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. HDACs 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. Studies 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 inhibitor. 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 reinvigorate 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 vitro: CLL cell lines Mec2 and OSU-CLL were plated with HDAC6 inhibitor (ACY-738) and Cell Tox 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 25mpk in chow. An accelerated CLL model was used to confirm findings where 25x10^6 leukemic 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.

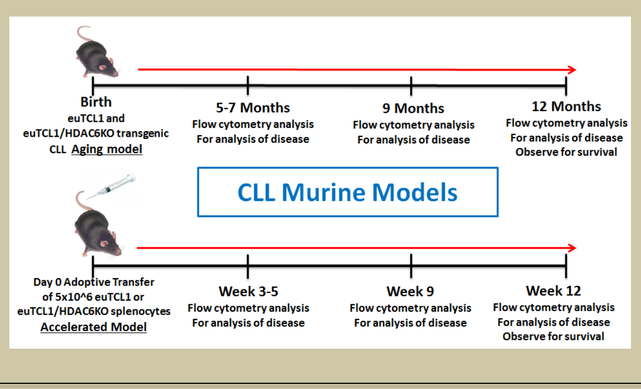

Figure 1

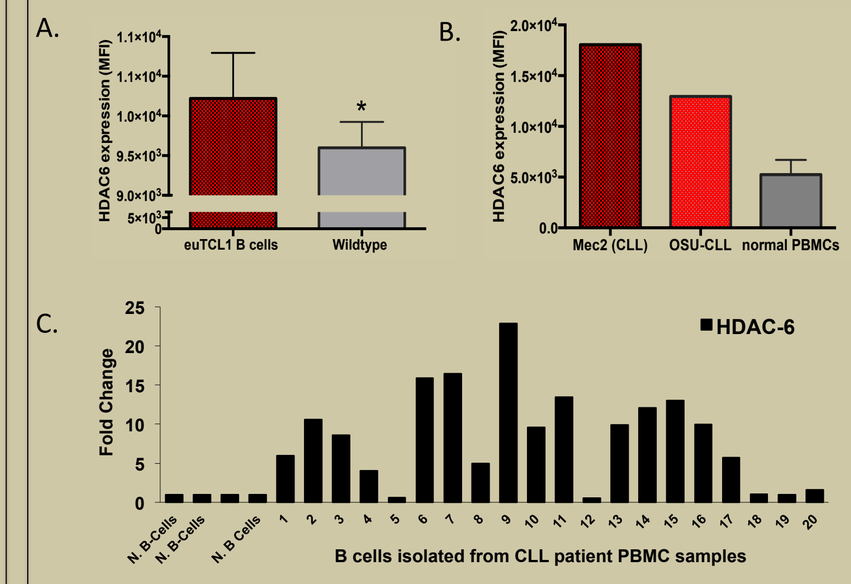

Figure 1. HDAC6 is over-expressed in CLL. HDAC6 protein expression was quantified by flow cytometry using anti-HDAC6-AF488 antibody in B cells of euTCL1 transgenic CLL murine model or wildtype mice (A) and CLL cell lines or normal peripheral blood mononuclear cells (PBMCs, n=3) (B) mRNA expression of HDAC6 was also quantified in a cohort of primary CLL patients' samples, n=20 or normal PBMCs, n=4 (C).

Figure 2

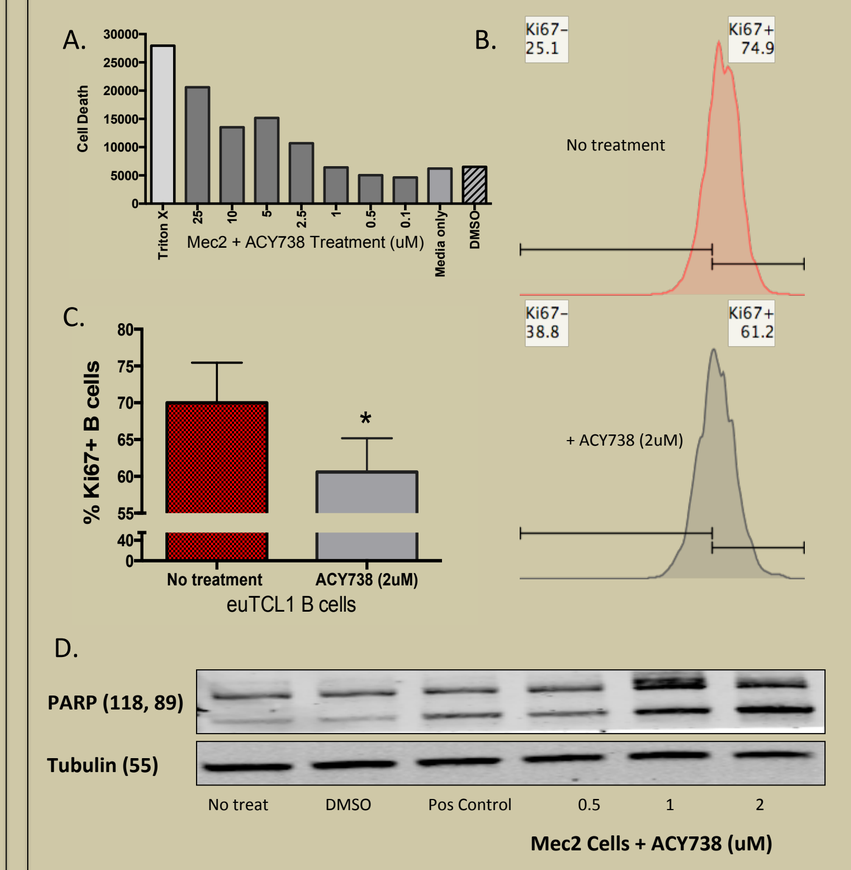

Figure 2. Selective HDAC6 inhibition with ACY738 reduces viability, proliferative capacity and induces apoptosis in CLL B cells. Cell death was measured via fluorescence in Cell Tox assay after treating Mec2 cell line or primary CLL patients' samples with ACY738 in increasing doses (A). Ex vivo treatment with ACY738 for 24h reduced proliferative capacity of euTCL1 B cells by ~10% after IgM-stimulation as demonstrated by Ki67 staining (B-C). Apoptosis is dose-dependently induced in Mec2 cells upon ACY738 treatment as demonstrated by PARP cleavage via immunoblotting (D).

Figure 3

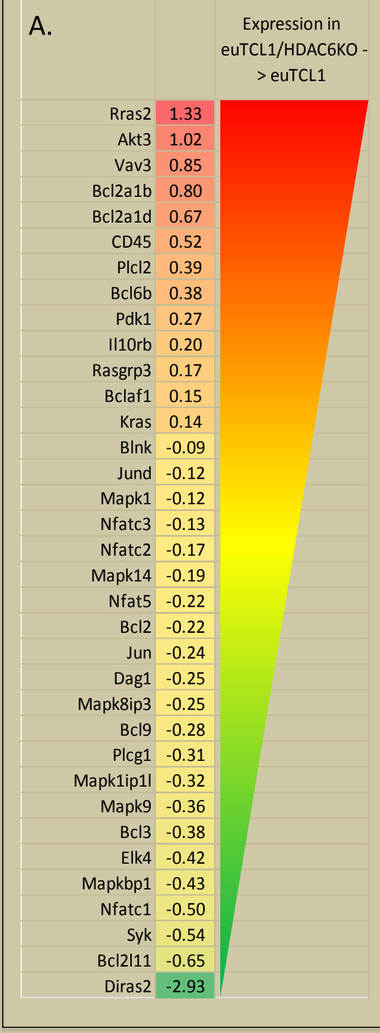

Figure 3. Genetic HDAC6 silencing alters activation of BCR signaling pathways in CLL. mRNA was isolated from B cells and RNA-seq analysis was performed followed by pathway analysis. Shown here are differentially expressed molecules involved in BCR signaling comparing euTCL1-H6KO mice to euTCL1 mice (A-B). Immunoblot showing reduced phosphoryation of BTK, AKT and ERK proteins after stimulation in B cells of euTCL-H6KO mice compared to euTCL1 mice (C).

Figure 4

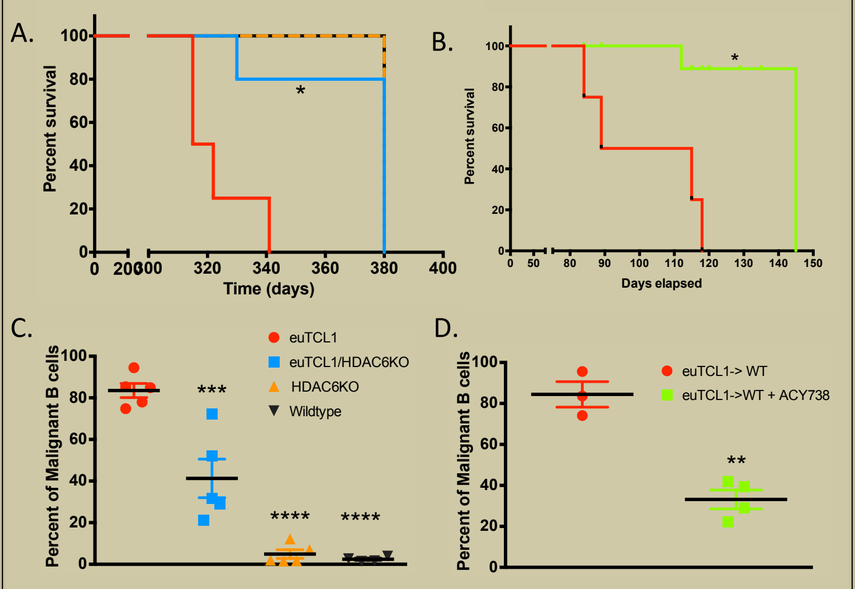

Figure 4. Pharmacological or genetic HDAC6 ablation prolongs survival and reduces tumor load in a murine model of CLL. Age-matched mice of indicated strains were followed over time and survival was recorded, n=5 per group. *Comparing euTCL1 to euTCL/HDAC6KO group (A). Survival was followed for the accelerated CLL murine model with or without ACY738 treatment, n=4 per group. (B) Tumor load was quantified by flow cytometry. Graph displays percent of malignant B cells in spleen at end point for indicated groups (C-D).

Figure 5

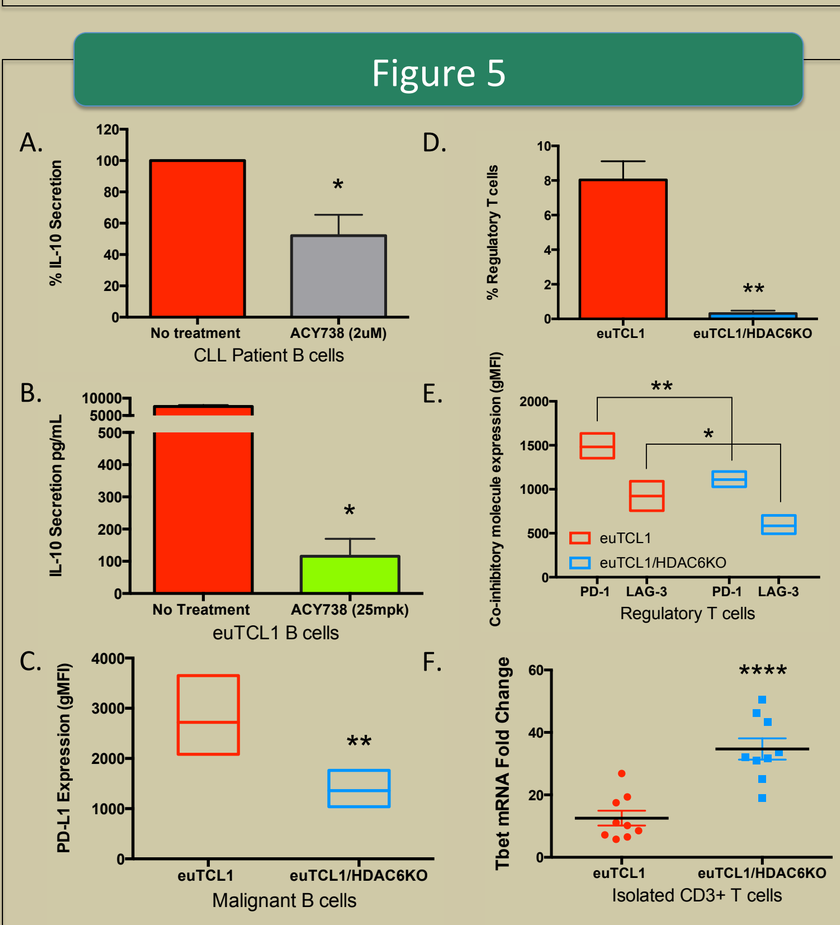

Figure 5. HDAC6 inhibition alleviates tumor-induced immune suppression in the CLL murine model. Secretion of suppressive cytokine IL-10 is reduced after ACY738 treatment of CLL patient B cells *ex vivo* and euTCL1 mice *in vivo* (A-B) Expression of inhibitory molecule PD-L1 is decreased on euTCL1-H6KO B cells (C) Total Treg % (D) and expression of co-inhibitory molecules is decreased on Tregs in euTCL-H6KO mice (E). Transcription of Tbet is increased in euTCL1-H6KO CD3+ T cells indicating greater TH1 response post-stimulation (F).

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 CLL 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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