Histone Deacetylase 6 (HDAC6) As A Therapeutic Target in Chronic Lymphocytic Leukemia

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Background

Chronic lymphocytic leukemia (CLL) is the most prevalent leukemia in the western world and is characterized by an accumulation of CD5+ B cells in circulation and lymphoid tissue. There is a need to identify novel therapies for CLL patients: Partial responses and relapsed/refractory disease are currently prevalent even with administration of clinically available therapeutic regimes and older, weaker patients cannot tolerate harsh chemotherapy.

Histone Deacetylase Inhibitors have a potential role in modulating the immunobiology of CLL. HDAC6 may therefore represent viable targets to develop new immunomodulating therapies for CLL.

In this study, we aimed to investigate the role of histone deacetylase 6 (HDAC6) in immunobiology of CLL and determine how HDAC6 may regulate malignant B cell survival pathways. Further, we aimed to determine efficacy of HDAC6 inhibition in a CLL murine model as a single agent.

Abstract

Our lab has demonstrated that expression of HDAC6 is increased in primary CLL cell lines, primary CLL patients’ samples & murine model compared to normal controls at both transcript and protein levels. Study with selective HDAC6 inhibitor (ACY-738) showed dose-dependent cell kill in CLL cell lines. Reduced growth kinetics and differences in viability, proliferation and apoptosis were also noted after treatment with HDAC6 inhibitors. These results prompted us to examine the role of HDAC6 in CLL biology and determine whether HDAC6 could be an appropriate therapeutic target for treatment of this disease.

Mechanistically, we found that HDAC6 silencing reduced signaling of B-cell receptor (BCR) and PI3K-AKT pathways, both constitutively activated and crucial for survival in malignant B cells. Next, HDAC6 inhibition or knockdown modulated CLL immunobiology as follows: 1) Reduced secretion of interleukin-10, an important cytokine in contributing to malignant B cell survival. 2) Increased MHCII expression on CLL cells leading to increased visibility of malignant B cells to helper T cells, eliciting a greater type 1 allogeneic T cell response. 3) Reduction of co-inhibitory molecule PD-L1 expression on CLL cells, which may counter immune-evasion strategies of the malignant cells. These immunomodulating effects of HDAC6 inhibition seem to reinstate a beneficial immune response to malignant cells.

To determine in vivo effects of HDAC6 inhibition we utilized the euTCL1 and euTCL1-HDAC6KO murine CLL models. Interestingly, we demonstrated overall survival advantage, reduction in tumor burden, reduction in PD-L1 expression and reduction of immunosuppressive regulatory T cells.

Materials & Methods

In vivo: CLL cell lines Mec2 and OSU-CLL were plated with HDAC6 inhibitor (ACY-738) and Cell Tax assay was used to determine viability. Phospho-specific antibodies were used to detect signaling proteins via immunoblotting. RNA-seq was performed on RNA extracted from isolated CLL B cells. Flow cytometry was performed to detect expression of immunoregulatory cell surface markers and cell cycle on an LSRII.

In vivo: Aged euTCL1 and euTCL1-HDAC6KO mice which spontaneously develop CLL were treated systemically with ACY-738 at 25mg in kinase. An accelerated CLL model was used to confirm findings where 25x10^6 leukemia splenocytes were transferred to C57Bl/6 wildtype mice followed by drug treatment as described above. Disease burden was quantified in circulation and spleen at end point with flow cytometry. Immunophenotyping was also performed by flow cytometry to quantify ratios of immune cells and functional markers in the CLL microenvironment.

Conclusions & Acknowledgements

Collectively our data confirms the importance of HDAC6 to CLL disease progression. We have found that HDAC6 inhibition regulates CLL immunobiology to deter tumor cell immune evasion mechanisms and reinvigorate a beneficial immune response against CLL disease. Results from our preclinical models suggest that therapy with HDAC6 inhibitor reduces disease progression likely via alteration of BCR signaling survival signals. As such, HDAC6 represents a viable therapeutic target in CLL as a single agent. In addition, we are currently exploring combinatorial effect of HDAC6 inhibition with several approved therapies to impact durable response and possibly overcome resistance disease. This target may therefore be developed with the intention to treat relapsed/refractory CLL patients or weaker, aged patients.

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