Targeting Epigenetics through Histone Deacetylase Inhibitors in Acute Lymphoblastic Leukemia

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Abstract

Epigenetics play a critical role in controlling normal gene expression and altered epigenetics can lead to abnormal cellular differentiation, proliferation and survival. Acute lymphoblastic leukemia (ALL) is the most common malignancy in children and is characterized by numerous epigenetic abnormalities. These epigenetic changes correspond to repressed activity of some genes and inappropriate activation of others. In contrast to genetic alterations stemming from mutations, deletions or translocation, epigenetic changes are relatively reversible when treated with certain small molecule-based anticancer agents. Histone deacetylase inhibitors (HDI) are a class of drugs capable of modifying the epigenetic status of ALL cells. Several recent preclinical and clinical studies have demonstrated the potential of HDI as therapeutic agents in ALL. This review summarizes recent studies on (1) the principles of epigenetics and their importance in ALL tumorigenesis; (2) the structure, mechanism of action and anti-tumor activity of HDI; (3) the first comprehensive summary of data from preclinical and clinical studies for HDI as the therapeutic agents for ALL; and (4) novel directions for future research on HDI and ALL.

Keywords

Leukemia; epigenetics; histone deacetylase inhibitors; therapeutic agent; anticancer agent; small molecule inhibitors

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common malignancy in children and is characterized by the clonal expansion of blast cells of the hematopoietic compartment [1]. These blast cells present with a wide array of molecular aberrations and phenotypic markers and require distinct treatment modalities. With current treatment protocols, a positive response is seen in more than 80% of these patients [1]. However, the cure rate in infants, adolescents as well as adult patients remains significantly lower [2]. To improve the efficacy of cancer therapies, clinicians are shifting from pan therapies to personalized remedies by

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stratifying patients according to the genetic signature of their tumors. A number of studies have investigated the critical growth regulatory pathways in ALL cells with the aim of identifying novel targets and therapeutic agents for more effective treatment strategies. This approach has led to the development of several exciting drugs such as Imatinib which inhibits constitutively active BCR-ABL kinase and successfully treats Philadelphia-chromosome positive leukemias [3–6].

It is becoming clear that cancer development may also be influenced by inheritable gene expression patterns that are not directly linked to DNA sequence alterations. Such epigenetic mechanisms are often involved in the regulation of chromatin packing as well as in the activation of promoters of various genes that are instrumental in the process of malignant transformation [7]. In contrast to genetic lesions, epigenetic changes are relatively susceptible to small molecule-based anticancer agents. Histone deacetylase inhibitors (HDI) are a class of drugs capable of modifying the epigenetic status of ALL cells. Thus, several preclinical and clinical studies have examined HDI as potential therapeutic agents in ALL. This review summarizes recent studies on the principles of epigenetics and their importance in ALL tumorigenesis. Furthermore, we will discuss the structure, mechanism of action and anti-tumor activity of HDI and provide the first comprehensive summary of data from preclinical and clinical studies of new HDI as therapeutic agents for ALL. Finally we will identify novel directions for future research on HDI and ALL.

Epigenetics

A historic landmark was achieved in 2001 when the International Human Genome Sequencing Consortium published a draft sequence of the human genome [8]. In the following decade it has become clear that genes alone are not sufficient to explain human development, physiology, and medicine. Epigenetics has evolved as a bridge between genotype and phenotype. Epigenetics describes phenomena that change gene function without changing the underlying DNA sequence [9].

Within the cell, epigenetic control of gene function occurs through chromatin organization. In the eukaryotic cell there is roughly 2 meters of DNA [10] that must be organized in order for the cell to function correctly. Chromatin is the structural polymer that is created when DNA wraps itself around histones to form an ordered ‘beads on a string’ structure. These “strings” can then be further folded into higher-order chromatin. There are numerous proteins besides histones that are contained in chromatin and these proteins play a role in determining the level of chromatin coiling. Within each cell, different regions of chromatin have different levels of compaction. This compaction level determines the transcriptional activity of the region. For instance, euchromatin is chromatin that is relatively uncoiled and therefore accessible to proteins that regulate genetic activity. In contrast, heterochromatin remains tightly coiled and therefore, is mostly genetically inactive.

It is now clear that chromatin structure is dynamic. In order for critical processes such as DNA replication, transcription, repair and recombination to occur, the chromatin structure must shift. This shift can result from biochemical modifications of the DNA or of the protein complexes that organize chromatin and thereby alter gene expression [11]. These modifications can include: acetylation or methylation of arginines and lysines; ubiquitylation

or sumoylation of lysines; phosphorylation of threonines or serines; and ribosylation. Most of these post-translational modifications occur on the carboxy-terminal and amino-terminal histone tail domains but several modifications of the central histone domains have also been uncovered [12]. Of all these histone modifications, acetylation is one of the most heavily studied.

The acetylation level of histones is determined by the balance between the actions of histone acetyltransferases (HATs) that acetylate histones and histone deacetylases (HDACs) that remove acetyl groups from histones. A shift in the balance of activity between these two enzyme groups determines the transcription of DNA. Acetylation of histones will neutralize the positive charge of lysine residues and disrupt histone interaction with negatively charged DNA. Therefore, acetylation creates a chromatin structure that promotes the recruitment of other histone-modifying and chromatin-remodeling enzymes that support transcriptional activation [13]. A shift to deacetylated histones allows enzymes called histone methyltransferases (HMT) to add a methyl group to the cytosine in a cytosine guanine pair (CpG) in the DNA. Methylated DNA may then form highly repressive structures, such as heterochromatin.

CpG pairs often combine to form CpG islands. CpG islands are found around gene promoters and also in the highly repetitive elements of noncoding DNA [14]. In healthy cells CpG islands in the noncoding DNA are densely methylated while most CpG islands around gene promoters are hypomethylated. However, when a cell undergoes malignant transformation the global methylation pattern reverses such that CpG islands of noncoding regions become hypomethylated and those of gene promoters become hypermethylated [15]. Aberrant methylation of promoter-associated CpG islands can result in the silencing of tumor suppressor genes and lead to cancer formation or progression, and therefore, epigenetic alterations may play an important role in the development of ALL. For an excellent review of how epigenetic changes contribute to cancer formation and can be targeted therapeutically (see reference)[11].

Aberrant Epigenetics in Acute Lymphoblastic Leukemia

Analyses of single genes and entire genomes have demonstrated that aberrant epigenetics are common in both adult and pediatric ALL (Table 1). It is believed that methylation of multiple gene promoters leads to down-regulation of tumor suppressor genes or pathways thereby promoting the formation and progression of ALL [16]. Methylation profiles have been identified that distinguish ALL cells from normal cells [17, 18] and from the other leukemia cell types including acute myeloid leukemia (AML) [19], chronic lymphocytic leukemia (CLL) [20], and myeloid cell leukemia (MCL) [20]. Epigenetic changes can also aid in the differentiation of different subtypes of ALL. For example, the methylation status of DDX51 alone is able to differentiate patients with B-cell and T-cell ALL [18]. For an extensive review of epigenetic alterations in ALL and their translational potential (see reference) [21].

Mixed lineage leukemia (*MLL*) gene-rearranged ALL provides an exciting example of the importance of epigenetics in ALL. *MLL* translocations are found in more than 70% of infant leukemia cases [22] and in approximately 5% of ALL cases [23]. Patients with *MLL*-

rearranged ALL have a far worse prognosis than patients with other ALL subtypes [24]. It appears that the *MLL* protein MLL-AF4 can deregulate gene expression by recruiting the histone H3 lysine79 (H3K79) methyltransferase DOT1L. Methylation of H3K79 by DOT1L is increased at many loci in leukemia cells from both MLL-AF4 ALL murine models and human patients. This elevation in H3K79 methylation correlates with reduced expression of genes that have this histone modification, implying that altered epigenetics may be a potential therapeutic target in the treatment of *MLL*-rearranged ALL [25].

Further evidence for the importance of epigenetic alterations in ALL comes from a recent study by Kuang *et al.* (2008) [17] that used a MCA/RDA and a MCA/DNA promoter microarray to identify genes that may have aberrant DNA methylation in ALL. Both techniques independently identified a set of thirty-one genes as aberrantly methylated in ALL. Only 15 genes of these genes, however, were methylated in primary ALL samples, implying that inactivation of these 15 genes may play a key role in ALL. Certainly, analysis of gene expression revealed an inverse relationship with methylation in primary ALL samples [17]. Furthermore, those patients with methylation of more than four genes appeared to have a significantly lower overall survival. The link between increased gene methylation and diminished prognosis has been confirmed by several other studies [26, 27].

The clinical importance of epigenetics has been implicated in a recent study [28] that linking epigenetic modifications with increased risk of relapse in ALL patients. Milani's group identified 20 DNA methylated genes that predicted relapse of leukemia. Intriguingly, methylation of some of the genes identified in their study has actually been associated with favorable prognosis in patients. Specifically, high methylation levels of ROR1 contributed to a lower probability of relapse in B-cell precursor (BCP) ALL. Furthermore, high methylation levels of NOTCH3 contributed to a lower probability of relapse in t(12;21) ALL. Consistent with the earlier finding of an inverse relationship between gene expression and methylation in primary ALL samples [17], the expression levels of ROR1 and NOTCH3 inversely correlated with methylation levels [28]. Thus, epigenetic analysis has the potential to improve stratification of ALL patients.

It is important to note that different studies have used different techniques to determine methylation status, and the criterion for "methylation positivity" also differs between studies. Further research could lead to a more efficient universal methylation criterion that will shed greater light on the importance of epigenetic modifications to certain genes in ALL.

Although most studies have focused on methylation of histones to assess the importance of epigenetics in ALL, new studies point to the importance of acetylation as well. In newly diagnosed ALL patients without poor risk cytogenetics a higher level of histone H4 acetylation was associated with increased rates of complete remission, extended relapse-free survival, and better overall survival [29].

Despite some limitations, current studies clearly show that epigenetic modifications in ALL are prevalent and have important pathogenic, prognostic and therapeutic significance. Certainly, epigenetic alterations can be reversed pharmacologically unlike the genetic

alterations that are also prevalent in cancer. This exciting prospect has attracted significant research and investment.

PHARMACOLOGICAL MODIFICATIONS OF EPIGENETICS BY HDAC INHIBITORS

The formation of repressive epigenetic structures requires the actions of HDAC and HMT [30]. Therefore, much effort has gone into developing DNA methyltransferase inhibitors (DMTi) and histone deacetylase inhibitors (HDI). Indeed, targeting either HDAC or HMT for therapy has promising results in hematologic malignancies. Currently, the United States Food and Drug Administration has approved two DMTis (5-azacitidine and decitabine) for the treatment of myelodysplastic syndromes, and the HDI vorinostat has been approved for the treatment of cutaneous T-cell lymphoma [31].

Histone Deacetylase Inhibitors (HDI)

HDI are a group of structurally diverse molecules. Despite their varied appearance, most HDI follow a common “cap-linker-chelator” model [32]. The chelator is a metal-binding biasing element such as hydroxamate or *o*-aminoanilide that connects with zinc in the active site of HDAC. The linker region spans the narrow channel of HDAC to connect the chelator to a simple aromatic or macrocyclic cap [33]. This cap interacts with the rim of the active site to adjust the three-dimensional conformation of the HDAC and present the chelator to the active site [34].

Although HDI target many of the functional hallmarks of cancer, including the cell cycle, apoptosis, angiogenesis, cellular differentiation and cellular transformation [35], their effects in normal cells are minimal [36]. The specificity of HDI effects on cancer cells may be attributed to the relatively small number of genes whose transcription is directly affected by HDI. Previous studies revealed that HDI affect the transcription of less than 20% of genes and mainly affect genes related to cell growth and survival [32, 33, 37, 38]. Although many of the anticancer effects of HDI can be linked to HDI's direct regulation of gene expression through hyper-acetylation of histones, histones are not the only targets of HDAC (Fig. 1). Therefore, HDI can alter tumour cell biology in ways that do not directly involve histones. Non-histone substrates of HDAC include transcription factors, tumour suppressors, oncogenes and cytoskeletal proteins (Table 2).

It is important to note that HDAC are a diverse group of enzymes. Four HDAC classes have been identified and each class has specific functions within the cell [39]. Until recently, class I HDACs were believed to be exclusively responsible for tumor growth. However, HDAC6, a class II HDAC, deacetylates tubulin and promotes independent cell movement implicating HDAC6 in metastasis [35]. This interesting finding highlights the need for a better understanding of the roles of the specific types of HDAC in order to optimize the design and biological effects of HDI.

A greater knowledge of the roles of the specific types of HDAC is key to the development of type-specific HDI. The current assortment of HDI available for research are considered to be non-specific. However, the potential for the generation of distinct forms of HDI exists

because different chelator and cap combinations can modify the potency and selectivity of HDI [33]. It is hoped that future selective inhibitors will have lower toxicity while maintaining their biological anticancer effects [40].

PRECLINICAL STUDIES OF HDI IN ALL TREATMENT

HDAC inhibitors have been shown to induce cell cycle arrest, terminal differentiation and/or apoptosis *in vitro* and in animal models of ALL. Table 3 shows a summary of preclinical studies of HDI in ALL. These studies consistently revealed that treatment of ALL with HDI led to cancer cell apoptosis and/or cell cycle arrest while sparing healthy cells. The specific outcomes of each study implicate HDI in a wide variety of cellular responses including apoptosis *via* the mitochondrial and/or the death receptor pathways and immunomodulation. However, care should be taken in interpreting these results as the indicated studies utilized different HDI, ALL model systems and molecular endpoints and therefore, it is possible that the results obtained may not be generally applicable to ALL.

Mitochondrial Death Pathway

The mitochondria play a key role in the cellular response to death signals that originate within the cell, including DNA damage, oxidative stress and starvation [41]. Several studies have linked HDI treatment to activation of the mitochondrial death pathway in ALL through a variety of mechanisms. For example, the HDI LAQ824 down-regulates protein levels of the anti-apoptotic BCL-2 and activates caspase-9 in leukemia cell lines derived from patients with B-lineage ALL [42]. On the other hand, MS-275 induces the generation of reactive oxygen species (ROS) upstream of mitochondrial injury [43]. In addition, SAHA, MS275, and Valproic acid cause CEM-C1 cell death through a combination of the mitochondrial pathway and caspase-independent apoptosis [44]. Furthermore, sodium butyrate induces caspase cell death in the CEM-C7H2 human T-lineage ALL cell line [45].

Death Receptor Pathway

Extrinsic apoptosis signaling results from the activation of cell surface “death receptors” that transmit apoptotic signals after interaction with specific ligands [46]. SAHA, MS-275 and Valproic acid all triggered apoptosis in CEM-C7 through the death receptor pathway with caspase 10 as the initiator caspase [44]. Furthermore, both Trichostatin A and depsipeptide combined with TNF-related apoptosis-inducing ligand (TRAIL) activated the death receptor apoptosis pathway *via* caspase-8 in Jurkat T human lymphoblastic leukemia cells [47]. Finally, treatment of CEM-C3 and CCRF-CEM T-cell ALL cells with the HDIs SAHA or Valproic Acid in combination with Tumor Necrosis Factor-Related Apoptosis Inducing Ligand (TRAIL) or anti-Death Receptor 4 Antagonistic Monoclonal Antibody (AY4) caused these cells to undergo synergistic caspase-dependant apoptotic cell death [48].

Immunomodulatory Effects

Pro-apoptotic anti-tumour immunity can be initiated by making ALL cells into more appealing immune targets, altering immune cell activity and/or cytokine production [40]. Combination treatment of the REH ALL cell line with cytokines and Trichostatin A

enhanced the allostimulatory capacity of REH-DC and improved dendritic cell differentiation [49].

Angiogenesis and Invasion

While studies on other types of cancer have shown HDI to have anti-angiogenic and anti-invasive activities both *in vitro* and *in vivo* [40], there has been no such study in ALL. The importance of angiogenesis and invasion are less intuitive in hematological malignancies than solid tumors but both of these processes play an important role in ALL. Indeed, ALL cells can induce angiogenesis in the bone marrow and it is possible that ALL might be angiogenesis dependent [50]. Furthermore, invasion of ALL cells into the central nervous system is associated with a poor prognosis [51]. Future studies should examine what, if any, effects HDI have on angiogenesis and invasion in ALL.

CLINICAL STUDIES OF HDI IN ALL TREATMENT

The promising results from preclinical trials have resulted in several clinical trials to assess HDI as potential therapies for ALL. Table 4 contains a summary of the current status of these clinical trials. Several of these studies are still in the preliminary phases and results are yet to be published. However, it is clear from published results that HDI are less effective and more toxic *in vivo* than they appeared to be *in vitro* [52, 53].

There are several limitations of the current clinical studies of HDI on ALL. As many of the trials are phase I, patients with relapsed disease and poor prognosis are targeted. Therefore it is probable that the participants in these trials may not accurately represent the majority of ALL cases. Furthermore, most of the studies required participants to be 18 years or older while ALL primarily affects children. Since several differences exist between adult and pediatric ALL, the effect of HDI on the majority of ALL patients has yet to be accurately assessed.

The multitude of anticancer activities generated by HDI suggests that they could work with several pharmacological and biological anticancer agents to generate ALL cell apoptosis. This movement from monotherapy to combination therapy has generated promising results in other hematological malignancies [31]. Several interesting combinations of HDI with other anticancer agents are already under investigation [35] (see Table 4). There is also interest in developing “smart drug” HDI that are designed to target multiple ligands as an alternative to multi-drug combinations because single drugs have more predictable pharmacokinetics [35].

CONCLUSIONS AND PERSPECTIVES

In summary, HDI are exciting new anticancer agents capable of altering the epigenetics of ALL. Preclinical studies have shown tremendous promise as HDI stimulated cell death, differentiation and cell cycle arrest in ALL. However, clinical studies have also shown that these drugs have some dose limiting toxicities and only weak anti-leukemic activity. A number of questions must be addressed in order to develop novel, effective and non-toxic HDI for ALL treatment. Important areas for future research include: a better understanding

of the epigenetics of ALL; analysis of HDI effects on angiogenesis and invasion of ALL; studies of HDI in combination with other treatments for ALL; and the development and testing of new HDAC type-specific and multi-ligand HDI. Research in these areas has the potential to improve the treatment and survival of ALL patients of all ages.

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ABBREVIATIONS

ALL	Acute Lymphoblastic Leukemia
AY4	Anti-Death Receptor 4 Antagonistic Monoclonal Antibody
AML	Acute Myeloid Leukemia
BCP	B-cell Precursor
CLL	Choroid Lymphocytic Leukemia
CpG	Cytosine Guanine Pair
DTMi	DNA methyltransferase inhibitors
DNA	Deoxyribonucleic Acid
H3K79	Histone 3 Lysine 79
HAT	Histone Acetyltransferase
HDAC	Histone Deacetylase
HDI	Histone Deacetylase Inhibitor
HMT	Histone Methyl Transferases
MCL	Myeloid Cell Leukemia
MLL	Mixed Lineage Leukemia Gene
ROS	Reactive Oxygen Species
SAHA	Suberoylanilide Hydroxamic Acid
TRAIL	Tumor Necrosis Factor-Related Apoptosis Inducing Ligand

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Fig. 1.

Potential Mode of Action of HDI. HDI inhibits HDACs resulting in (i) accumulation of acetylated histone proteins and leading to relaxation of chromatin and altered gene transcription, and (ii) increased number of acetylated non-histone substrates (listed in Table 2). Together these changes lead to cell cycle arrest, apoptosis and cell differentiation.

Table 1

A list of Aberrantly Methylated Genes in ALL

Gene	Chromosomal Location (Homo Sapiens)	Function	Reference
GIPC2	1p31	Prostanoid signaling	[17, 21]
RSPO1	1p34	Wnt signaling	[17, 21]
P73	1p36	Transcription factor	[21, 54–57]
KCNK2	1q41	K ⁺ channel	[18, 21]
LRP1B	2q21	LDL complex	[18, 21]
DCL-1	2q24	GTPase negative regulator	[18, 21]
CAST1	3p14	CNS synapsis	[17, 21]
MAGI1	3p14	Guanylate kinase	[17, 21]
WntA5	3p21	Wnt signaling	[21, 57]
ADCY5	3q13	Adenylyl cyclase	[17, 21]
CD10	3q25	Peptidase	[21, 56]
NKX6-1	4q21	Transcription factor	[18]
HSPA4L	4q28	Heat shock protein	[17, 21]
sFRP2	4q31	Wnt signaling	[21, 57]
OCLN	5q13	Tight junction	[17, 21]
EFNA5	5q21	Ephrin signaling	[17, 21]
PCDHGA12	5q31	Potential calcium-dependent cell-adhesion protein	[18]
MSX2	5q34	Transcriptional repressor	[17, 21]
GFPT2	5q34	Aminotransferase	[17, 21]
LATS1	6q24	Kinase	[21, 27]
ER	6q25	Estrogen receptor	[21, 58]
PARKIN	6q25	Proteosomal degradation	[21, 27]
THSBS2	6q27	Cell adhesion	[21, 56]
sFRP4	7p14	Wnt signaling	[17, 21]
ABCB1	7q21	Energy-dependent efflux pump	[18]
MDR1	7q21	Transmembrane transporter	[21, 56]
RPIB9	7q21	Rap2-binding protein	[18]
sFRP1	8p12	Wnt signaling	[21, 57]
P15	9p21	Cell cycle control	[21, 56, 59–61]
GNA14	9q21	G protein	[17, 21]
DBC1	9q32	Inhibitor of Sirt1	[21, 62]
c-ABL	9q34	Kinase	[21, 56]
DAPK	9q34	Apoptosis	[21, 27]
PTEN	10q23	Phosphatase	[21, 27]
sFRP5	10q24	Wnt signaling	[21, 57]
BNIP3	10q26	Apoptosis	[21, 63]
Calcitonin	11p15	Calcium metabolism	[21, 64–66]

Gene	Chromosomal Location (Homo Sapiens)	Function	Reference
Dkk3	11p15	Wnt signaling	[21, 57]
P57	11p15	Cell cycle control	[21, 56, 57]
SLC2A14	12p13	Glucose transport	[18, 21]
WIF1	12q14	Wnt signaling	[21, 57]
APAF-1	12q23	Apoptosis	[21, 27]
DDX51	12q24	ATP-binding RNA helicase	[18, 21]
HDPR1	14q23	Wnt signaling	[21, 57]
THSBS1	15q15	Cell adhesion	[21, 56]
IGDCC4	15q22	Member of immunoglobulin superfamily	[18, 21]
SALL1	16q12	Zinc finger protein	[17, 21]
E-cadherin	16q22	Cell adhesion	[21, 67]
CDH-13	16q24	Cell adhesion	[21, 27]
DCC	18q21	Putative tumour suppressor gene	[18, 21]
MYO5B	18q21	Protein traffic	[17, 21]
ZNF382	19q13	Zinc finger protein	[17, 21]
NES-1	19q13	Serine protease	[21, 27]
Hck	20q11	Kinase	[21, 68]
SERINC3	20q13	Apoptosis	[21, 27]
MN1	22q12	Involved in meningioma	[17, 21]

Table 2

Non-Histone Proteins Known to be Direct Substrates of HDAC Enzymes

Substrate	Function	Reference
α -Tubulin	Cytoskeleton	[69]
β -Catenin	Cell adhesion/transcription regulator	[69]
Bcl6	Oncogene	[69]
c-Myc	Oncoprotein	[69]
Cortactin	Actin binding protein	[69]
E2F	Transcription factor/cell cycle	[69]
ER	Steroid hormone receptor	[69]
ETS	Transcription Factor	[39]
GATA1	Transcription factor	[69]
HIF1 α	Transcription factor	[69]
HMG1 and HMG2	Non-histone chromosomal proteins	[69]
HSP90	Chaperone	[69]
Importin	Nuclear import factors	[69]
Ku70	DNA damage response	[39]
MEF2	Transcription factor	[69]
p53	Tumor suppressor	[69]
pRb	Tumor suppressor	[69]
RUNX	Transcription factor	[39]
SMAD7	Transcription factor	[39]
STAT3	Signal transduction and transcription activation	[39]
SRBEP	Transcription Factor	[39]
TCF	DNA binding	[69]
UBF	Transcription factor	[69]
YY1	Transcription factor	[69]

Table 3

Summary of Preclinical Studies on HDI in ALL

Drug	Model System	Cellular Alterations Resulting from HDI	End Result of HDI Treatment	Ref.
FK228	Clinically Isolated ALL cells and hematopoietic progenitor cells	Histone acetylation was significantly increase in both clinically Isolated ALL cells and hematopoietic progenitor cells	Apoptosis and cell cycle arrest of ALL cells at IC 50 levels lower than for hematopoietic progenitor cells	[70]
	TEL- AML1-negative and positive leukemic cells of children with ALL	-FK228 caused decreases in CD19, TdT, and CD10; and a slight increases in CD20, CD22 and CD45 expression. This corresponds to differentiation to a more mature B-cell stage.	Cytotoxicity of FK228 was the same between TEL-AML1 positive and TEL- AML1-negative ALL	[71]
LAQ824	Four different pre-B lymphoblastic cell lines: Sup-B15 and TMD-5, both t(9;22) positive, SEM, t(4;11) positive, and NALM-6 cells	Histone acetylation was ten times higher when cells were treated with LAQ824 as compared to TSA treatment Upregulation of p21WAF1/Cip1 Down-regulation of BCL-2 Disruption of γ PARP cleavage. Activation of Caspases 3 and 9	ALL cell growth inhibition by G0/G1 phase arrest followed by apoptosis LAQ824 induces apoptosis in cell lines harboring translocations which define high risk ALL patients in the clinical setting.	[42]
Trichostatin A	REH, an ALL cell line with a (12;21)- chromosomal translocation	Combination treatment of REH with cytokines and Trichostatin A up-regulated CD83 expression Combination treatment of REH with cytokines and Trichostatin A enhanced the allostimulatory capacity of REH-DC	Combination treatment of REH with cytokines and Trichostatin A improved dendritic cell differentiation	[49]
MS-275	Jurkat T human lymphoblastic leukemia cells	MS-275 robustly induced p21CIP1/WAF1	MS-275 inhibits ALL cell proliferation and induces cell death in a dose-dependent manner. MS-275 induces the generation of ROS upstream of mitochondrial Injury.	[43]
Vorinostat	Notch1-induced mouse model of T-ALL, human T-ALL cell lines and human T-ALL primary samples	Vorinostat down-regulated MYC, GRWD1, PUS7, WDR74, PPRC1, POLR3E and IFRD2 genes and up-regulated the ZNF292 and PDCL gene in both T-ALL cell lines	T-ALL cell lines more sensitive to vorinostat than other leukemia/cancer cell lines, and GSI-sensitive T-ALL cell lines were more sensitive to vorinostat than GSI-resistant cell lines IC50 of vorinostat was similar for leukemic mouse DP cells and human T- ALL cell lines Vorenistat induced a modest increase in G1-phase cells when used in combination with GSI	[72]
Valproic acid	Human Philadelphia chromosome-positive (Ph+) ALL cell lines	HDAC activity was slightly, but significantly, reduced by valproic acid alone and in combination with imatinib mesylate Crkl phosphorylation was not inhibited by valproic acid	Valproic acid caused proliferation inhibition and apoptosis induction in ALL cells Combination of valproic acid with SCH66336, imatinib enhances anti-cancer effects.	[73]
LBH589	Two model human Ph(-)ALL cell lines (T-cell MOLT-4 and pre-B-cell Reh) and cultured human primary Ph(-) ALL cells	Hyperacetylation of histone H3K9 and H4K8 Depletion of c-Myc Up-regulation of the cyclin-dependent kinase inhibitors p21 (Waf1/CIP1) and p27 (Kip1) protein levels LBH589 up-regulates expression of apoptosis and DNA damage response-related genes, such as TP73, CHEK2, RBBP8, TREX1, BRCA1, FANCG, FOXO3A, GADD45A, GADD45B, and GADD45G. Phosphorylation of H2AX	LBH589 induced apoptosis, inhibition of cell proliferation, and cell-cycle arrest in G2/M phase in both the Ph- ALL cell lines and the primary patient samples	[74]

Drug	Model System	Cellular Alterations Resulting from HDI	End Result of HDI Treatment	Ref.
Sodium butyrate	CEM-C7H2 cell lines	ALL cell death involved zVAD-sensitive proteases/caspases with participation of DEVD/VEID-sensitive effector caspases	Sodium butyrate induced typical apoptotic cell death, inhibited cell proliferation, and accumulated cells in the G2/M phase of the cell cycle	[45]
SAHA, MS275, and Valproic acid	CEM-C1, Reh, CEM- C7, RS4;11 and TOM-1 ALL cell lines. Leukemic blasts from individuals with ALL	CEM-C7 cells die through activation of the extrinsic pathway involving mainly caspase 10 as initiator caspase CEM-C1 cells die by a combination of the intrinsic pathway (cytochrome c release, Ψ m) and caspase-independent (z-VAD resistant) apoptosis	Both B and T-ALL cells were much more efficiently killed by SAHA and VPA than by MS275	[44]
Trichostatin A and depsipeptide	Jurkat T human lymphoblastic leukemia cells	HDI combined with TRAIL activated the extrinsic apoptosis pathway <i>via</i> caspase-8	Low concentrations of HDI markedly sensitize cells to TRAIL-induced apoptosis	[47]
Sodium Butyrate and SAHA	CCRF-CEM (T-ALL), SupB15 (Bp-ALL t(9;22)) and REH (Bp- ALL t(12;21))	HDI induced the expression of folypoly- γ - glutamate synthetase and increased the accumulation of methotrexate long-chain polyglutamates	Combination of HDI and methotrexate enhanced cytotoxicity in ALL cells, indicating a potential means of combination therapy	[75]
SAHA and Valproic Acid	CEM-CM3 and CCRF- CEM T-ALL Human cell lines	HDI treatment in combination with TRAIL or AY4 activated caspase-dependant apoptotic cell death.	Most leukemia cell lines have intrinsic resistance to TRAIL or AY4 but HDI treatment sensitized ALL cells to TRAIL and AY4 induced apoptosis.	[48]

For the structure of HDIs listed, see references [39, 78, 79].

Table 4

Clinical Studies of HDI and ALL [79]

HDI Drug	Identifier	Phase	Ages	Status	Response	Toxicity	Ref.
PXD101 and Azacitidine	NCT00351975	I	18 Years and older	Recruiting	-	-	-
Decitabine, Vorinostat, and Combination Chemotherapy	NCT00882206	II	2 Years to 60 Years	Recruiting	-	-	-
Dasatinib and Vorinostat	NCT00816283	I	18 Years and older	Recruiting	-	-	-
Vorinostat and Decitabine	NCT00275080	I	18 Years and older	Ongoing, but not recruiting	-	-	-
Vorinostat, Cytarabine, and Etoposide	NCT00357305	I	18 Years and older	Recruiting	-	-	-
Flavopiridol and Vorinostat	NCT00278330	I	18 Years and older	Completed	-	-	-
Vorinostat and Idarubicin	NCT00331513	I	18 Years and older	Completed	No patients with ALL achieved a clinical response, however, seven of 41 patients (17%) studied did. Increased histone-H3 acetylation was detected in 15 of 33 (46%) available patient samples	13 instances including: prolonged myelosuppression, mucositis, fatigue, uncontrollable diarrhea, tumour lysis syndrome and left ventricular dysfunction	[80]
Vorinostat With or Without Isotretinoin	NCT00217412	I	1 Year to 21 Years	Completed	-	-	-
Vorinostat and Decitabine	NCT00357708	I	18 Years and older	Completed	-	-	-
Pyroxamide	NCT00042900	I	18 Years and older	Completed	-	-	-
MS-275 and GM-CSF	NCT00462605	II	18 Years and older	Recruiting	-	-	-
MS-275	NCT00015925	I	18 Years and older	Completed	No responses by classical criteria were seen	Dose limiting infections and neurologic toxicity	[52]
LBH589	NCT00918333	I/II	18 Years and older	Recruiting	-	-	-
LBH589	NCT00723203	II	18 Years and older	Recruiting	-	-	-
LBH589	-	I	Median age, 63 years; range, 42–87 years	Completed	Transient antileukemic activity at all doses tested.	Cardiotoxicity, nausea, diarrhea, vomiting, hypokalemia, loss of appetite, and thrombocytopenia	[53]
NSC706995	NCT01132573	I	40–59 years	Recruiting	-	-	-