

Expert Opinion

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Histone deacetylase inhibitors: possible implications for neurodegenerative disorders

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During the past six years numerous studies identified HDAC inhibitors as candidate drugs for the treatment of neurodegenerative disorders. Two major neuroprotective mechanisms of HDAC inhibitors have been identified, namely the transcriptional activation of disease-modifying genes and the correction of perturbations in histone acetylation homeostasis, which have been shown to be intimately involved in the neurodegenerative pathomechanisms of Huntington's, Parkinson's and Kennedy disease, amyotrophic lateral sclerosis, Rubinstein-Taybi syndrome as well as stroke. Based on the promising *in vitro* and *in vivo* analyses, clinical trials have been initiated to evaluate the safety and efficacy of HDAC inhibitors for the treatment of devastating diseases such as Huntington's disease, amyotrophic lateral sclerosis and spinal muscular atrophy. Here, the authors summarize and discuss the findings on the emerging field of epigenetic therapy strategies in neurodegenerative disorders.

Keywords: adrenoleukodystrophy, Alzheimer's disease, amyotrophic lateral sclerosis, dentatorubral-pallidolusian atrophy, depsiptide, histone acetyltransferase, histone deacetylase, histone deacetylase inhibitor, phenylbutyrate, romidepsin, sodium butyrate, spinal muscular atrophy, spinocerebellar ataxia, suberoylanilide hydroxamic acid, valproic acid

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1. Introduction

DNA within the eucaryotic nucleus is compacted through its association with two copies of the highly basic histone proteins H2A, H2B, H3 and H4 to form nucleosome core particles that, together with linker DNA and linker histone, assemble into a dynamic structure known as chromatin. Histones are subjected to extensive post-translational modifications that involve the addition or removal of acetyl, methyl or phosphate groups as well as the reversible transfer of the ubiquitin and sumo proteins. The acetylation and deacetylation of histones has shown to play an important role in transcription regulation of eukaryotic cells and is determined by histone deacetylases (HDACs) and histone acetyltransferases (HATs) which exert opposite activities. HATs add acetyl groups to lysine residues, whereas HDACs remove the acetyl groups. In general, histone acetylation promotes a more relaxed chromatin structure, allowing transcriptional activation. HDACs can act as transcription repressors, due to histone deacetylation, and consequently promote chromatin condensation. At present, there are no reports of small molecules that augment HAT function but numerous small-molecule HDAC inhibitors belonging to different chemical classes have been developed that allow the modulation of chromatin structure in a transient manner. HDAC inhibitors are by no means a brand new group of compounds, considering the fact that the first compound bearing an HDAC inhibitory function, sodium butyrate (SB), was identified in the late 1970s. However, the development of

1 potent and well-tolerated HDAC inhibitors gained a
considerable momentum due to the observation that these
drugs have a substantial antitumor activity. In 2006, the
5 drug development efforts culminated in the FDA granting
approval for the potent HDAC inhibitor vorinostat
(suberoylanilide hydroxamic acid, SAHA), whereas numerous
other compounds such as romidepsin (FK-228, depsipeptide)
and SNDX-275 (MS-275) are under clinical investigation
10 for the treatment of various types of cancer. However,
intriguing preclinical evidence accumulated during the past
six years indicating that HDAC inhibitors may be used for
the treatment of numerous neurodegenerative disorders and
based on these findings, clinical trials have been initiated to
15 evaluate the safety and efficacy of HDAC inhibitors for
the treatment of devastating disorders such as Huntington's
disease (HD), amyotrophic lateral sclerosis and spinal muscular
atrophy (SMA).

2. Histone deacetylases

20 The family of HDACs has already been extensively reviewed
and is thus only briefly described [1]. In humans, 18 HDAC
enzymes have been identified and classified dependent on
cofactor dependency and sequence similarity. Generally, two
25 HDAC families are discriminated: the classical HDACs
which require Zn^{2+} for deacetylase activity and the sir2-related
HDACs (sirtuins) which require NAD^+ as cofactor. Based
on their homology to yeast HDACs, the classical HDACs
have been further subdivided into class I, IIa, IIb and IV.
30 The class I HDACs include HDAC1, HDAC2, HDAC3
and HDAC8, which are related to the yeast RPD3 deacetylase.
Although HDAC3 is able to shuttle between the nucleus
and the cytoplasm, the other three class I family members
are found primarily in the nucleus [2,3]. Class II HDACs are
35 related to yeast HDAC1 and include six HDAC isoenzymes.
This class is further subdivided into class IIa, consisting of
HDAC4, HDAC5, HDAC7 and HDAC9, and class IIb,
consisting of HDAC6 and HDAC10 which contain two
catalytic sites as unique feature. Class II HDACs can be
40 primarily cytoplasmic and/or migrate between the cytoplasm
and nucleus similar to the class I family member HDAC3.
Several class II HDACs have been shown to interact with
class I HDAC3 indicating a tight functional connection
between class I and II isoenzymes. Class IV is represented
45 by HDAC11 which contains conserved residues in the
catalytic core regions shared by both class I and class II
HDAC enzymes [4]. The NAD^+ -dependent sirtuin family of
HDACs (class III) is composed of seven members (SIRT1
to SIRT7) which are homologs of the yeast SIR2. Sirtuins
50 have gained considerable attention as they may provide
novel targets for diseases associated with ageing [5]. During
recent years intriguing evidence accumulated that showed
that predominantly the classical HDACs represent highly
promising target proteins for the potential treatment of
55 numerous neurodegenerative conditions. These insights are

mainly driven by the finding that small-molecule inhibitors
of classical HDACs exhibit definite neuroprotective properties
in vitro and *in vivo*, although the specific role of each
HDAC isoenzyme remains elusive in most cases.

3. Histone deacetylase inhibitors

With a single exception, as discussed later, small-molecule
HDAC inhibitors investigated for the potential treatment of
neurodegenerative disorders do not affect sirtuin (class III
HDAC) activities. These compounds, which considerably
differ in potency and HDAC isoenzyme selectivity, belong
to four different classes, namely the short chain fatty acids,
hydroxamic acids, benzamides and cyclic tetrapeptides.
The fatty acid group of HDAC inhibitors comprises the
compounds SB, phenylbutyrate (PB) and valproic acid
(VPA). In 1977, Riggs and co-workers [6] linked the
previously reported antiproliferative action of SB to histone
hyperacetylation suggesting an HDAC inhibitory function.
Today, the SB derivative PB is an orphan drug which
achieved FDA approval for the treatment of urea cycle
disorders. VPA, a commonly used anticonvulsant and mood
stabilizer, achieved FDA approval in 1987 and its HDAC
inhibitory function was discovered in 2001 [7,8]. Using
HDACs isolated from rat liver, SB and VPA inhibit total
HDAC activity incompletely and at comparatively high
millimolar concentrations (Figure 1A), which is in line with
a pronounced class I selectivity shown for VPA (Table 1).
This is somewhat in contrast to the observation that VPA
and SB alter histone acetylation levels already at submillimolar
doses in single cell cultures [7,8], which suggests that
mechanism(s) other than direct interference with the catalytic
activity of HDAC isoenzymes are involved. Indeed, Kramer
and co-workers [9] have shown that VPA and SB (but not
TSA and SNDX-275, see below) reduce HDAC2 (class I)
protein levels. For VPA, this effect has been shown to be
based on proteosomal degradation of HDAC2 but not of
other class I HDAC isoenzymes [9]. Thus, the fatty acids
VPA and SB might be considered as class I-selective HDAC
inhibitors with a pronounced activity against HDAC2 at
submillimolar doses. Prominent members of the hydroxamic
acid group of HDAC inhibitors are trichostatin A (TSA)
and vorinostat. TSA, a natural product isolated from
a *Streptomyces hygroscopicus* strain, was identified as a
fungistatic antibiotic in 1976, and its activity was linked to
HDAC inhibition by Yoshida and co-workers in 1990 [10,11].
TSA is a highly potent pan-HDAC inhibitor active already
at low nanomolar doses (IC_{50} : 12 nM, [12]). Vorinostat
(SAHA) [13] achieved FDA approval in 2006 for the
treatment of cutaneous T-cell lymphoma. Like several other
hydroxamic acids (such as scriptaid, oxamflatin,
carboxycinnamic acid bishydroxamic acid and suberic
bishydroxamic acid), vorinostat is a pan-HDAC inhibitor
active at submicromolar concentrations (Figure 1B), whereas
neither TSA nor vorinostat appear to show profound HDAC

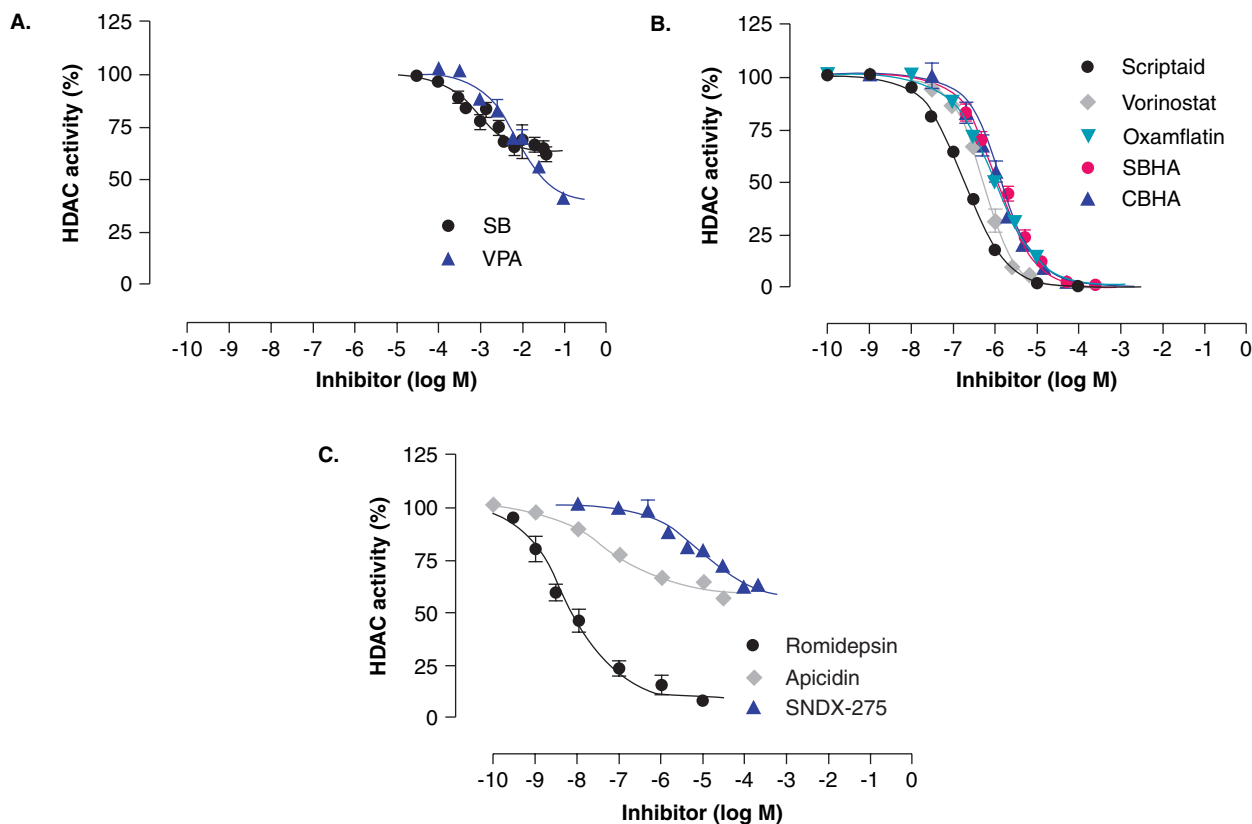


Figure 1. HDAC inhibition by the test compounds was investigated *in vitro* as described [84,118] using histone deacetylases purified from rat liver. Detailed quantitative analysis confirmed a concentration-dependent inhibition of total HDAC activity in all experiments, though with considerable variations in potency and efficacy. **Fattyacids (1A):** Inhibition of total HDAC activity by sodium butyrate (SB) and valproic acid (VPA) plateaued at $64 \pm 2\%$ and $40 \pm 3\%$ of control HDAC activity, respectively, suggesting a pronounced HDAC isoenzyme selectivity (means \pm SE, $n = 3 - 6$ experiments performed in duplicate). For both compounds half maximum inhibition (IC_{50}) was observed at millimolar doses. Given are $(-\log)$ mean IC_{50} values \pm SE throughout, the corresponding molar concentration is stated in brackets: SB: 3.10 ± 0.09 (0.80 mM); VPA: 2.14 ± 0.07 (7.24 mM). **Hydroxamic acids (1B):** The hydroxamic acids investigated inhibited HDAC activity completely at comparatively low concentrations, with a half maximum inhibition observed at μ molar doses (scriptaid: 6.73 ± 0.02 (0.19 μ M); vorinostat (SAHA): 6.30 ± 0.02 (0.50 μ M); oxamflatin: 6.01 ± 0.02 (0.98 μ M); carboxycinnamic acid bishydroxamic acid: 5.94 ± 0.02 (1.15 μ M) suberic bishydroxamic acid: 5.91 ± 0.03 (1.23 μ M). **Cyclic tetrapeptides/benzamides (1C):** The cyclic tetrapeptide romidepsin (FK-228) inhibited HDAC activity almost completely (bottom: $9 \pm 3\%$) with a half maximum inhibition observed 8.25 ± 0.09 (5.62 nM). In contrast, inhibition of total HDAC activity by apicidin (cyclic tetrapeptide) and SNDX-275 (MS-275, benzamide) plateaued at $57 \pm 3\%$ (apicidin) and $54 \pm 5\%$ (SNDX-275) of control HDAC activity, respectively, suggesting a pronounced HDAC isoenzyme selectivity. Half maximum inhibition of HDAC activity was observed at 7.26 ± 0.21 (0.06 μ M) for apicidin and 5.03 ± 0.21 (9.33 μ M) for SNDX-275. Romidepsin was kindly provided by Gloucester Pharmaceuticals, Cambridge, MA, USA. HDAC: Histone deacetylases.

1 isoenzyme selectivities (Table 1). The cyclic tetrapeptide
romidepsin (FK-228, depsipeptide), also a natural drug,
is active at low nanomolar doses (Figure 1C) showing
5 little activity against HDAC6 (Table 1), while the cyclic
tetrapeptide apicidin appears to be highly selective with a
pronounced activity against class I HDAC2, HDAC3 and
HDAC8 (Table 1). Similar to apicidin, the benzamide
SNDX-275 (MS-275) represents a highly selective
HDAC inhibitor (Figure 1C) and does not affect HDAC8
10 (class I) or HDAC 4/6/7 (class II) activities (Table 1) [14].
These data demonstrate that the HDAC inhibitors
frequently used in experimental settings differ in their

potency and HDAC isoenzyme selectivity. Regarding the
published patent literature of the last years, a plethora of
new HDAC inhibitors has been developed and numerous
HDAC inhibitors belonging to all four chemical classes
(including PB, VPA, SAHA, FK-228, MS-275) are under
clinical development for cancer treatment [1]. However,
with respect to the potential treatment of neurodegenerative
conditions, the panel of HDAC inhibitors investigated is
limited and mainly focussed on the well-established
experimental drug TSA and the clinically used HDAC
inhibitors SB, PB, VPA and vorinostat, which are all known
to penetrate the blood-brain barrier [15,16].

Table 1. HDAC inhibitors differ in potency and HDAC isoenzyme selectivity.

Inhibitor	Class I IC ₅₀					Class IIa IC ₅₀					Class IIb IC ₅₀			Ref.
	HDAC1	HDAC2	HDAC3	HDAC8	HDAC9	HDAC4	HDAC5	HDAC7	HDAC9	HDAC6	HDAC10	HDAC6	HDAC10	
TSA	0.002 μM	0.003 μM	0.004 μM	0.456 μM	0.006 μM	0.006 μM	*	0.005 μM	0.006 μM	0.003 μM	*	0.003 μM	*	[14]
Vorinostat (SAHA)	0.068 μM	0.164 μM	0.048 μM	1.524 μM	0.101 μM	0.101 μM	*	0.104 μM	0.107 μM	0.090 μM	*	0.090 μM	*	[14]
Romidepsin (FK-228)	0.036 μM	0.047 μM	*	*	0.510 μM	0.510 μM	*	*	*	‡	*	‡	*	[12]
redFK-228*	0.002 μM	0.004 μM	*	*	0.025 μM	0.025 μM	*	*	*	0.790 μM	*	0.790 μM	*	[12]
Apicidin	‡	0.120 μM	0.043 μM	0.575 μM	‡	‡	*	‡	‡	‡	*	‡	*	[14]
SNDX-275 (MS-275)	0.181 μM	1.155 μM	2.311 μM	‡	‡	‡	*	‡	0.505 μM	‡	*	‡	*	[14]
VPA	1.6 mM	3.1 mM	3.1 mM	7.4 mM	*	*	*	‡	‡	‡	*	‡	*	[14]

*Not determined.

*On entering the cell, romidepsin (FK-228) is reduced to its active form, redFK-228.

‡No complete inhibition found at doses of 10 μM (romidepsin, apicidin, SNDX-275) or 100 mM (VPA).

HDAC: Histone deacetylases; IC₅₀: Half maximal inhibitory concentration; TSA: Trichostatin A; VPA: Valproic acid.

4. Histone deacetylase inhibition

Transcriptome-wide studies mainly performed using neoplastic cells have shown that HDAC inhibitors can affect the transcription levels of 7 – 10% of all genes [1]. However, there is increasing evidence that these changes are not solely based on histone hyperacetylation due to the observation that HATs and HDACs are not just for histones. Numerous non-histone protein targets of HDACs including transcription factors and regulators, signal transduction mediators, DNA repair enzymes, nuclear import regulators, chaperone proteins, structural proteins, inflammation mediators and viral proteins [1] have been identified which are likely to contribute to the hitherto reported effects induced by HDAC inhibitors. An additional mechanism of action is given by the finding that HDAC inhibitors counteract gene silencing by DNA methylation mediated by methyl-CpG-binding protein 2 (MeCP2). MeCP2 binds tightly to chromatin in a methylation-dependent manner and associates with a corepressor complex containing HDAC1 and HDAC2, suggesting that the fundamental mechanisms of epigenetic gene regulation, DNA methylation and histone acetylation, are linked by MeCP2 [17]. Jones and co-workers [18] demonstrated that gene silencing conferred by MeCP2 and methylated DNA can be relieved by HDAC inhibition using the pan-HDAC inhibitor TSA. A striking observation is that the consequences of HDAC inhibition are not limited to changes in protein acetylation but may also bring about changes in the state of DNA methylation. In single cell cultures, TSA and VPA have shown to trigger DNA-demethylation in a replication-independent manner [19-21] and DNA-demethylating activity of VPA, SNDX-275 and TSA has subsequently been confirmed *in vivo* [22,23]. However, Cameron and co-workers [24] reported that TSA alone is neither sufficient to modulate DNA methylation nor to transcriptionally reactivate heavily hypermethylated genes in neoplastic cells, indicating that CpG island methylation is the dominant mechanism of epigenetic gene silencing. Even though the exact mechanism by which HDAC inhibitors affect DNA methylation remains to be clarified, there is increasing evidence that HDAC inhibition may have manifold consequences, including the hyperacetylation of histone and non-histone proteins and the alleviation of DNA methylation.

5. Polyglutamine disorders

Polyglutamine (polyQ) expansion diseases [25] are a class of nine inherited neurodegenerative disorders that are known to be caused by mutations in polyglutamine-encoding CAG tracts in different genes that result in degeneration of different populations of neurons. These conditions include HD, Dentatorubral-Pallidoluysian atrophy (DRPLA), spinal and bulbar muscular atrophy (SBMA) and six spinocerebellar ataxias (types 1, 2, 3, 6, 7 and 17). All these disorders are

caused by expansions of polymorphic (CAG)_n repeats in the coding regions of the disease genes leading to long polyQ stretches, which confer a gain-of-function to the mutant proteins. As outlined subsequently, the polyQ disorders have many features in common and thus might be treatable by common pharmacologic interventions.

5.1 Huntington's disease

HD (MIM+143100) is caused by neuronal dysfunction and progressive neuronal cell death that is especially severe in the striatum. The disease is characterized by choreic movements, neuropsychiatric symptoms and severe cognitive deficits. HD follows an autosomal dominant mode of inheritance and is caused by expansion of a polymorphic (CAG)_n repeat in the coding region of the *huntingtin* (*HD*) gene. Although the range of CAG repeat numbers in exon 1 of the *HD* gene is < 35 in unaffected individuals, the CAG repeat number is expanded to > 40 and unstable in HD patients, with repeat length inversely correlating with age of disease onset [15,26]. The polyQ expansion in the mutant huntingtin protein (*htt*) leads to its aberrant proteolytic cleavage, resulting in the release of N-terminal fragments that readily enter the nucleus and form aggregates in brain tissue from affected patients [27,28]. As loss of neuronal huntingtin function may also contribute to HD [29], the precise mechanisms leading to neurodegeneration in HD have not been fully elucidated. However, there is increasing evidence that transcriptional dysregulation plays a pivotal role in HD pathogenesis [30-32]. In line with these findings, mutant *htt* fragments bearing polyQ expansions have shown to directly interact and impair the function of several nuclear proteins involved in the transcription machinery [33], including the transcription factor Sp1, its co-activator TAF_{II}130 [34,35] as well as HATs [36,37]. *In vitro* studies revealed that mutant *htt* fragments inhibit the HAT activity of CREB-binding protein (CBP), its close homolog p300 and CBP/p300-associated factor (P/CAF) [37] and reduce overall H3/H4 acetylation levels *in vitro* and *in vivo* [37-39]. Concordant with these findings, histones associated with downregulated genes have been recently shown to be hypoacetylated in HD models [40]. *In vitro* studies gave the first evidence that HDAC inhibitors (SB, TSA, vorinostat) are able to counteract H3/H4 hypoacetylation induced by mutant *htt* [37]. Even though the involvement of p300 appears to be questionable [41,42], these data highlight changes in chromatin texture to be intimately involved in HD pathogenesis and identified HDAC inhibitors as candidate drugs for HD therapy. By employing a *Drosophila* model, Steffan and co-workers [37] showed that SB and vorinostat decelerated rhabdome degeneration observed in transgenic flies expressing mutant *htt* fragments or polyQ peptides, whereas subsequent studies demonstrate neuroprotective effects of TSA in *Caenorhabditis elegans* models for HD [43,44]. The potential applicability of HDAC inhibitors for HD therapy was consecutively confirmed in transgenic mice which mimic

HD-like features. R6/2 mice express an N-terminal portion of human huntingtin with a polyQ stretch of approximately 150. R6/2 mice show spatial learning deficits starting at 3 – 4 weeks of age, followed by abnormal performance in motor tests, tremor, gait disturbances and premature death at 13 – 15 weeks of age. Hockly and co-workers [45] demonstrated that oral application of vorinostat (100 mg/kg/day, starting at 4 weeks of age) improved motor impairment (rotarod performance) in R6/2 mice. Using the same mouse model, intraperitoneal (i.p.) injection of SB (400 – 1200 mg/kg/day, starting at 3 weeks of age) increased lifespan and rotarod performance in a dose-dependent manner [38]. In addition, high doses of SB (1200 mg/kg/day) mitigated brain atrophy and bilateral ventricular hypertrophy observed in untreated R6/2 animals. By using a different mouse model expressing an N-terminal *htt* fragment containing 82 CAG repeats (N171-82Q), Gardian and co-workers [39] confirmed that administration of PB (100 mg/kg, 6 days/week, i.p., starting at 11 weeks of age) improved survival and attenuated gross brain atrophy and ventricular enlargement. In contrast to the vorinostat and SB studies, effects of PB on rotarod performance have not been observed which might be due to the late drug application after onset of symptoms [39]. Interestingly, none of the HDAC inhibitors tested so far attenuate mutant *htt* aggregation [38,39,45]. In all, these studies may suggest that the administration of HDAC inhibitors corrects global histone hypoacetylation induced by mutant *htt* and ameliorates the HD phenotype *in vivo*. Based on these findings a clinical Phase II trial has been initiated to assess the safety and tolerability of PB for potential HD treatment (clinicaltrials.gov; identifier: NCT00212316). A pilot trial using VPA for the adjuvant therapy of HD patients suffering from myoclonic hyperkinesias revealed improved motor scores following treatment. Even though myoclonus is rare in HD, these findings may further emphasize the potential use of HDAC inhibitors for HD therapy [46].

5.2 Dentatorubral-Pallidoluysian atrophy

DRPLA (MIM#125370) is an autosomal dominant disease characterized by a number of symptoms that include ataxia, chorea, seizure, myoclonus, incoordination and dementia. DRPLA is caused by CAG expansion within the *Atropin-1* gene. The polyQ stretch in the Atropin-1 protein ranges from 6 to 35 in normal individuals and expands from 48 to 88 in DRPLA patients. Neuropathological analyses of DRPLA patients identified widespread nuclear inclusion bodies in affected neurons with concomitant loss in specific brain regions such as the dentate cerebellar nucleus, red nucleus, globus pallidus and subthalamic nucleus. Even though different brain regions are affected in HD and DRPLA, both disorders show striking similarities on a molecular level. Comparative microarray analyses of cerebellar gene expression in HD and DRPLA mouse models indicated that most of the mRNAs that changed in response to the mutant *htt* transgene (N171-82Q mice) changed

1 similarly in response to the mutant *atropin-1* transgene
 (At-65Q mice) [31]. In line with these findings, mutant
 atropin-1 interacts and impairs the function of nuclear
 proteins involved in the transcription machinery, including
 5 the transcriptional co-activator TAF_{II}130 and the HAT CBP,
 suggesting both proteins to be crucially involved also in
 DRPLA pathogenesis [42,47]. Mutant atropin-1 co-aggregates
 with CBP in human DRPLA postmortem brain tissue,
 inhibits CBP-mediated transcription and causes cell death
 10 *in vitro*. Interestingly, overexpression of CBP rescued cells
 from mutant atropin-1 as well as mutant htt toxicity,
 providing further evidence that the impairment of CBP
 activity represents a common pathomechanism in HD and
 DRPLA [31]. Ying and co-workers [48] reported that SB
 15 ameliorates the DRPLA phenotype *in vivo* using transgenic
 mice expressing expanded (118Q) full-length *atropin-1*
 under the control of a neuron-specific promoter. Atro-118Q14
 mice developed a neurodegenerative phenotype including
 feet-clasping, tremor, ataxic gait and premature death with a
 20 mean lifespan of 22 weeks. Daily injection of SB (0.5 and
 1.5 mg/kg/day, i.p., starting from 4 weeks of age) mitigated
 motor impairments (rotarod performance, hanging wire)
 and increased average lifespan, while the nuclear accumulation
 of mutant atropin-1 was not altered. Interestingly, drug
 25 application after disease onset (12 weeks of age) extended
 the average lifespan of Atro-118Q14 mice but did not
 improve motor performance. Together with the *in vivo*
 data presented by Gardian and co-workers using a HD
 mouse model (N171-82Q), these data indicate that HDAC
 30 inhibitors may be effective in antagonising polyQ toxicity
 even after disease onset. Similar to HD, histone H3
 has shown to be hypoacetylated in brains of DRPLA mice,
 while H3 acetylation could be restored to control levels
 by SB treatment [48].

5.3 Spinal and bulbar muscular atrophy

35 SBMA (Kennedy disease, MIM#313200) is a slowly
 progressing polyQ disease that exclusively affects adult males.
 SBMA is characterized by proximal muscle weakness, atrophy
 and fasciculations of bulbar, facial and limb muscles mainly
 40 due to a loss of lower motor neurons in the anterior horn of
 the spinal cord. The disease is caused by CAG expansion
 in the first exon of the *androgen receptor (AR)* gene located
 on the X chromosome. In unaffected individuals, the
 CAG number ranges from 11 to 35, whereas repeat sizes
 45 of 40 – 62 cause SBMA. Very similar to HD and DRPLA,
in vitro studies revealed that a truncated *AR* construct bearing
 an expanded polyQ tract associates with the HAT CBP
 and induces H3 hypoacetylation associated with polyQ
 50 toxicity [49]. The toxicity of mutant AR fragments could be
 mitigated by either HDAC inhibitor treatment (TSA,
 vorinostat, PB) or CBP overexpression, further supporting
 the hypothesis that transcriptional dysregulation plays a
 pivotal role also in SBMA pathology [49]. H3 hypoacetylation
 55 has subsequently been confirmed *in vivo* using spinal cord

lysates of transgenic SBMA-like (AR-97Q) mice, although
 no differences were detected in H2A, H2B or H4
 acetylation levels [50]. By employing the AR-97Q mouse
 model expressing a full-length AR containing 97 CAGs,
 Minamiyama and co-workers [50] demonstrated that oral
 application of SB (4 and 8 g/l in drinking water, starting
 from 5 weeks of age) ameliorated the neuromuscular pheno-
 type (gait disturbances, rotarod performance) and survival
 rate of SBMA-like mice but did not inhibit nuclear aggregation
 of the mutant AR protein observed in the residual motor
 neurons and non-neuronal cells. Histologic analyses revealed
 that SB treatment restored H3 acetylation levels in nuclei
 of spinal cord motor neurons and increased the diameter
 of muscles, spinal roots and motor neurons as compared
 with non-treated SBMA mice [50].

5.4 Spinocerebellar ataxia 3

Spinocerebellar ataxia type 3 (SCA3, MIM#109150),
 also known as Machado–Joseph disease, is an autosomal
 dominant disorder characterized by progressive gait and limb
 ataxia and ocular movement abnormalities. SCA3 is caused
 by CAG repeat expansions in the *ataxin 3 (ATXN3)* gene
 resulting in polyQ stretches in the C-terminus of the
 encoded protein ataxin-3 (AT3). Very similar to the disease
 mechanism proposed for HD, DRPLA and SBMA, AT3
 binds to the HAT CBP as well as p300 and P/CAF and
 represses CBP, p300 and P/CAF-mediated transcription
 when overexpressed [51]. In contrast to HD, DRPLA and
 SBMA, these properties of AT3 appear to be not restricted
 to mutant AT3. Li and co-workers demonstrated that the
 C-terminus containing either a normal or pathological
 polyQ co-immunoprecipitated with CBP, p300 and
 P/CAF [51]. Interestingly, wild-type (Q28) and mutant
 (Q78) full-length AT3 were equally effective at inhibiting
 histone acetylation, a feature restricted to the respective
 pathogenic polyQ-expanded protein in HD, DRPLA and
 SBMA [51]. Evert and co-workers [52] provide *in vitro*
 evidence that ectopic expression of normal (Q23) but not
 mutant AT3 (Q70) induces histone H3 hypoacetylation,
 suggesting mechanistic differences in polyQ toxicity between
 HD, DRPLA and SBMA compared with SCA3 pathogenesis.
 Furthermore, the finding that histone H3 is hyperacetylated
 in pontine tissue of SCA3 patients may argue against HDAC
 inhibitors as potential drugs for SCA3 treatment [52].

5.5 Spinocerebellar ataxia 7

Autosomal dominant spinocerebellar ataxia 7 (SCA7,
 MIM#164500) is clinically characterized by progressive
 incoordination of gait and limb movements due to neuronal
 loss within the cerebellum and brainstem. A unique feature
 that distinguishes SCA7 from other polyQ disorders and
 hereditary ataxias is the degeneration of cone and rod
 photoreceptor neurons in the retina leading to blindness [53].
 The hypothesis that also SCA7 is caused by transcriptional
 dysregulation was supported by the finding that the disease

1 gene *ataxin-7* is an integral component of GCN5-containing
 5 protein complexes such as TFTC and STAGA [54]. GCN5 is
 a well-characterized HAT closely related to P/CAF and
 consequently TFTC/STAGA complexes contain HAT
 10 activity [54]. Even though normal and mutant ataxin-7 are
 found in GCN5-containing protein complexes, *in vitro* studies
 demonstrated that incorporation of polyQ-expanded
 ataxin-7 (92Q) into STAGA dramatically reduced its ability
 15 to acetylate free histone H3 [55]. By employing a *sca7Δ* yeast
 strain, McMahon and co-workers [56] show that the loss of
sca7 reduces the HAT activity of the yeast protein complex
 homologous to STAGA (SAGA) and demonstrate that
 20 normal (Q10) but not polyQ-expanded (Q60) human
 ataxin-7 is able to restore the yeast SAGA HAT activity
in vitro [56]. Moreover, ataxin-7 interacts with the photoreceptor-
 specific transcription factor CRX (cone-rod homeobox
 protein) while polyQ-expanded ataxin-7 antagonised CRX
 25 transactivation [57]. This finding and the observation that
 CRX mutations cause a cone-rod dystrophy phenotype
 suggest that retinal degeneration observed in SCA7 patients
 is mediated by CRX [57]. Concordantly, decreased transcript
 levels of CRX-regulated genes and genes with a retina-
 30 restricted expression have been observed in SCA7 mice
 expressing mutant ataxin-7 (Q92) [57,58]. In agreement with
 these findings, Palhan and co-workers [55] demonstrated a
 marked reduction of acetylated histone H3 in the promoter
 regions of the CRX-dependent photoreceptor genes in
 35 retinæ of SCA7-92Q mice. Thus, an increasing body of
 evidence suggests that SCA7 is caused by transcriptional
 dysregulation due to reduced HAT activity and argues for
 HDAC inhibition as promising pharmacologic intervention.
 However, Helmlinger and co-workers [58] presented opposing
 40 results. Using mice expressing normal (Q10) or mutant
 (Q92) ataxin-7 in rod photoreceptors, TFTC/STAGA
 complexes immunopurified from SCA7 mouse retina showed
 normal levels of HAT activity. Unexpectedly, genes which
 were downregulated in the retinæ of SCA7 mice have shown
 to be hyperacetylated, indicating that the exact causal
 45 relationship between histone acetylation and SCA7
 pathogenesis and, consequently, the potential use of HDAC
 inhibitors require further investigation.

5.6 HDAC inhibitors in polyQ disorders: does isoenzyme selectivity matter?

45 The outlined studies provide preclinical *in vitro* and *in vivo*
 evidence for the hypothesis that HDAC inhibitors might be
 further exploited to treat polyQ disorders such as HD,
 DRPLA and SBMA. In numerous *in vitro* and *in vivo*
 50 paradigms, HDAC inhibition reduced polyQ toxicity and
 ameliorated neurodegeneration. However, inhibition of HDAC6
 may have opposite effects. By employing a *Drosophila* SBMA
 model which ectopically expressed a mutated human AR
 in photoreceptor neurons, Pandey and co-workers
 55 demonstrated that overexpression of HDAC6 suppressed the
 degenerative phenotype in flies with polyQ-expanded AR,

whereas knockdown of endogenous HDAC6 enhanced
 neurodegeneration [59]. Kawaguchi and co-workers [60] identified
 HDAC6 as a crucial player in the cellular management of
 misfolded protein-induced stress. In addition, Iwata and
 co-workers [61] have shown HDAC6 activity to be required
 for autophagic degradation of aggregated huntingtin,
 suggesting a role for endogenous HDAC6 activity in
 protecting cells from polyQ toxicity. Isoenzyme selectivity of
 HDAC inhibitors becomes thus a vitally important issue.
 HDAC6 has shown to be nearly resistant to VPA, PB,
 SNDX-275 and apicidin, and displays a comparatively low
 susceptibility against romidepsin [8,14,62,63]. However, it
 remains elusive whether HDAC inhibitors that do not affect
 HDAC6 activity show superior neuroprotective effects
 compared with neuroprotective pan-HDAC inhibitors such
 as vorinostat and TSA [14]. Dompierre and co-workers have
 shown that inhibition of HDAC6 activity by vorinostat and
 TSA rescues vesicular transport defects observed in HD [64],
 suggesting a two edged role of HDAC6 in neurodegenerative
 disorders which might be further clarified *in vivo* using
 selective HDAC6 inhibitors such as tubacin [65]. Bates and
 co-workers [43] confirmed a neuroprotective effect of TSA
 by employing a *C. elegans* model for HD. Interestingly,
 RNAi-mediated knockdown of most *C. elegans* HDACs
 enhanced mutant htt toxicity whereas knockdown specifically
 of *hda-3* suppressed toxicity, supporting the idea that neuro-
 protective effects of HDAC inhibitors are mediated by
 specific HDAC isoenzymes only. In summary, these data
 suggest that further analysis of the specific role(s) of each
 HDAC isoenzyme in polyQ disorders and the decoding of
 the HDAC inhibitor selectivity represent important steps
 to identify and to develop optimized HDAC inhibitors
 for the potential treatment of polyQ disorders and other
 neurodegenerative conditions.

6. Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative
 disorder characterized by the death of motor neurons in the
 brain, brainstem and spinal cord, resulting in fatal paralysis.
 ALS usually begins with asymmetric involvement of the
 muscles in middle adult life. Approximately 10% of ALS
 cases are familial with an autosomal dominant mode of
 inheritance of most entities. About 20% of the familial cases
 are caused by gain of function mutations in the *SOD1* gene
 encoding the Cu/Zn superoxide dismutase 1. The mutant
 SOD1 forms intracellular aggregates in the brain and spinal
 cord of patients and mouse models showing ALS-like
 phenotypes. Even though the primary pathogenic event(s)
 are still a matter of debate, the toxic pathway has been
 linked to the secretion of mutant SOD1 indicating that the
 motor neuron death is not cell autonomous and may be
 caused by cell types in the vicinity of motor neurons [66,67].
 Albeit most ALS cases occur sporadically, the phenotypes of
 mice overexpressing human SOD1 missense mutations

1 closely mimic human ALS and thus serve as valuable disease
 models. Interestingly, disturbance of histone acetylation
 homeostasis is associated with the ALS pathogenesis similar to
 polyQ disorders, suggesting that transcriptional dysregulation
 5 may play a central role in the pathogenesis of ALS. This
 hypothesis is supported by the finding that the immunore-
 activities of acetylated histone H3 and the histone
 acetyltransferase CBP were severely reduced in motor
 neuron nuclei in the lumbar spinal cord of ALS-like
 10 mice [68]. Using SOD1/G93A mice, injection of PB
 (400 mg/kg/day, i.p.) starting before or at symptom onset
 significantly prolonged survival, improved motor performance
 and reduced motor neuron loss [69,70], and oral application
 of VPA (530 mg/kg/day) prolongs the lifespan of affected
 15 SOD1/G93A mice [71]. Consistent with the hypothesis that
 transcriptional dysregulation may play a role in the ALS
 pathogenesis, Ryu and co-workers [70] observed hypoacetylation
 of histones H2A, H2B, H3 and H4 in spinal cord of
 affected SOD1/G93A mice, while PB treatment restored
 20 histone acetylation to near-normal levels. Based on these
 promising findings, a clinical Phase I/II trial has been initiated
 to assess the safety of PB for potential ALS treatment
 (clinicaltrials.gov; identifier: NCT00107770). By employing
 a different ALS mouse model (G86R), Rouaux and
 25 co-workers [72] demonstrate that histone acetylation levels were
 dramatically decreased in motor neuron nuclei from affected
 mice, while proper histone acetylation and Cbp levels were
 maintained in mice treated with VPA (250 mg/kg/day, i.p.).
 Interestingly, VPA reactivated *Cbp* transcription *in vivo*,
 30 indicating that HDACs combat neurodegeneration at least
 in part through modulation of the *Cbp* transcriptional
 pathway. However, the data presented by Rouaux and
 co-workers partially contradict previous *in vivo* studies.
 Although VPA-treatment counteracts motor neuron death,
 35 delays disease onset and prevents from muscular atrophy at
 the early stage of the disease, the lifespan remained
 unaltered. Irrespective of its neuroprotective effects, the
 authors demonstrate that VPA does not mitigate neuromuscular
 denervation which might be the reason that motor neuron
 40 protection alone is not sufficient for ALS therapy [72].

7. Spinal muscular atrophy

45 SMA is an autosomal recessive inherited α -motor neuron
 disorder causing weakness and atrophy of voluntary muscles.
 The disease determining *survival motor neuron gene 1* (*SMN1*)
 is homozygously absent in 96% of all SMA patients and
 intragenic *SMN1* mutations are correspondingly rare [73].
 Within the SMA region on chromosome 5q, the human
 50 *survival motor neuron gene* exists in two copies, *SMN1* and
SMN2, which are ubiquitously expressed and encode identical
 proteins [74]. Even though all SMA patients lacking *SMN1*
 carry at least one *SMN2* gene copy, the amount of
 functional SMN protein produced by *SMN2* is not sufficient

to prevent progressive α -motor neuron degeneration. This
 finding has been assigned to a single translationally silent
 mutation within exon 7, affecting the splicing of *SMN*
 transcripts [75]. As a consequence, the disease determining
SMN1 gene produces full-length transcripts only (FL-*SMN*),
 whereas the majority of *SMN2* transcripts lack exon 7 due
 to alternative splicing ($\Delta 7$ -*SMN*). Truncated $\Delta 7$ -SMN
 proteins are reduced in their ability to self-oligomerise which
 is essential for proper SMN function [76,77] and have been
 shown to ameliorate but not to prevent the SMA phenotype
in vivo [78]. The disease-modifying property of the *SMN2*
 gene has been verified in transgenic mouse models, confirming
SMN2 as therapeutic target [79]. Consequently, transcriptional
SMN2 activation and/or modulation of the *SMN2* splicing
 pattern to increase FL-*SMN* levels may be an effective strategy
 for SMA treatment. Several HDAC inhibitors have shown
 to increase *SMN2*-derived FL-SMN protein levels *in vitro*
 by transcriptional activation and/or by modulation of the
SMN2 splicing pattern. These compounds include the fatty
 acids SB [80], PB [81] and VPA [82-84], the benzamide
 M344 [84,85] as well as the hydroxamic acids vorinostat and
 TSA [16,84,86]. The potential applicability of HDAC inhibitors
 for SMA therapy was confirmed in transgenic mice which
 mimic SMA-like features. By employing a knockout transgenic
 mouse model (*Smn*^{-/-} *SMN2*) Chang and co-workers [80]
 demonstrated that oral application of SB (0.8 or 8 g/l in
 drinking water, starting after disease onset) increased lifespan
 and FL-SMN protein levels in motor neurons of affected
 mice. In agreement with these findings, Tsai and co-workers [87]
 demonstrated that oral application of VPA (0.2 g/l in drinking
 water) attenuates motor neuron death, increases spinal SMN
 protein levels and partially normalizes motor function in
 SMA-like mice. By employing a different mouse model
 (*Smn*^{-/-} *SMN2*^{+/+} *SMN Δ 7*^{+/+}), Avila and co-workers [16]
 provide evidence that injection of the hydroxamic acid TSA
 (10 mg/kg/day, i.p., starting after disease onset) improved
 survival and motor function of SMA-like mice. Considering
 the apparent positive therapeutic responses in SMA model
 mice and the relatively few adverse effects, clinical Phase II
 trials have been initiated to evaluate the efficacy of PB and
 VPA for SMA therapy (clinicaltrials.gov; identifier:
 NCT00528268; NCT00481013; NCT00227266). Pilot
 trials with small numbers of patients treated with PB [88,89]
 or VPA [90,91] revealed increased quantitative muscle strength
 and subjective muscle function which might further emphasize
 the potential use of HDAC inhibitors for SMA treatment.
 These trials provide the first *in vivo* evidence that oral
 administration of PB and VPA increases *SMN2* expression in
 SMA patients. However, only 7 out of 20 patients displayed
 elevated *SMN2* transcript levels following VPA treatment,
 suggesting that some patients might be non-responders [91].
 Similar to polyQ disorders, the selectivity of HDAC inhibitors
 represents an important issue for SMA therapy. The observed
SMN2 gene activation by the HDAC class I selective inhibitor

1 VPA initially identified class I HDACs (1, 2, 3 and 8) as
 potential therapeutic targets. The authors have shown that
 the HDAC1-specific inhibitor SNDX-275 [14] fails to
 increase *SMN2* expression levels, suggesting that *SMN2*
 5 activation by less selective drugs occurs independently of
 HDAC1 [84]. Kernochan and co-workers demonstrated that
 the human *SMN2* promoter is associated with HDAC1 and
 more pronounced with HDAC2 but not with HDAC3,
 -4 and -5 [86]. In all, these data point toward HDAC2 as
 10 a major target for epigenetic SMA therapy.

8. Adrenoleukodystrophy

15 X-linked adrenoleukodystrophy (X-ALD, MIM#300100) is an
 inherited disorder characterized by pathologic accumulation
 of very long chain fatty acids (VLCFA) in all tissues of the
 body, progressive demyelination of the CNS and adrenal
 insufficiency. The disease is due to mutations in the *ABCD1*
 20 gene encoding the adrenoleukodystrophy protein (ALDP).
 ALDP, a peroximal member of the ATP-binding cassette
 family, is thought to participate in the entry of VLCFA into
 the peroxisome where VLCFA are β -oxidized. ALDP is supposed
 to act in concert with three other peroxisomal ATP-binding
 25 cassette transporters encoded by *ABCD2*, *ABCD3* and *ABCD4*
 genes. Among those, *ABCD2* is the closest paralog of the
 disease gene *ABCD1* and appears to have a redundant function.
 Overexpression of the adrenoleukodystrophy-related protein
 (ALDRP), the *ABCD2* gene product, has been demonstrated
 30 to compensate for ALDP deficiency in *Abcd1^{-/-}* mice by
 preventing VLCFA accumulation and delaying disease
 onset [92]. Thus, *ABCD2* appears to be a promising target
 for X-ALD therapy. Interestingly, PB substantially increases
 ALDRP expression and corrects VLCFA levels in cultured
 35 fibroblasts from X-ALD patients [93]. By employing X-ALD
 model mice (*Abcd1^{-/-}*), Kemp and co-workers [93]
 demonstrate that oral application of PB mitigates VLCFA
 accumulation in brain and adrenal glands, indicating that
 PB and related compounds may be relevant as a treatment
 40 option for patients with X-ALD. This hypothesis is further
 supported by the finding that SB as well as PB dose-
 dependently activate mouse *Abcd2* in cultured glial cells [94].
 McGuinness and co-workers [95] demonstrated that although
 PB and TSA increase VLCFA beta-oxidation in cultured
 45 mouse ALD fibroblasts, the beneficial effect of TSA appears
 to be independent of an increase in *Abcd2*/ALDR expression.
 These data suggest that the correction of the biochemical
 abnormality in X-ALD by HDAC inhibitors might not be
 dependent on pharmacologic induction of a redundant gene.
 Even though the exact underlying mechanism(s) remain
 50 elusive, further studies using HDAC inhibitors might be
 worthwhile to explore how the *ABCD2* gene is epigenetically
 regulated and to evaluate the potential applicability of HDAC
 inhibitors for X-ALD therapy by either *ABCD2*-dependent
 or *ABCD2*-independent mechanisms.

9. Rubinstein-Taybi syndrome

Mental impairment syndromes are diagnosed based on
 below-average intellectual function originated during
 developmental periods. Intellectual abilities rely on the
 capability of the brain to obtain, process, store and retrieve
 information. One of these disorders is the autosomal dominant
 Rubinstein-Taybi syndrome (RSTS, MIM#180849) character-
 ized by severe mental retardation, postnatal growth
 deficiency, microcephaly, facial characteristics, and broad
 thumbs and big toes [96]. RSTS can be caused by deletion or
 mutation of the histone acetyltransferase CBP or in rare
 cases by mutation of its close homolog p300 [97], suggesting
 that perturbation of histone acetylation is involved also in
 the pathogenesis of RSTS. RSTS mouse models heterozygous
 for either a CBP null allele or a mutation leading to the
 expression of a truncated CBP protein exhibit phenotypes
 similar to certain clinical aspects of RSTS, including growth
 retardation and skeletal abnormalities. Interestingly, behavioral
 studies of RSTS-like animals revealed long-term memory
 impairments (fear conditioning, object recognition) associated
 with histone hypoacetylation in the hippocampi of affected
 mice [98-101]. Alarcón and co-workers [101] show that applica-
 tion of the pan-HDAC inhibitor vorinostat before training
 (20 μ g, intraventricular injection) augments histone H2B
 acetylation and most strikingly restores long-term memory
 in a fear conditioning paradigm. Simultaneously, Korzus and
 co-workers [102] generated a tetracycline-inducible mouse
 model expressing a dominant-negative CBP transgene that
 lacks HAT activity. The transgene expression was restricted
 to the hippocampal formation and induced 7 days before
 starting an experiment. Concordant with the data presented
 by Alarcón and co-workers, behavioral tests revealed that
 stabilization of short-term into long-term memory is
 impaired in these mice (recognition memory, spatial memory).
 Importantly, these defects were reversible either on
 termination of transgene expression or administration of
 TSA before training (2 mg/kg, i.p.). These studies highlight
 a pivotal role of CBP acetyltransferase activity in controlling
 memory consolidation, and based on these studies one might
 speculate that HDAC inhibitors may be useful to counteract
 the histone acetylation imbalance caused by CBP haploin-
 sufficiency to alleviate some RSTS symptoms. Strikingly,
 HDAC inhibitors (SB, TSA) have subsequently been
 shown to enhance long-term memory formation also in
 non-diseased animals [103,104], suggesting that HDAC
 inhibition might represent a viable route also for the
 treatment of disturbances in memory formation such as
 age-related memory impairment.

10. Alzheimer's disease

Alzheimer's disease (AD) is the most common form of
 progressive dementia in the elderly. It is a neurodegenerative

1 disorder characterized by the neuropathological findings of
 intracellular neurofibrillary tau aggregation and extracellular
 amyloid plaques accumulating in vulnerable brain regions.
 Several studies revealed also an accumulation of the protein
 5 fragment p25 in sporadic AD. The neurotoxic p25 derives
 from proteolysis of p35, and overactivates the tau kinase
 cyclin-dependent kinase 5. Transgenic mice expressing high
 levels of p25 exhibit hyperphosphorylation of tau as seen in
 AD, and neurodegeneration. By employing a tetracycline-
 10 inducible mouse model (CK-p25) expressing p25 under con-
 trol of a neuron-specific promoter, Fischer and co-workers [105]
 demonstrate that recovery of learning and memory is
 associated with chromatin remodeling. In CK-p25 mice,
 p25 causes severe cognitive defects (fear conditioning, spatial
 15 learning) associated with neuronal loss and tangle formation
 (cortex, hippocampus) similar to AD pathology. Very similar
 to the RSTS mouse model, application of an HDAC inhibitor
 (SB, 1200 mg/kg/day, i.p.) mitigated learning and memory
 defects in CK-p25 mice. These effects were not restricted to
 20 diseased CK-p25 animals. Application of either SB (100 ng)
 or TSA (50 ng) into the brain ventricles of wild-type mice
 following fear conditioning significantly improved their
 long-term memory abilities when tested 24 h later. An
 intriguing observation of Fischer and co-workers is that
 25 environmental enrichment (large cages where exploratory
 activity is promoted by the presence of toys, tunnels and
 climbing devices) induces histone H3/H4 hyperacetylation
 in wild-type mice and normalizes memory performance in
 diseased CK-p25 mice. In line with these findings environ-
 30 mental enrichment has been demonstrated to be beneficial
 in reducing cognitive deficits and disease progression in
 several models of neurodegenerative pathologies such as
 HD [106] and AD [107], which might at least in part be
 triggered by increased histone acetylation. Even though the
 underlying mechanism(s) remain elusive, the link between
 35 environmental enrichment and chromatin remodeling
 might be exploited to develop a 'behavioral therapy' for
 human neurodegenerative conditions in which the histone
 acetylation homeostasis is disturbed.

11. Parkinson's disease

45 Parkinson's disease (PD) is the second most common
 neurodegenerative disorder after AD, affecting ~ 1% of the
 population > 50 years of age. PD is characterized by relatively
 selective depletion of dopaminergic neurons in the substantia
 nigra and nucleus coeruleus. Though the majority of PD
 cases are sporadic in origin, mutations in several proteins,
 including α -synuclein, have been linked to PD. Kontopoulos
 50 and co-workers [108] demonstrate that wild-type α -synuclein
 binds histone H3 *in vivo* and induces histone H3 hypoacety-
 lation when overexpressed in SH-SY5Y cells. Even though
 no direct binding of α -synuclein to histone acetyltransferases
 was found, wild-type as well as mutant α -synuclein proteins
 55 (A30P, A53T) inhibit histone acetylation mediated by CBP,

p300 and P/CAF *in vitro*. Based on these findings, the
 authors suggest that α -synuclein decreases acetylation by
 histone 'masking' and hypothesise that in case α -synuclein
 promotes neurotoxicity by inhibiting histone acetylation,
 increasing histone acetylation should ameliorate α -synuclein
 toxicity. Indeed, both SB and vorinostat diminished apoptotic
 cell death in response to α -synuclein expression *in vitro* and
 rescued dopaminergic neuron degeneration in α -synuclein
 transgenic flies. Very similar to the perturbation in histone
 acetylation homeostasis observed in spinocerebellar ataxia 3,
 these properties appear to be not restricted to mutant
 proteins. Kontopoulos and co-workers [108] demonstrate that
 mutant α -synuclein displays increased nuclear targeting,
 which underlines their finding that mutant α -synuclein
 toxicity is at least in part mediated by histone hypoacetylation.
 Most recently, Outeiro and co-workers [109] identified SIRT2,
 a member of the sirtuin HDAC family (class III), as a potential
 target for PD treatment. The authors demonstrate that
 pharmacologic SIRT2 inhibition rescues neuronal α -synuclein
 (A53T) toxicity *in vitro* and *in vivo* by employing a
Drosophila model of PD. Interestingly, pharmacologic SIRT2
 inhibition affects α -synuclein aggregation. *In vitro* studies
 revealed that SIRT2 inhibition results in accumulation of
 larger α -synuclein aggregates which appear to be protective,
 whereas smaller aggregates correlate with toxicity. Even
 though the underlying mechanisms remain elusive, these
 data suggest that, in addition to classical HDACs, SIRT2
 targeting may be therapeutically beneficial in PD and
 other diseases where aggregation of misfolded proteins is
 central to disease pathogenesis.

12. Stroke

Stroke, also referred to as cerebral ischemia, is the third
 leading cause of death in the western world and a major
 cause of disability [110]. Besides polyQ disorders such as
 HD, DRPLA and SBMA, perturbation in histone acetylation
 homeostasis is considered as a central event also in the
 pathogenesis of stroke [111]. Faraco and co-workers [111] show
 that histone H3 acetylation is severely decreased in ischemic
 brain tissue in mice following transient middle cerebral artery
 occlusion (MCAO). Interestingly, injection of vorinostat
 (25 and 50 mg/kg, i.p., immediately and 6 h after MCAO)
 significantly decreased the infarct volume and blocked
 ischemia-induced H3 hypoacetylation. By employing a rat
 MCAO model, Ren and co-workers [112] demonstrated that
 subcutaneous injection of VPA (300 mg/kg; every 12 h
 starting immediately after MCAO) decreased infarct volume
 and reduced ischemia-induced neuronal deficit scores. In
 both transient MCAO studies, HDAC inhibitor treatment
 resulted in a 30 – 40% reduction of infarct volumes which
 implies that HDAC inhibitors efficiently counteract
 postischemic brain damage. By using a permanent rat
 MCAO model in which the cerebral blood flow in the ischemic
 brain area is permanently reduced (pMCAO), Kim and

co-workers [113] confirmed a histone H3 hypoacetylation in the ischemic tissue as well as the neuroprotective effects of pharmacologic HDAC inhibition. Subcutaneous injection of VPA (300 mg/kg), SB (300 mg/kg) or TSA (0.5 mg/kg) reduced infarct volumes ranging from 34% (TSA) to 50% (SB) and improves neurologic performance in ischemic rats. Strikingly, delayed administration of VPA or SB (3 or 6 h after ischemic onset) still reduced infarct volumes and improved neurologic scores suggesting a comparatively long therapeutic window for potential stroke treatment. The pathophysiology leading to cell death during cerebral ischemic injury is complex and includes fundamental mechanisms such as excitotoxicity and ionic imbalance, oxidative stress, apoptotic-like cell death and inflammation [110]. Using the rodent MCAO models, HDAC inhibition suppressed ischemia-induced p53 expression as well as neuronal caspase-3 activation and superinduced the expression of HSP70 and Bcl-2 which both have shown to protect from ischemic neuronal death [111-113]. In addition, HDAC inhibitors exhibit anti-inflammatory effects which might contribute to the beneficial effects observed in the MCAO paradigms [113]. Even though the HDAC isoenzymes involved in HDAC-inhibitor-induced neuroprotection against cerebral infarction remain to be identified, the finding that the class I selective HDAC inhibitor VPA [14] mitigated ischemia-induced brain damage suggests that class I HDACs (1, 2, 3, 8) represent promising future targets for pharmacologic intervention.

13. Other possible indications

Besides the above-mentioned diseases, other indications such as multiple sclerosis, Friedreich's ataxia (FRDA) and the fragile-X mental retardation syndrome may also be considered. TSA has shown to reduce inflammation, demyelination, neuronal and axonal loss in experimental autoimmune encephalomyelitis which is regarded as a model for multiple sclerosis [114]. Most recently, HDAC inhibition has shown to reverse *FXN* gene silencing that is causative for autosomal recessive FRDA (MIM#229300). FRDA is due to a defect in transcription resulting from expansion of GAA triplet repeats in the first intron of the essential mitochondrial protein frataxin, which results in progressive spinocerebellar neurodegeneration. Herman and co-workers [115] demonstrated transcriptional *FXN* downregulation to be associated with histone H3 and H4 hypoacetylation of the genomic region surrounding the expanded GAA repeat. Interestingly, although all HDAC inhibitors tested so far (including the pan-HDAC inhibitors TSA and vorinostat) induced global histone H3/H4 hyperacetylation *in vitro*, only the novel experimental HDAC inhibitor BML-210 has shown to activate *FXN* gene expression, which might suggest that BML-210 inhibits a yet unidentified HDAC enzyme associated with the *FXN* gene [115]. The fragile-X mental retardation syndrome (MIM#300624) is caused by a CGG expansion in the *FMRI* gene promoter. This alteration triggers DNA

methylation of the repeat plus local changes in histone modifications leading to the clinical features of fragile X syndrome, the most common form of inherited mental retardation. *In vitro* studies revealed that TSA, SB and PB are able to transcriptionally activate the *FMRI* gene [116]. With respect to the observation that HDAC inhibitors promote DNA demethylation of specific genes *in vitro* and *in vivo* it would be interesting to evaluate whether HDAC inhibitors affect *FMRI* promoter methylation.

14. Conclusions

Despite the heterogeneous nature of neurodegenerative conditions presented here, HDAC inhibitor treatment appears to target two major neuroprotective mechanisms, namely: i) the transcriptional activation of disease-modifying genes in disorders such as spinal muscular atrophy or adrenoleukodystrophy; and ii) the correction of perturbations in histone acetylation homeostasis which have shown to be intimately involved in the neurodegenerative pathologies of Huntington's, Parkinson's and Kennedy disease, ALS, RSTS as well as stroke.

15. Expert opinion

Numerous neurodegenerative disorders have been identified as potential targets for HDAC inhibitor treatment and the number of diseases is likely to increase rapidly. Even though the neuroprotective effects of HDAC inhibitors are highly promising in preclinical experiments, the proof of their clinical efficacy for the treatment of neurodegenerative disorders is pending. Ongoing clinical trials for the potential treatment of HD, ALS and SMA are focussed on PB and VPA which are generally well-tolerated drugs and likely to be suitable for long-term treatment but comparatively weak HDAC inhibitors. In animal trials, high drug concentrations have been applied, ranging from 250 to 530 mg/kg for VPA and 100 to 1200 mg/kg for SB and PB. Despite the clear neuroprotective properties of the fatty acid class of HDAC inhibitors, one might argue whether neuroprotective drug concentrations are achievable in the patient's CNS. Highly potent HDAC inhibitors which penetrate the blood-brain barrier have been developed for cancer treatment but only little information is available concerning their efficacy in long-term therapy regimens. Thus, the results of presently ongoing clinical trials are eagerly awaited and will provide a significant impetus for future studies. Although considerable progress has been made in elucidating the disease-specific roles of HDACs and the effects of HDAC inhibitors, these areas are still in early stages of discovery. Thus, prospective efforts will very likely be focussed on the further decoding of the HDAC selectivity of known inhibitors and the further development of selective HDAC inhibitors. It remains elusive, however, whether selective HDAC inhibitors show superior neuroprotective effects compared with

1 neuroprotective pan-HDAC inhibitors such as vorinostat. Recently, novel mercaptoacetamide-based HDAC inhibitors have shown to possess superior neuroprotective properties compared with pan-HDAC inhibitors such as TSA and scriptaid [117]. Besides the well-recognized inhibitors of classical HDACs, the finding that the inhibition of sirtuin activity rescues neuronal cell death in models of Parkinson's disease [109] might further boost the drug screening efforts to develop new or optimized sirtuin inhibitors for the treatment of neurodegenerative and age-related disorders. A remarkable observation by Fischer and co-workers [105] is that *in vivo* histone acetylation levels can either be modulated by drug treatment or environmental factors, which allows the assumption that the further examination of gene-environment relationships might open new avenues to ameliorate the progression of some neurodegenerative disorders. In respect of the highly complex chromatin 'language' consisting of DNA methylation and manifold

post-translational histone modifications, the field of chromatin regulation in neurodegenerative disorders is still poorly understood. Thus, the further deciphering of chromatin modifications which correlate with either positive or negative transcriptional states might reveal novel therapeutic options to counteract transcriptional dysregulation observed in neurodegenerative disorders.

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