengual, MD
Mentor: Owen O'Connor, MD, PhD

**Therapeutic Targeting of the Unfolded Protein Response (UPR) with selective HDAC6 inhibitor (ACY1215) for the Treatment of Lymphoma:
Understanding the Relationship Between UPR and Apoptosis**

Abstract:

The emergence of epigenetic therapies has identified histone deacetylase (HDAC) inhibitors as effective therapeutic agents for the treatment of refractory lymphoma. While pan-class I/II HDAC inhibitors have led to treatment ramifications, recently goals have shifted to the development of discrete HDAC selective inhibitors to further define and better target pathways germane to specific subtypes of lymphoma. We have investigated the potential therapeutic impact of the selective HDAC6 inhibitor ACY-1215, in a panoply of lymphoma cell lines. HDAC6 is a class IIb histone deacetylase that binds polyubiquitinated, misfolded protein aggregates and facilitates their transport to the aggresome. The aggresome then sequesters these aggregates for degradation. The aggresomal pathway is proteasome-independent and is a key outlet for the unfolded protein response (UPR). Inhibition of HDAC6 with ACY-1215 leads to an accumulation of misfolded proteins, activates the UPR stress response and ultimately induces apoptosis through induction of CHOP. We evaluated the single agent activity of ACY-1215, its synergistic potential when combined with bortezomib, and the mechanism of action of this combination in lymphoma with respect to inhibiting two separate protein degradation pathways. Treatment of the Diffuse Large B-cell Lymphoma cell lines OCI-Ly10 and Su-DHL6 with ACY-1215 led to acetylation of α -tubulin, increased poly-ubiquitinated proteins, GRP78, p-IRE1, p-eif2 α , and spliced XBP-1 as detected by immunoblotting. These effects were enhanced by treatment with bortezomib. This confirms that accumulation of misfolded proteins activates the UPR response triggering apoptosis, and substantiates blocking two protein degradation systems simultaneously. The UPR pathway is known to induce apoptosis via CHOP upregulation of JNK and BIM. Notably, some cell lines demonstrated activation of the apoptotic cascade with cleaved PARP and caspase prior to UPR activation, indicating that ACY-1215 may have other mechanisms of action. In addition, lymphoma cell lines not “primed” for apoptosis, expressing high Bcl2 (anti-apoptotic protein) and low BIM (pro-apoptotic protein), responded poorly to HDAC6 inhibition. If activation of the Unfolded Protein Response and Apoptotic Pathways could be measured simultaneously in real-time in lymphoma cells, then the sequence and interaction of these two pathways could be determined with respect to treatment with the HDAC6 inhibitor ACY-1215. Identification of “primed” cell lines may predict response to therapy, and thus may be used as a biomarker allowing for a tailored approach to treatment of lymphoma patients with ACY-1215.