HDAC6

Subjects: Oncology

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Histone deacetylase (HDAC) 6 is a zinc-dependent enzyme of HDAC class IIb. HDAC6 is unique within the HDAC family due to a particular structure giving it unique biological functions implicated in all major cell pathways. This isoenzyme is mainly active in the cytoplasm and possesses two functional catalytic sites and an ubiquitin-binding domain. The deacetylase functions of HDAC6 targets multiple substrates including essentially α-tubulin and heat shock protein (HSP)90α which are key factors in cell regulatory networks through the regulation of the microtubule network and many protein functions, respectively. Accordingly, several studies have highlighted the role of HDAC6 in various pathological conditions. For instance HDAC6 overexpression frequently correlates with tumorigenesis and favor cell survival and metastasis. Therefore, HDAC6 represents an interesting potential therapeutic target.

Keywords: histone deacetylase 6 inhibitor ; personalized treatment ; heat shock protein 90α ; leukemia stem cells ; imatinib resistance ; targeted therapy

1. Introduction

Carcinogenesis is a multistep process whereby normal cells are transformed into malignant cells. The process is characterized by major biological changes shared by most neoplastic cells called hallmarks of cancer. These transformational events relies on multiple alterations at genetic and epigenetic levels leading to abnormal cell growth [1].

Over the past years protein lysine acetylation has emerged as a key post-translational modification in the coordination of tightly regulated biological functions and alterations of the acetylome profiles are associated with various pathological conditions such as cancer.

The acetylation status of lysine residues within histone and non-histone proteins is finely tuned by the concert action of histone acetyltransferases (HATs) and histone deacetylases (HDACs) catalyzing the addition and removal of the acetyl groups, respectively. Recently, there was a particular focus on HDAC6 coming from its unique properties to control multiple cellular pathways linked to cell growth, survival, and migration. Accordingly, the use of HDAC6 inhibitors alone or in combination with additional chemotherapeutic agents appear as a promising strategy to treat various cancers.

2. Histone Deacetylase 6

The HDAC6 protein is part of the HDAC family, which are enzymes catalyzing the deacetylation of proteins, which corresponds to the removal of an acetyl group from lysine residues [2]. The 18 HDACs found in mammals are divided into four classes according to their sequence homology. For classes I (HDAC1, 2, 3, and 8), IIa (HDAC4, 5, 7, and 9), IIb (HDAC6 and 10), and IV (HDAC11), the deacetylation of lysine occurs through a transfer of charge, and their essential component is a zinc ion (Zn^{2+}) present at the bottom of the catalytic pocket of HDAC enzymes [3]. For class III [sirtuins (SIRT) 1-7] HDACs, the presence of a cofactor, nicotinamide adenine dinucleotide (NAD^+), is essential for the reaction [4][5].

Class I HDACs are ubiquitously present in many human cell lines and tissues, while class II HDACs exhibit a specific expression profile in certain human tissues such as the heart (HDAC5), the breast (HDAC6), the ovary (HDAC7 and 9), and the kidney (HDAC10) [6][7].

2.1. Structure

Here, we will focus more specifically on HDAC6 belonging to class IIb. This enzyme is the only HDAC to possess two functional active catalytic sites, and has a nuclear localization sequence, a nuclear export sequence, and a repetitive region of eight consecutive serine-glutamic acid tetradecapeptides, a cytoplasmic retention signal, and is mainly present in the cytoplasm [8]. HDAC6 also has a C-terminal ubiquitin-binding domain required when binding to poly-ubiquitinated proteins (Figure 1).

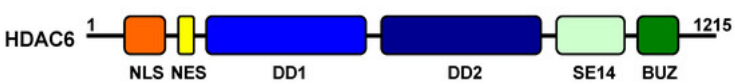

Figure 1. Protein structure of histone deacetylase 6 (HDAC6). The HDAC6 protein consists of 1215 amino acids. It has a nuclear localization sequence (NLS), a nuclear export sequence (NES), two functional active catalytic (SC) sites, a

cytoplasmic retention signal of eight consecutive serine-glutamic acid tetradecapeptides (SE14), and a ubiquitin-binding domain (BUZ) at the C-terminal.

2.2. Function

The HDAC6 protein deacetylates many substrates [9] (Table 1) including α-tubulin, cortactin, and heat shock protein (HSP)90α, and is thus involved in many cell processes, some of which are described below [10].

Table 1. List of substrates specifically deacetylated by HDAC6.

Substrates	Localization of the Substrate	Deacetylated Lysine(s)	Function of the Deacetylated Substrate	Interaction Domains of HDAC6	Reference
14-3-3ζ	Cytoplasm and nucleus	49, 120	Regulation of protein binding Bad and AS160	ND	[11]
β-catenin	Cytoplasm and nucleus	49	Epidermal growth factor-induced nuclear localization and decreased expression of c-Myc	ND	[9]
Cortactin *	Cytoplasm	87, 124, 161, 189, 198, 235, 272, 309, 319	Regulation of cell migration and actin filament binding	DD1 and DD2	[9]
DNAJA1	Cytoplasm	ND	Protein folding	ND	[12]
ERK1	Cytoplasm and nucleus	72	Proliferation, mobility, and cell survival	ND	[13]
Foxp3 *	Nucleus	ND	ND	ND	[14]
HDAC9	Cytoplasm and nucleus	ND	Modulation of cell survival and arrest of cellular movement	DD2	[15]
HDAC11	Nucleus	ND	Transcriptional activation of interleukin 10	ND	[16]
HMGN2	Nucleus	2	Increased transcription of STAT5	ND	[17]
HSC70	Cytoplasm	ND	Protein folding	ND	[12]
HSPA5	Cytoplasm	353	Ubiquitination of HSPA5 mediated by GP78	ND	[18]
HSP90α	Cytoplasm	294	Degradation and elimination of misfolded proteins and regulation of glucocorticoid receptors	DD1, DD2 et BUZ	[9]
K-RAS *	Cytoplasm	104	Cell proliferation	ND	[19]
Ku70	Cytoplasm	539, 542	Suppression of apoptosis	ND	[9]
LC3B-II*	Cytoplasm	ND	Regulation of autophagy	ND	[20]
MSH2	Cytoplasm and nucleus	845, 847, 871, 892	Reduced cellular sensitivity to DNA damaging agents and reduced DNA mismatch repair activities by downregulation of MSH2	DD1	[21]
MYH9	Cytoplasm	ND	Regulation of binding to actin filaments	ND	[12]
PrxI	Cytoplasm and nucleus	197	Antioxidant activity	ND	[22][23]
PrxII	Cytoplasm and nucleus	196	Antioxidant activity	ND	[22][23]
RIG-I	Cytoplasm	858, 909	Recognition of viral RNA	ND	[24]
Sam68	Nucleus	ND	Alternative splicing	ND	[25]
Survivin	Nucleus	129	Anti-apoptotic function	DD2	[9]
Tat	Cytoplasm	28	Suppression of HIV transactivation	DD2 and BUZ	[26]
α-tubulin *	Cytoplasm	40	Formation of immune synapses, viral infection, cell migration and chemotaxis	DD1 or DD2	[9][27]

* Cortactin and LC3B-II are also deacetylated by SIRT1, K-RAS and α-tubulin are also deacetylated by SIRT2, and Foxp3 is also deacetylated by HDAC9 and SIRT1. AS160: Akt substrate of 160 kDa; Bad: Bcl-2 associated agonist of cell death; BUZ: binding-of-ubiquitin zinc; DD: deacetylase domain; DNAJA1: dnaJ homolog subfamily A member 1; ERK1: extracellular signal-regulated kinase 1; Foxp3: forkhead box P3; GP: glycoprotein; HDAC: histone deacetylase; HIV: human immunodeficiency virus; HMGN2: high mobility group nucleosomal binding domain 2; HSC: heat shock cognate;

HSP (A): heat shock protein [family A (HSP70) member 5]; LC3B-II: microtubule-associated protein 1 light chain 3; MSH2: MutS protein homolog 2; MYH9: myosin heavy chain 9; ND: non determined; Prx: peroxiredoxin; RIG-I: retinoic acid-inducible gene I protein; Sam: Src-associated substrate in mitosis; STAT: signal transducer and transcriptional activator; Tat: twin-arginine translocation protein.

The HDAC6 protein plays an important role in the dynamism of two components of the cytoskeleton, actin filaments (or F-actin) and microtubules, α- and β-tubulin polymers, which are involved in particular in cell mobility and division. Cortactin, which improves the polymerization of actin filaments, and α-tubulin, a constituent of microtubules, are substrates of HDAC6. The deacetylation of cortactin by HDAC6 and SIRT1 leads to its binding to F-actin, improving its polymerization, and thus contributing to cytoskeletal dynamics (Figure 2A). The deacetylation of α-tubulin by HDAC6 and SIRT2 is associated with microtubule depolymerization, thus contributing to the dynamism of microtubules (Figure 2B), and to proteasome-independent protein degradation. When the proteasome is degraded, the polyubiquitinated misfolded proteins are transported to the microtubule-organizing center and are supported by HDAC6 via its ubiquitin binding domain, leading to the formation of aggresomes through deacetylation of cortactin. The aggresomes thus formed are subsequently removed after autophagosome fusion with lysosomes via autophagy (Figure 2C). A decrease in the acetylation of microtubule-associated protein 1 light chain 3 by HDAC6 was observed during autophagic degradation [28] [29]. The HDAC6 protein is also involved in proteasome-dependent protein degradation via its interaction with HSP90α, a chaperone that stabilizes other proteins when deacetylated by HDAC6. In its acetylated form, HSP90α loses its chaperone function, which leads to the degradation of its client proteins by the proteasome (Figure 2D). An accumulation of misfolded proteins causes dissociation of the complex containing HSP90α, heat shock factor 1 (HSF) 1, chaperone valosin-containing protein/ATPase, and HDAC6. In complex in inactive form, during dissociation, the release of HSF1 induces the transcription of many HSPs (Figure 2E), and HDAC6 will allow its binding to misfolded proteins [28].

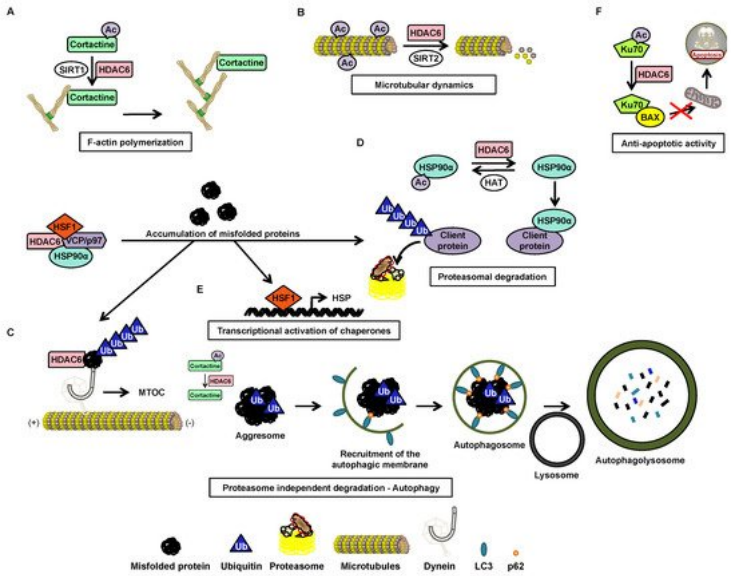

Figure 2. The HDAC6 protein is involved in many cellular processes. HDAC6 is involved in F-actin polymerization (**A**), microtubule dynamics (**B**), anti-apoptotic activity (**C**), proteasome-dependent and -independent degradation (**D**), transcriptional activation of chaperone proteins (**E**), and autophagy (**F**). Ac: acetylated; HAT: histone acetyltransferase; HDAC: histone deacetylase; HSF: heat shock factor; HSP: heat shock protein; MTOC: microtubule organizing center; SIRT: sirtuin; VCP: valosin-containing protein/ATPase.

HDAC6 is also involved in apoptosis by deacetylating the Ku70 protein, which then forms a complex with BAX, a proapoptotic protein, allowing the inhibition of apoptosis (Figure 2F). Similarly, inhibition of the catalytic activity of HDAC6 promotes the dephosphorylation of AKT and ERK, associated with decreased cell proliferation and death of cancer cells [28].

Furthermore, HDAC6 regulates endocytosis and exocytosis vesicles. When the epidermal growth factor receptor (EGFR) receptor is bound to its ligand, it interacts with HDAC6 and inactivates it by phosphorylation, which then leads to the hyperacetylation of microtubules and finally the internalization of the receptor. The inhibition of HDAC6 induces the increase of the acetylation of peroxiredoxins 1 and 2, which are antioxidant enzymes, increasing their activity and causing a reduction in cell resistance to chemotherapy [28]. HDAC6 is involved in the process of autophosphorylation of tau protein, giving it the ability to form aggregates called neurofibrillary tangles that can cause neurotoxicity [30].

2.3. Post-Transcriptional Regulation

There is a lack of current data explaining the post-transcriptional regulation of HDAC6 protein. Nevertheless, some microRNAs stimulating cancer cell proliferation and metastasis formation (miR-22, miR-221, miR-433, and miR-548) [10], and stem cell differentiation (miR-26a) [31], are predicted to interact with HDAC6 protein, thus inducing a destabilization or repression of the translation of its mRNA.

2.4. Post-Translational Regulation

Post-translational modifications such as phosphorylation and acetylation have a significant impact on HDAC6 functions. Indeed, although EGFR induces an inhibitory phosphorylation of HDAC6, in the majority of cases it is established that the phosphorylation of HDAC6 improves its deacetylase activity, whereas acetylation decreases its enzymatic activity, preventing the deacetylation of α-tubulin. Examples of post-translational modifications of the HDAC6 protein influencing its activity are shown in Table 2.

Table 2. Post-translational modifications regulating the activity of HDAC6.

Post-Translational Modification	Enzyme	Target Site	Consequences	Reference
Phosphorylation	GSK3β	Ser-22	Increased deacetylation activity of α-tubulin	[10]
	ERK1	Ser-1035	Regulation of cellular motility	[10]
	GRK2	ND	Increased deacetylation activity of α-tubulin	[32]
	Aurora	ND	Increased deacetylation activity of α-tubulin	[10]
	PKCζ	ND	Increased deacetylation activity of α-tubulin	[10]
	CK2	Ser-458	Improved formation and elimination of aggresomes	[10]
	EGFR	Tyr-570	Inhibition of deacetylation activity	[33]
Acetylation	p300	Lys-16	Inhibition of deacetylation activity	[10]

CK2: casein kinase 2; EGFR: epidermal growth factor receptor; ERK1: extracellular signal-regulated kinase; GRK2: G protein-coupled receptor kinase 2; GSK3: glycogen synthase kinase 3; Lys: lysine; ND: non determined; PKCζ: protein kinase C isoform ζ; Ser: serine; Thr: threonine.

In addition to these known post-translational modifications, there are proteins interacting directly with the HDAC6 protein and inducing its inhibition by direct interaction (Table 3).

Table 3. Proteins that interact directly with the HDAC6 protein.

Protein Inhibiting HDAC6 by Direct Interaction	Protein Function	Protein Region Required for Interaction with HDAC6	HDAC6 Domain Interacting with the Protein	Cellular Impact	References
CYLD	Deubiquitinase	ND	DD1/DD2	Cell proliferation, ciliogenesis	[10]
Dysferlin	Skeletal muscle membrane repair, myogenesis, cell adhesion, intercellular calcium signaling	Domain C2	ND	Myogenesis	[34]
Mdp3	Stabilization factor of microtubules	Amino-terminal region	ND	Cell motility	[35]
Paxillin	Focal adhesion	Region rich in proline	ND	Polarization and cell migration	[10]
p62	Transport of misfolded proteins	Between the ZZ domain and the TRAF6 link area	DD2	Aggresome formation	[36]
RanBPM	Apoptosis, proliferation and cell migration		ND	Aggresome formation	[37]
Tau	Stabilization factor of microtubules	Tubulin binding region	SE14 domain	Aggresome formation	[36][38]
TPPP1	Polymerization and acetylation of microtubules		ND	Regulation of microtubule acetylation and β-catenin expression	[39]

DD: deacetylase domain; Mdp3: microtubule-associated protein (MAP) 7 domain-containing protein 3; ND: non determined; RanBPM: Ran-binding protein microtubule-organizing center; tau: tubulin-associated unit; TPPP1: tubulin polymerization-promoting protein-1.

3. HDAC6 in cancer

Several studies have demonstrated the influence of HDAC6 in neurodegenerative, cardiovascular and renal diseases, as well as in inflammation [40] and viral response [10]. The role of the HDAC6 protein in cancer is also now well better understood. Although its oncogenic or tumor suppressor potential is dependent on the type of cancer [28], its involvement in oncogenic cell transformation, tumor development, and cancer immunity regulation makes a strong therapeutic candidate [41].

HDAC6 is overexpressed in many types of cancer (Table 4) and may be implicated in disease progression.

Table 4. Deregulation of HDAC6 expression in different types of cancers.

Cancer Type	Cancers	Expression of HDAC6-Comments	References
Solid tumors	Bladder	Overexpressed	[41]
	Melanoma	Overexpressed	[41]
	Lung	Overexpressed	[41]
	Oral squamous cell carcinoma	Overexpressed-Enhanced expression in advanced stages	[28][42]
	Ovarian carcinoma	Overexpressed-Enhanced expression in advanced stages	[28][42]
	Breast	Overexpressed-Prediction of a good or bad prognosis	[28][43]
	Hepatocytic carcinoma	Overexpressed-Enhanced expression in advanced stages	[28]
		Under-expressed-HDAC6 suggested as a tumor suppressor	[28][44]
Hematological	Chronic lymphocytic leukemia	Overexpressed-Observation on patient samples, cell lines and a transgenic mouse model	[42]
	Acute myeloid leukemia	Overexpressed	[28][42]
	Acute lymphoblastic leukemia	Overexpressed-Enhanced expression in advanced stages	[28]
	Chronic lymphocytic leukemia	Overexpressed-Correlated with longer survival	[28]
	T-cell cutaneous lymphoma	Overexpressed-Correlated with longer survival	[28]
	Chronic myeloid leukemia	Overexpressed-Increased expression in $CD34^+$ cells	[45]
	Multiple myeloma	Overexpressed	[46]
	Mantle cell lymphoma	Overexpressed	[46]
	Diffuse large B cell lymphoma	Overexpressed	[46]
	Peripheral T-cell lymphoma	Overexpressed	[46]

CD: cluster of differentiation; HDAC6: histone deacetylase 6.

3.1. HDAC6 Inhibitors

The ability to specifically target HDAC6 would have valuable clinical utility in the treatment of these cancers. However, despite a large number of pan-HDAC inhibitors, very few compounds are capable of selectively inhibiting HDAC6 (Table 5). This type of inhibitor can be divided into 2 groups according to their chemical structure: benzamides and hydroxamates [28].

Table 5. List of HDAC6 inhibitors.

Class	HDAC6 Inhibitor	Binding Domain	CI_{50} (nM) of the HDAC6 Activity *in Vitro*	Selectivity Ratio for HDAC6 Compared to (Other HDACs)	Inhibition of HDAC6 *in Cellulo* (μM)[$]	Effect on Cancer Cell Lines or Cancer Type	Ref
Benzamides	Trithiocarbonate derivative (12ac)	ND	65	19 (HDAC1)	10 (lung cancer)	CI_{50} = 8.2 μM (cervical cancer)	

Class	HDAC6 Inhibitor	Binding Domain	CI_{50} (nM) of the HDAC6 Activity *in Vitro*	Selectivity Ratio for HDAC6 Compared to (Other HDACs)	Inhibition of HDAC6 *in Cellulo* (μM)[$]	Effect on Cancer Cell Lines or Cancer Type	Ref
	NQN-1 (2-benzyl-amino-naphthoquinone)	ND	5540	Values non available (HDAC1, 2, 3, 4, 5, 7, 8, 9, 10, 11)	4 (chronic myeloid leukemia)	CI_{50} = 0.8 μM (leukemia)	
Hydroxamates	Hydroxamic acid containing a phenylalanine (4n)	His215, His216, Tyr386, Phe283, and Tyr255 of DD1 and His610, His611, Tyr782, Phe620, and Phe680 of an HDAC6 homology model	1690	14 (HDAC1)	1 (colorectal carcinoma)	IC_{50}: 3 to > 50 μM (various cancer cell lines)	
	Hydroxamic acid containing a pyridylalanine (5a)	Phe566 of DD2 of an HDAC6 homology model	3970	25 (HDAC1)	ND	IC_{50}: 104 μM (breast cancer)	
	ACY-738	ND	1.7	55 (HDAC1), 75 (HDAC2), 128 (HDAC3)	2.5 (neural cells)	ND	
	ACY-775	ND	7.5	283 (HDAC1), 343 (HDAC2), 1496 (HDAC3)	2.5 (neural cells)	ND	
	ACY-1083	His573 and His574 of DD2	3	260 (HDAC1)	0.03 (neuroblastoma)	ND	[5
	Bavarostat	Ser568 of DD2	60	>10000 (HDAC1, 2, 3), 188 (HDAC4), 317 (HDAC5), 78 (HDAC7), 142 (HDAC8), 87 (HDAC9), >17 (HDAC10), 167 (HDAC11)	10 (neural progenitor cells derived from induced pluripotent stem cells)	ND	

Class	HDAC6 Inhibitor	Binding Domain	CI_{50} (nM) of the HDAC6 Activity *in Vitro*	Selectivity Ratio for HDAC6 Compared to (Other HDACs)	Inhibition of HDAC6 *in Cellulo* (μM)[$]	Effect on Cancer Cell Lines or Cancer Type	Ref
	BRD9757	ND	30	21 (HDAC1), 60 (HDAC2), 23 (HDAC3), 727 (HDAC4), 611 (HDAC5), 420 (HDAC7), 36 (HDAC8), >1000 (HDAC9)	10 (cervical cancer)	ND	
	Cay10603	His499 of DD2 of an HDAC6 homology model	0.002	ND	<1 to 1 μM (several pancreatic cancer cell lines)	ND	[5]
	Citarinostat (ACY-241)	ND	2.6	14 (HDAC1), 17 (HDAC2), 18 (HDAC3 and 4), >7000 (HDAC4, 5,9), 2808 (HDAC7), 53 (HDAC8),	0.3 (ovarian cancer)	IC_{50}: 4.6 to 6.1 μM (ovarian and breast cancer)	
	α3β-cyclic tetrapeptide (23)	ND	39	3 (HDAC1), 4 (HDAC3), 6 (HDAC8)	2 (acute lymphoblastic leukemia)	IC_{50}: 9 to > 20 μM (various cancer cell lines)	
	Compound containing a phenylisoxazole group as a surface recognition group (7)	His499 of HDAC7	0.002	>100000 (HDAC1), >100000 (HDAC2), 210 (HDAC3), >3000000 (HDAC8), 45350 (HDAC10)	ND	IC_{50}: 0.1 to 1 μM (various prostate cancer cell lines)	
	Compound containing a triazolylphenyl group (6b)	ND	1.9	52 (HDAC1), 155 (HDAC2), 7 (HDAC3), 420 (HDAC8), 59 (HDAC10)	ND	IC_{50}: <0.5 to 22 μM (several prostate cancer lines)	
	Compound containing a peptoid (2i)	Tyr301 of DD2 of an HDAC6 homology model	1.59	126 (HDAC2), >6000 (HDAC4), 40 (HDAC11)	N	IC_{50}: 0.34 to 2.7 μM (various cancer cell lines)	
	3-aminopyrrolidinone derivative (33)	ND	17	4359 (HDAC1), 11 (HDAC8)	0.3 (multiple myeloma)	Good oral bioavailability	

Class	HDAC6 Inhibitor	Binding Domain	CI_{50} (nM) of the HDAC6 Activity *in Vitro*	Selectivity Ratio for HDAC6 Compared to (Other HDACs)	Inhibition of HDAC6 *in Cellulo* (μM)[$]	Effect on Cancer Cell Lines or Cancer Type	Ref
	4-aminomethylaryl acid derivative (1a)	ND	19	305 (HDAC1), 842 (HDAC2), 237 (HDAC3), 790 (HDAC4), 174 (HDAC5), 242 (HDAC7), 36 (HDAC8), 195 (HDAC0)	0.46 (cervical cancer)	ND	
	4-hydroxybenzoic acid derivative (7b)	ND	200	>50000 (HDAC1, 2, 8), >500000 (HDAC3, 10, 11)	50 (prostate cancer)	IC_{50}: 41 to 130 (several prostate and breast cancer cell lines)	
	4-hydroxybenzoic acid derivative (13a)	ND	20000	25 (HDAC1), >5000 (HDAC2, 3, 4, 8, 10), >2500 (HDAC11)	50 (prostate cancer)	IC_{50}: 19 to 127 (several prostate and breast cancer cell lines)	
	Aminoteraline derivative (32)	Phe620 and Phe680 of an HDAC6 homology model	50	126 (HDAC1), 2 (HDAC8)	2 (neuroblastoma)	IC_{50} = 5.4 μM (neuroblastoma)	
	Benzothiophene derivative (39)	ND	14	ND	Same effect as tubastatin A	Does not target NF-κB and AP-1 at the transcriptional level	
	2,4-imidazolinedione derivative (10c)	ND	4.4	218 (HDAC1), 63 (HDAC2), 53 (HDAC3), > 20000 (HDAC4, 7, 8, 9, 11), 3386 (HDAC5), 37 (HDAC10)	1.6 (acute myeloid leukemia)	IC_{50}: 0.2 to 0.8 μM (various cancer cell lines)	
	Mercaptoacetamide derivative (2)	ND	95.3	34 (HDAC1), 77 (HDAC2), 64 (HDAC8), 112 (HDAC10)	ND	At 10 μM protects cortical neurons from oxidative stress inducing death	

Class	HDAC6 Inhibitor	Binding Domain	CI_{50} (nM) of the HDAC6 Activity *in Vitro*	Selectivity Ratio for HDAC6 Compared to (Other HDACs)	Inhibition of HDAC6 *in Cellulo* (μM)[$]	Effect on Cancer Cell Lines or Cancer Type	Ref
	N-Hydroxycarbonylbenylamino quinoline derivative (13)	ND	0.291	32817 (HDAC1), 42955 (HDAC2), 26632 (HDAC3), 15250 (HDAC4), 10694 (HDAC5), 2436 (HDAC7), 4089 (HDAC8), 5258 (HDAC9), 33646 (HDAC10), 1292 (HDAC11)	0.1 (multiple myeloma)	IC_{50}: 9.1 to 40.6 μM (multiple myeloma)	
	Isoxazole-3-hydroxamate derivative (SS-208)	His463, Pro464, Phe583, and Leu712 of DD2	12	116 (HDAC1), 1625 (HDAC4), 576 (HDAC5), 695 (HDAC7), 103 (HDAC8), 3183 (HDAC9), 427 (HDAC11)	5 (melanoma)	ND	
	Phenothiazine derivative (7i)	Phe620 and Phe680 of DD2	5	538 (HDAC1)	0.1 (acute myeloid leukemia)	ND	
	Phenylhydroxamate derivative (2)	Phe464 and His614 of DD2	3	27 (HDAC1)	ND	CI_{50}: 0.65 to 2.77 (ovarian cancer and squamous cell carcinoma of the tongue)	[
	Phenylsulfonylfuroxan derivative (5c)	ND	7.4	33 (HDAC1), 51 (HDAC2), 45 (HDAC3), 4 (HDAC4), 46 (HDAC8), 82 (HDAC11)	0.013 (acute myeloid leukemia)	IC_{50}: 0.4 to 5.8 μM (various cancer cell lines)	
	Pyridone derivative (11e)	Phe155 and Phe210 of HDAC2	2.46	8 (HDAC1), 52 (HDAC2), 127 (HDAC3), 2329 (HDAC4), 785 (HDAC5), 1512 (HDAC7), 77 (HDAC8), 2268 (HDAC9), 21 (HDAC10), 22 (HDAC11)	ND	IC_{50}: 0.14 to 0.38 μM (various cancer cell lines)	

Class	HDAC6 Inhibitor	Binding Domain	CI_{50} (nM) of the HDAC6 Activity *in Vitro*	Selectivity Ratio for HDAC6 Compared to (Other HDACs)	Inhibition of HDAC6 *in Cellulo* (μM)[$]	Effect on Cancer Cell Lines or Cancer Type	Ref
	Pyrimidinedione derivative (6)	ND	12.4	138 (HDAC1), 444 (HDAC2)	ND	Induces arrest of the cell cycle in subG1 phase and death by apoptosis (colon cancer)	[
	Quinazolin-4-one derivative (3f)	ND	29	65 (HDAC1), 222 (HDAC2), 60 (HDAC18), 141 (HDAC11)	Increases acetylation levels of α-tubulin and histone H3 at 10 μM	ND	
	Sulfone derivative (36)	ND	8	138 (HDAC8), 300 (HDAC11)	0.01 (unspecified)	ND	
	Trichostatine A derivatives (M344, 16b)	ND	88	3 (HDAC1)	ND	ND	
	Tubacin derivative (WT-161)	Phe200, Phe201, Leu270, Arg194 of HDAC7	0.4	129 (HDAC3)	0.3 (multiple myeloma)	IC_{50} = 3.6 μM (multiple myeloma)SangtingTaoCI_{50}: 1.5 to 4.7 μM (multiple myeloma cell lines)	
	Tubastatin A derivative (Marbostat-100)	Asp649, His651 et Asp742 of DD2	0.7	1106 (HDAC2), 247 (HDAC8)	0.05 (acute monocytic leukemia)	Non-cytotoxic	
	Indolylsulfonylcinnamic hydroxamate (12)	ND	5.2	60 (HDAC1), 223 (HDAC2)	0.1 (colon cancer)	IC_{50}: 0.4 to 2.5 μM (multiple cancer cell lines)	
	MAIP-032	DD2	58	38 (HDAC1)	ND	CI_{50}: 3.87 μM (squamous cell carcinoma line of the tongue)	
	MPT0G211	ND	0.291	ND	0.1 (neuroblastoma)	ND	
	N-hydroxy-4-[(N(2-hydroxyethyl)-2-phenylacetamido)methyl)-benzamide)] (HPB)	His573 and His574 of DD2	31	37 (HDAC1)	8 (prostate cancer)	ND	[
	N-hydroxy-4-(2-[(2-hydroxyethyl) (phenyl)amino]-2-oxoethyl)benzamide (HPOB)	Binding to zinc ion only via its OH group but does not displace the zinc-bound water molecule	56	52 (HDAC1)	16 (prostate cancer, adenocarcinoma, glioblastoma)	Increases the effect on cell viability in combination with etoposide, dexamethasone or SAHA	[
	N-hydroxy-4-(2-methoxy-5-(methyl(2-methylquinazolin-4-yl)-amino)phenoxy)butanamide (23bb)	Tyr298 and Glu255 of an HDAC6 homology model	17	25 (HDAC1), 200 (HDAC8)	0.051 (cervical cancer)	IC_{50}: 14 to 104 nM (various cancer cell lines)	
	Nexturastat A	DD2 of an HDAC6 homology model	5	604 (HDAC1)	0.01 (murine melanoma)	IC_{50} = 14.3 μM (melanoma)	[

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	Oxazole hydroxamate (4g)	Phe620, Phe680, Leu749, and Tyr782 of DD2 of an HDAC6 homology model	59	237 (HDAC1, 8)	10 (cervical cancer)	IC_{50} = 10.2 μM (acute myeloid leukemia)	
	Ricolinostat (ACY-1215)	DD2 of an HDAC6 homology model	4.7	12 (HDAC1), 10 (HDAC2), 11 (HDAC3), 1490 (HDAC4), 1064 (HDAC5), 298 (HDAC7), 21 (HDAC8), >2000 (HDAC9, 11)	0.62 (multiple myeloma)	CI_{50}: 2 to 8 μM (multiple myeloma cell lines)	[57
	Sahaquine	ND	ND	ND	0.1 (glioblastoma)	CI_{50}: 10 μM (glioblastoma)	
	TC24	Ser568, His610, Phe679 and Tyr782 of HDAC6	ND	ND	1 et 10 (gastric cancer)	CI_{50}: 10.2 to 17.2 μM (several gastric cancer cell lines)	
	Tetrahydroisoquinoline (5a)	ND	36	1250 (HDAC1), >1000 (HDAC2, 4, 5, 7, 10, 11), 1278 (HDAC3), 58 (HDAC8)	0.21 (cervical cancer)	ND	
	Thiazole	ND	52	ND	ND	ND	
	Tubacin	DD2 of an HDAC6 homology model	4	350 (HDAC1)	5 (prostate cancer)SangtingTao2.5 (acute lymphoblastic leukemia)	IC_{50}: 1.2 to 2 μM (acute lymphoblastic leukemia)	[57
	Tubastatin A	His610, His611, Phe679, Phe680 and Tyr782 of HDAC6	15	1093 (HDAC1)	2.5 (unspecified)	ND	[5
	Tubathian A	ND	1.9	5790 (HDAC1)	0.1 (ovarian cancer)	ND	
Other	3-hydroxypyridine-2-thione (3-HPT)	Tyr306 of HDAC8	681	5 (HDAC8)	ND	Inactive against two prostate cancer cell lines and one acute T cell leukemia cell line	

Class	HDAC6 Inhibitor	Binding Domain	CI_{50} (nM) of the HDAC6 Activity *in Vitro*	Selectivity Ratio for HDAC6 Compared to (Other HDACs)	Inhibition of HDAC6 *in Cellulo* (μM)[$]	Effect on Cancer Cell Lines or Cancer Type	Ref
	1-hydroxypyridine-2-thione (1HPT)-6-carboxylic acid	DD	150	287 (HDAC1), 4733 (HDAC2), 473 (HDAC4), 233 (HDAC5), 1933 (HDAC7), 22 (HDAC8), 313 (HDAC9)	ND	CI_{50}: 18 to 75 μM (leukemia)	
	Adamantylamino derivative (20a)	ND	82	46 (HDAC1), 51 (HDAC4)	ND	ND	
	Mercaptoacetamide derivative (2b)	ND	1.3	3615 (HDAC1)	10 (primary rat cortical culture)	ND	
	Sulfamide derivative (13e)	ND	440	>23 (HDAC1)	1 (bladder cancer)	ND	
Undefined structure	CKD-506	ND	5	>400 (HDAC1, 2, 7, 8)	0.03 (Human PBMCs)	ND	

Arg: arginine; Asp: aspartic acid; CI_{50}: concentration inhibiting 50% of cell viability; DD: deacetylase domain; Glu: glutamic acid; HDAC: histone deacetylase; His: histidine; IC_{50}: concentration inhibiting 50% of cell growth; Leu: leucine; ND: non determined; PBMC: peripheral blood mononuclear cell; Phe: phenylalanine; Pro: proline; SAHA: suberoylanilide hydroxamic acid; Ser: serine; Tyr: tyrosine.

The compounds ACY-241 (Citarinostat) and ACY-1215 (Ricolinostat) are derivatives of hydroxamic acid, which shows a specific inhibitory activity against HDAC6 with IC_{50} values of 2.6 and 5 nM, respectively. They are the only HDAC6 inhibitors in currently clinical trials (Table 6) [58][88]. To date, no HDAC6 inhibitor has yet been approved by the FDA, unlike pan HDAC inhibitors such as romidepsin, suberoylanilide hydroxamic acid (SAHA, vorinostat), PXD101 (belinostat), and LBH589 (panobinostat) [101].

Table 6. HDAC6 Inhibitors in Clinical Trials in Cancer. Clinical studies include four phases. Phase I is performed on healthy volunteers to determine the maximum tolerated dose in humans. Phase II is performed on a limited patient population to determine the optimal dosage. Phase III is performed on several thousand patients and will demonstrate the therapeutic value of the drug and assess its benefit/risk. Phase IV is performed once the drug is marketed and allows to better characterize its adverse effects.

HDAC6 Inhibitor	Clinical Trial Identification	Phase of the Clinical Trial	Pathology
ACY-241	NCT02400242	Ia/Ib	Multiple myeloma
	NCT02935790	Ib	Stage III and IV unresectable melanoma
	NCT02551185	Ib	Advanced solid tumors
	NCT02635061	Ib	Non-resectable non-small cell lung cancer
ACY-1215	NCT02632071	Ib	Unresectable or metastatic breast cancer
	NCT02787369	Ib	Relapsed chronic lymphocytic leukemia
	NCT02091063	Ib/II	Relapsed or refractory lymphoid malignancies
	NCT01997840	Ib/II	Recurrent and refractory multiple myeloma
	NCT01583283	I/II	Multiple myeloma recurrent or recurrent and refractory
	NCT02189343	Ib	Recurrent and refractory multiple myeloma
	NCT01323751	I/II	Multiple myeloma recurrent or recurrent and refractory
	NCT02856568	Ib	Unresectable or metastatic cholangiocarcinoma

HDAC6 Inhibitor	Clinical Trial Identification	Phase of the Clinical Trial	Pathology
	NCT02661815	Ib	Ovarian cancer, primary peritoneal cancer or platinum-resistant fallopian tubes

It is important to note that inactivation of HDAC6 protein in mice does not result in abnormal development or major organ problems [94], suggesting that HDAC6 inhibition would have few side effects, unlike pan-HDAC inhibitors.

3.2 HDAC6 inhibitors in solid cancers

Despite the observation of a moderate overexpression of the HDAC6 protein in urothelial cancerous tissues, the inhibition of the protein had limited efficacy compared to the use of inhibitors targeting several HDACs [102]. On the other hand, HDAC6 inhibitors have notable anti-cancer properties in prostate cancer [64], breast cancer [103], melanoma [66], and ovarian cancer [29]. These effects could be explained by the implication of HDAC6 in metastasis formation by epithelial-mesenchymal transition induction via its recruitment by TGFβ [104], in cell migration via α-tubulin deacetylation and in angiogenesis via cortactin deacetylation [105]. In contrast to some selective HDAC6 inhibitors, currently approved pan-HDAC inhibitors failed to show any clinical benefits in solid tumors [94]. The reasons of such therapeutic failures, compared to the treatment of leukemia and lymphoma, are not fully understood; however, some hypotheses have been raised. For example, the hypothesis of some researchers is based on the cellular composition of solid tumors, which tend to arise from more differentiated cells with reduced epigenetic reprogramming capacity [106]. In addition, solid tumor complexity including genomic, epigenomic, and phenotypical changes, can be a part of the explanation [107]. Moreover, the lack of response of solid tumors treated with HDAC inhibitors could be due to the pharmacokinetic profile of those drugs, which generally have a short half-life [108]. For such reasons, some researchers are investigating new methods and routes of administration of these inhibitors. Accordingly, Wang et al. have demonstrated that the use of nanoparticles to administrate HDAC inhibitors allowing a slow release of the drug directly in solid tumors could induce a higher therapeutic efficacy than classic administration routes [109].

3.3. HDAC6 inhibitors in hematological malignancies

Similar to pan-HDAC inhibitors approved for the treatment of hematological cancers, specific HDAC6 inhibitors showed anti-cancer properties in various cancer types such as multiple myeloma [110], chronic lymphocytic leukemia [42], acute myeloid leukemia (AML) [111], acute lymphoblastic leukemia (ALL) and chronic myeloid leukemia (CML) [112].

3.3.1. Nuclear HDAC6 and Its Implication in Leukemia

The cytoplasmic localization of HDAC6 is well described. In leukemia cells, a significant amount of nuclear HDAC6 was revealed, considering the interaction between the nuclear localization sequence (NLS) of HDAC6 and importin-α, which translocates HDAC6 into the nucleus. This region can be heavily acetylated resulting in a reduction of the NLS-importin-α interaction [113]. Consequently, the presence of HDAC6 in the nucleus of leukemia cells could be explained by red levels of acetylation within the NLS region, compared to other cell types. After nuclear translocation, HDAC6 interacts with nuclear proteins, transcriptional repressors, and transcription factors to regulate gene expression. For example, HDAC6 inhibition has been linked to increased expression of the pro-apoptotic protein BIM in acute myeloid leukemia cells [114]. Moreover, in a model exhibiting significant nuclear HDAC6 levels, chemical HDAC6 inhibition reduces its nuclear localization and p53-HDAC6 interactions inducing cell cycle arrest and apoptosis via changes of p53 target gene expression [115]. The specific nuclear localization of HDAC6 in leukemia cells might offer a therapeutic advantage to specifically target those cells.

3.3.2. HDAC6 in CML

3.3.2.1.Degradation of BCR-ABL via Deacetylation of HSP90α by HDAC6 in the Cytoplasm

Although little research exists on HDAC6 in the context of CML, this protein has a function that makes it particularly interesting in the context of such pathology. HDAC6 deacetylates heat shock protein (HSP90)α, which is involved in the stabilization of the oncogenic tyrosine kinase *breakpoint cluster region-Abelson* (BCR-ABL) protein [114] protein. In the acetylated form, HSP90α loses its chaperone function, which leads to the degradation of its client proteins by the proteasome (Figure 3A). The importance of the acetylation status of HSP90α in the protein degradation of BCR-ABL makes HDAC6 inhibitors potentially promising molecules for the treatment of CML. Pan-HDAC inhibitors are capable of inducing the inhibition of HDAC6, as well as the downregulation of HDAC6 using si-RNA, which increases the acetylation of HSP90α, and in turn increases the ubiquitination of the BCR-ABL protein, decreasing its expression in K562 cells [116] [117].

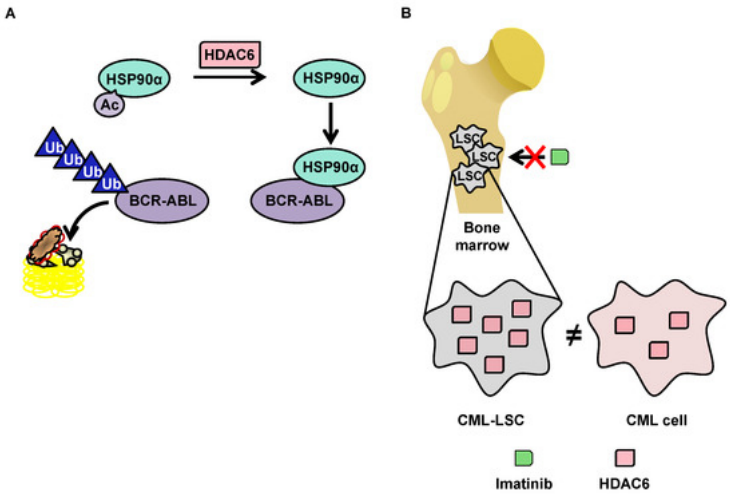

Figure 3. Role of HDAC6 in chronic myeloid leukemia. (**A**) HDAC6 is implicated in proteasome-dependent protein degradation by its interaction with HSP90α, a chaperone protein which, when deacetylated by HDAC6, is involved in the stabilization of BCR-ABL. In its acetylated (Ac) form, HSP90α loses its chaperone function, which leads to ubiquitination (Ub) and subsequent degradation of BCR-ABL by the proteasome. (**B**) Imatinib-insensitive chronic myeloid leukemia (CML) leukemic stem cells (LSCs) overexpress HDAC6 compared to CML cells.

3.3.2.2. OverExpression of HDAC6 in CML Stem Cells

LSCs that are not targeted by TKI and are characterized by a capacity for self-renewal play a crucial role in CML relapse. Although HDAC6 is necessary for the repression of genes involved in the differentiation targeted by the Tip60-p400 complex in embryonic stem cells (ESCs) [118], no study has provided evidence for this in LSCs, more differentiated. In contrast, studies have shown that several proteins in the HDAC family are overexpressed in LCSs of CML. Indeed, SIRT1 is activated by BCR-ABL via STAT5 and its expression is increased in LSCs compared to in CML cells [119]. Finally, overexpression of isoforms of HDAC (HDAC1, HDAC2, HDAC3, HDAC4, and HDAC5) and in particular HDAC6 was more frequently observed in LSCs ($CD34^+$ $CD38^-$) isolated from patients with CML than in K562 cells [45] (Figure 3B), making it a protein of interest in the search for treatments to prevent relapse in patients with CML.

3.3.3 HDAC6 inhibitors in CLL

HDAC6 is upregulated in CLL patient samples, cell lines, and euTCL1 transgenic mouse models compared to normal controls. Accordingly, this pathology could be an interesting target for selective HDAC6 inhibitors, as genetic silencing of HDAC6 improves the survival of euTCL1 mice. Moreover, the chemical inhibitor ACY738 reduces the proliferation of CLL B cells leading to their apoptosis. Together with ibrutinib, this HDAC6 inhibitor triggers synergistic cell death in vivo [120]. Beyond the direct effect on pathological B cells, HDAC6 inhibition improves CLL-induced immunosuppression of CLL T cells. HDAC6 inhibitors enhances immune checkpoint blockade in CLL so that combination treatment with ACY738 potentializes the in vivo antitumor effect of anti-PD-1 and anti-PD-L1 antibody treatments with increased cytotoxic CD8+ T cells [120].

3.3.4 HDAC6 inhibitors in AML

Several HDAC6 inhibitors were assessed as single agents in AML. The HDAC6 inhibitor ST80 shows potent antileukemic activity in myeloid cell lines and primary AML blasts at low micromolar concentrations, leading to preferential acetylation of a-tubulin [121]. HDAC inhibitors with a central naphthoquinone structure selectively inhibit HDAC6 in the AML cell line MV4-11, further decreasing mutant FLT-3 protein and constitutively active signal transducer and activators of transcription (STAT)5 levels and reducing extracellular regulated kinase (ERK) phosphorylation [122]. HDAC inhibitors with the 2-(oxazol-2-yl)phenol moiety as a novel zinc-binding group exhibited selective inhibition against HDAC1 and class IIb HDACs (HDAC6 and HDAC10) in the MV-4-11 AML cells [123]. The in vivo potency of the selective and orally-available HDAC6 inhibitor N-Hydroxy-4-(2-methoxy-5-(methyl(2-methylquinazolin-4-yl)amino)phenoxy)butanamide 23bb was better against MV4-11 AML cells compared to SAHA or ACY-1215 [124]. The O-aminobenzamide-based HDAC inhibitor compound 13e down-regulates HDAC6 in MV4-11 cells. 13e induces apoptotic cell death and cycle arrest most likely mediated by a p53-dependent pathway [125]. The parthenolide-SAHA hybrid compound 26 more potently reduces the viability of the resistant HL-60/ADR AML cell line compared to SAHA, triggering intrinsic apoptosis and reducing the protein expression levels of HDAC1, HDAC6 and the multidrug resistance-associated protein 1 (ABCC1) leading to an intracellular accumulation of drugs [126]. The HDAC6-selective inhibitor PTG-0861 induces apoptosis in MV4-11 AML cells with limited cytotoxicity against non-malignant cells [127].

In AML, inhibition of HDAC6 was essentially investigated in combination with other pharmacologically active compounds at a pre-clinical level. For instance, a combination of 17-(allylamino)-17-demethoxygeldanamycin (17-AAG), a synthetic derivative of the ansamycin benzoquinone antibiotic geldanamycin, with the HDAC6 inhibitor tubacin reduces the viability of primary AML samples, validating HDAC6 as a HSP90 client protein also in AML and that its hyperacetylation facilitates the anticancer potential of 17-AAG [128]. LBH-589 and PXD101 inhibit HDAC1 and HDAC6 and synergize with cytarabine to induce cell death in pediatric AML, accompanied by DNA damage induction and increased Bim expression levels [129].

Similarly, Bim protein induction and inhibition of nuclear factor-kappa B (NF-kB) pathway were identified as a mechanistic basis for the synergistic anti-cancer effects of belinostat in combination with the proteasome inhibitor bortezomib in AML and ALL cells [130]. The selective JAK2/HDAC6 dual inhibitor 20a shows excellent in vivo antitumor efficacy in HEL AML mouse xenograft assays and synergizes with the antifungal drug fluconazole [131]. The selective HDAC6 inhibitor MPT0G211 combined with doxorubicin displays anti-cancer effect by inducing a DNA damage response associated with increased Ku70 acetylation and BAX activation in HL-60 and MOLT-4 AML cell lines. Accordingly, ectopic expression of HDAC6 successively reverses the apoptosis triggered by the combined treatment [132].

3.3.5 HDAC6 inhibitors in ALL

The HDAC6 inhibitor tubacin enhances the anti-cancer effects of the Na+/K+-ATPase inhibitor ouabain or the proteasome inhibitor MG-13 against pre-B and T ALL cells in vitro and in vivo. These results suggest that selectively targeting HDAC6 alone or in combination with conventional chemotherapeutic drugs could provide a novel approach for ALL therapy [133]. Similarly, belinostat synergizes with the proteasome inhibitor bortezomib to kill ALL cells through Bim up-regulation and NF-kB inhibition. Altogether the perturbation of intracellular microtubular transport network, combined with the interference with protein homeostasis via proteasomal inhibition, could be a general and efficient mechanism explaining the synergistic effect observed [130]. MPT0G211 combined with vincristine interrupts ALL mitosis via interference with microtubular dynamics leading to apoptosis. In vivo, MPT0G211 plus doxorubicin or vincristine reduces tumor growth xenograft models [132].

Remarkably, it has been shown that the inhibition of HDAC6 using either the pan-HDAC inhibitor trichostatin, the selective HDAC6 inhibitor tubacin, or a genetic knock-down efficiently reduces Notch3 signaling through a post-translational-mediated protein down-regulation, leading to enhanced apoptosis in T-ALL cells and impairing leukemia growth in mice xenografted with T-ALL cell lines and primary human T-ALL cells. These results highlights the therapeutic potential of HDAC6 targeting in Notch3-addicted tumors [134].

3.3.6 HDAC6 inhibitors in other hematological malignancies

Inhibition of HDAC6 activity increases CD20 levels in B-cell tumor cell lines and malignant patient cells, potentializing the in vivo effect of anti-CD20 monoclonal antibodies like rituximab. Translation of CD20 mRNA is significantly enhanced after HDAC6 inhibition as CD20 mRNA was abundant within the polysomal fraction, indicating a post-transcriptional function of HDAC6. Collectively, these findings suggest HDAC6 inhibition is a rational therapeutic strategy to be implemented in combination therapies with anti-CD20 monoclonal antibodies and open up novel avenues for the clinical use of HDAC6 inhibitors [135].

The HDAC6 inhibitor A452 combined with the Bruton's tyrosine kinase inhibitor ibrutinib efficiently kills non-Hodgkin lymphoma cells, including follicular lymphoma [135].

The HDAC6 inhibitor KT-531 displays the highest anti-cancer potency against T-cell prolymphocytic leukemia (T-PLL) cells compared to other hematological neoplasms, together with safe differential toxicity compared to non-transformed cell lines. Accordingly, HDAC6 is overexpressed in primary T-PLL patient samples in which KT-531 exerts a potent anti-cancer activity. Moreover, a combination of KT-531 with various approved drugs including bendamustine, idasanutlin, and venetoclax shows promising synergistic effects against T-PLL patient cells [136].

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