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REVIEW

Histone deacetylase (HDAC) inhibitors in recent clinical trials for cancer therapy

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Abstract Heritable changes in gene expression that are not based upon alterations in the DNA sequence are defined as epigenetics. The most common mechanisms of epigenetic regulation are the methylation of CpG islands within the DNA and the modification of amino acids in the N-terminal histone tails. In the last years, it became evident that the onset of cancer and its progression may not occur only due to genetic mutations but also because of changes in the patterns of epigenetic modifications. In contrast to genetic mutations, which are almost impossible to reverse, epigenetic changes are potentially reversible. This implies that they are amenable to pharmacological interventions. Therefore, a lot of work in recent years has focussed on the development of small molecule enzyme inhibitors like DNA-methyltransferase inhibitors or inhibitors of histone-modifying enzymes. These may reverse misregulated epigenetic states and be implemented in the treatment of cancer or other diseases, e.g., neurological disorders. Today, several epigenetic drugs are already approved by the FDA and the EMEA for cancer treatment and around ten histone deacetylase (HDAC) inhibitors are in clinical development. This review will give an update on recent clinical trials of the HDAC inhibitors used systemically that were reported in 2009 and 2010 and will present an overview of different biomarkers to monitor the biological effects.

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Epigenetics

Heritable changes in gene expression that are not based upon alterations in the DNA sequence are defined as epigenetics. The most common mechanisms of epigenetic regulation are the methylation of CpG islands within the DNA and the modification of amino acids in the N-terminal histone tails, especially reversible histone acetylation (Jones and Baylin 2007). While these modifications constitute the biochemical basis for epigenetics, not necessarily always, epigenetics is addressed when they are investigated. Thus, the term epigenetics is used in many studies, although, e.g., only transient changes of histone modifications or in gene regulation are monitored. This is also true for epigenetic therapy because in the strictest sense it has to be shown whether the daughter cell generation is cured. Especially in the clinical setting, it might be difficult to show whether immature cancer cells are induced to differentiate based upon epigenetic phenomena or whether they have been killed by cytotoxic effects.

It became increasingly evident that cancer formation and persistence may not only be caused by genetic mutations but also because of changes in the patterns of epigenetic modifications. In contrast to genetic mutations, which are basically irreversible, epigenetic changes are potentially reversible (Yoo and Jones 2006). This implies that they are amenable to pharmacological interventions (Santos-Rosa and Caldas 2005).

Histone acetylation and histone deacetylases

Modifications of the N-terminal tails of histone proteins play a crucial role in chromatin packaging and gene expression. Among these numerous modifications, reversible acetylation of lysine residues has been studied most extensively. Histone acetyltransferases transfer acetyl moieties to lysines in the N-terminal histone tails by use of the cofactor acetyl-CoA. This results in the neutralization of the negative charge of the nitrogen in the ε-amino-group of the lysine residue which in turn leads to a more open form of chromatin (euchromatin) that is associated with activation of gene expression. The acetyl groups are in turn cleaved off by histone deacetylases leading to a more condensed form of chromatin (heterochromatin) and gene silencing. In the last years, it became evident, that histone acetylation and histone deacetylases (HDACs) are not only linked with gene repression but also with transcriptional activation. Wang et al. (2009) could show that HDACs are also located at active gene loci. They suggest that the acetylation-induced gene expression needs to be reset before a new activation can be initiated and that HDACs play a role in the reset of active genes (2009).

The family of HDACs comprises of four classes, based on their homology to yeast proteins. Three (class I, II, IV) of these are zinc-dependent amidohydrolases, whereas class III requires NAD^+ for the deacetylation reaction. Until today, a total of 18 family members are known in humans, class I consists of HDAC 1, 2, 3, and 8 and is homologous to the yeast enzyme rpd3. This class is predominantly located in the nucleus. Class II shows homology to the yeast protein hda1 and comprises of six members (Verdin et al. 2004). They can be subdivided into class IIa with the subtypes HDAC 4, 5, 7, and 9 and the class IIb which covers HDAC 6 and 10. Class IIb enzymes have two catalytic sites, although one HDAC domain of HDAC10 is lacking the active pocket residues required for the enzymatic activity (Verdin et al. 2003). First studies suggested that the two HDAC domains of HDAC6 might function independently, but more recent data shows that both domains are required for the catalytic activity (Zhang et al. 2006). The class II subtypes shuttle between the cytoplasm and the nucleus (Yoo and Jones 2006). The only member of class IV known today is HDAC 11. The members of the class III HDACs are homologous to the yeast-silencing protein Sir2 (Sauve et al. 2006). Several members are located exclusively to the mitochondria.

Besides histones, many other proteins are substrates for reversible protein acetylation. Among the best studied are α-tubulin (target of HDAC6) and p53. From the target point of view, it might no longer be correct to call these enzymes histone deacetylases. Some researchers prefer protein deacetylases but most reports stick to the historical term (Buchwald et al. 2009; Glozak et al. 2005). This is due to tradition but also because the major phenotypic responses are thought to be mediated by histone deacetylation. Still, the relative roles of histone vs. non-histone effects on phenotypic response have to be dissected in further detail. While for transcriptional effects clearly histone acetylation is involved, apoptotic processes will likely also be influenced by inhibition of deacetylation of other protein substrates, e.g., p53, hsp90 or tubulin. In the clinical settings, more detailed analyses of unselective versus class I selective inhibitors should shed light on this in the future, Additionally, HDAC6 selective inhibitors allow to address some of these questions (Scott et al. 2008).

HDACs and cancer

In recent years, it became evident that HDACs are promising therapeutic targets with the potential to reverse aberrant epigenetic states associated with cancer (Bolden et al. 2006). Various studies in cancer cell lines and tumor tissue revealed changes in the acetylation levels and the expression of the HDAC enzymes (Bolden et al. 2006). In hematologic malignancies, the aberrant recruitment of HDACs to promoters plays a causal role in tumorigenesis (Pandolfi 2001). Chromosomal translocations, which are common in these diseases or overexpression of repressive transcription factors create oncogenic DNA-binding fusion proteins that physically interact with HDACs. Acute promyelocytic leukemia was the first model disease in which the involvement of HDACs in cancer onset was demonstrated on a molecular level (Minucci and Pelicci 2006). Here, 100% of the patients show formation of fusion proteins of the retinoic acid receptor-α with the promyelocytic leukemia, the promyelocytic zinc finger, or other proteins. These fusion proteins recruit HDAC-containing repressor complexes that constitutively repress the expression of specific target proteins (Pandolfi 2001). B-cell lymphoma 6 is an example for a transcriptional repressor which recruits complexes containing HDAC enzymes. These complexes cause activation of BCL-6 resulting in transcriptional silencing. BCL-6 is overexpressed in 40% of diffuse large B-cell lymphomas (Pasqualucci et al. 2003).

Moreover, the expression of the HDAC enzymes themselves can be up- or downregulated in various types of cancer. However, most studies show that there is a considerable variation in the expression levels between tumors of the same entity. In general, expression of class I HDACs tended to be higher in tumor samples compared to the corresponding normal tissue. In contrast, class II HDACs seemed to be downregulated and high expression correlated with a better prognosis (Weichert 2009). Increased HDAC activity leads to hypoacetylation of target

proteins, e.g., histones in the promoter area of tumor suppressor genes, thus resulting in transcriptional repression (Santos-Rosa and Caldas 2005). Interestingly, mutations in genes encoding for HDACs are rarely found in cancer (Lafon-Hughes et al. 2008). So far, only one truncating mutation of HDAC2 in colorectal and endometrial tumors has been described. Somatic HDAC4 mutations were found in breast and colorectal cancer and there are reports about germline polymorphisms in different HDACs. The functional significance for these sequence alterations is not clear yet (Ganesan et al. 2009).

Effects of HDAC inhibition

HDAC inhibitors cause changes in the acetylation status of chromatin and other non-histone proteins, resulting in changes in gene expression, induction of apoptosis, cell cycle arrest, and inhibition of angiogenesis and metastasis (Ma et al. 2009). In general, these small molecule inhibitors show a higher sensitivity towards transformed cells as compared to normal cells (Qui et al. 1999; Parsons et al. 1997). The overall number of genes regulated by HDACs is relatively small (Van Lint et al. 1996). The genes induced by HDAC inhibitors are mainly involved in cell growth, differentiation, and survival.

HDACi were first discovered as a result of their ability to induce cellular differentiation (Leder et al. 1975). This effect is associated with their ability to cause cell cycle arrest in G1 and/or G2 phase, thus leading to inhibition of cell growth (Bolden et al. 2006). The concentrations necessary to cause growth inhibition correlate very well with those needed to induce hyperacetylation of histones (Richon et al. 1998). G1 cell cycle arrest is, in most cases, a result of the induction of the CDKN1A gene, which encodes the cyclin-dependent kinase inhibitor WAF1 (also known as p21; Richon et al. 2000). Treatment of cells with HDACi can also lead to the induction of apoptosis. The inhibitors can initiate extrinsic (death receptor) and intrinsic (mitochondrial) pathways (Ma et al. 2009). It has been shown that various members of the TNF receptor super family and ligands become transcriptionally activated upon HDACi treatment (Bolden et al. 2006). The HDACi-induced activation of the intrinsic apopotic pathway is not fully understood today. One possibility is that HDACi cause global changes in gene expression that alter the balance of expression of pro- and antiapoptotic proteins. It is also possible that HDACi can activate a defined protein or signaling pathway; thus, inducing the intrinsic apoptotic pathway (Bolden et al. 2006).

Burgess et al. (2004) could show that histone deacetylase inhibitors also kill nonproliferating tumor cells, whereas normal cells remain unaffected. This is an advantage over most classical anti-cancer agents that only target the proliferating cell population of a tumor and thus bearing the risk of a relapse. In a mouse model of chronic myelogenous leukemia (CML), the HDACi Panobinostat in combination with Imatinib mesylate was able to deplete CML leukemia stem cells and thus preventing a relapse after therapy discontinuation (Zhang et al. 2010). A clinical trial of the above mentioned combination in CML patients is underway.

Furthermore, HDACi have been described to have antiangiogenic and antimetastatic effects. Their antiangiogenic properties result from a decrease in expression of proangiogenic genes like vascular endothelial growth factor and endothelial nitric oxide synthase (Deroanne et al. 2002; Michaelis et al. 2004; Rössig et al. 2002). These effects also contribute to a decreased nutrient supply of the metastasis, thus leading to an inhibition of the metastatic spread of the tumor (Bolden et al. 2006). Upregulation of gene expression of metastatic suppressors and downregulation of genes that promote metastasis were also described to be responsible for the antimetastatic effects of HDACi (Mudduluru et al. 2004; Mazières et al. 2007; Liu et al. 2003).

Growing evidence shows that HDACi have immunomodulatory effects. This can result in an increased recognization of malignant cells by the immune system due to an increased presence of surface antigens. For example, it has been shown that HDACi can upregulate the expression of major histocompatibility complex class I and II proteins (Magner et al. 2000). Additionally, HDACi can enhance immune cell activity by altering cytokine secretion. But the HDAC inhibitor suberoylanilide hydroxamic acid (SAHA, INN: Vorinostat) has also been demonstrated to suppress the production of pro-inflammatory cytokines that play a role in the pathogenesis of acute graft-versus-host disease (GVHD) (Reddy et al. 2004). These immunomodulatory effects may contribute to the anti-tumor activity of HDACi.

But some of the effects of HDAC inhibitors may also hamper therapeutic efficacy. Examples are the increased expression of multi-drug resistance proteins that lead to increased cellular efflux of chemotherapeutic agents (Tabe et al. 2006; Kim et al. 2008a, b).

HDAC inhibitors

A lot of effort has been put into the development of HDAC inhibitors in recent years. Seven structurally distinct classes of inhibitors are known today; inhibitors of four different classes are now in clinical development (see Table 1–6 for an overview). These classes comprise hydroxamic acids, cyclic peptides, short-chain fatty acids, and benzamides.

Vorinostat (Zolinza®), a hydroxamate-based inhibitor, was the first HDACi to be approved by the Food and Drug Administration (FDA) in October 2006 for the treatment of refractory cutaneous T-cell lymphoma for patients who had received two or more prior systemic therapies. It has long been considered to inhibit all zinc-dependent HDACs in the low nanomolar range. Recent studies suggest that it has only weak inhibitory effect on class IIa enzymes (Bradner et al. 2010). Vorinostat induces cellular differentiation, e.g., of erythroleukemia cells, causes increased levels of p21 and G1 cell cycle arrest. The compound inhibits cell growth in a variety of different tumor cell lines and animal models with little toxicity (Jones 2009).

With Romidepsin (Istodax®) (see Fig. 1), a structurally different cyclic peptide (also called depsipeptide or FK-228 in the early literature), a second HDAC inhibitor was

Table 1 Vorinostat trials reported in 2009 and 2010

Combination therapy	Type of cancer	Phase	*N*	Outcome (most relevant findings)	Literature
None	Acute myeloid leukemia	II	37	1 Response (group 3×200 mg)	Schäfer et al. (2009)
None	Follicular (FL) and mantle cell lymphoma (MCL)	I	10	40% Response; 2CRu, 1PR (FL); 1CRu (MCL)	Watanabe et al. (2010)
None	Solid tumors	I	18	MTD was not reached	Fujiwara et al. (2009)
None	Advanced prostate cancer	II	27	2SD; all patients off therapy before 6 months	Bradley et al. (2009)
None	Recurrent glioblastoma multiforme	II	52	9 Progression-free after 6 month	Galanis et al. (2009)
Bortezomib	Relapsed and refractory multiple myeloma	I	23	42% Response rate; 3PR	Badros et al. (2009)
Peg-Lip-Doxorubicin, Bortezomib	Relapsed/refractory Multiple Myeloma	I	7	1CR, 1vgPR, 4PR	Voorhees et al. (2009)[a]
Idarubicin, Cytarabine	Acute myelogenous leukemia	II	45	Response rate 80%; 35CR, 1CRP	Garcia-Manero et al. (2009)[a]
Lenalidomide, Dexamethasone	Relapsed/refractory multiple myeloma	I	25	Response rate 64%; 1CR, 1nCR, 2vgPR, 8PR, 4MR, 5SD	Siegel et al. (2009)[a]
Decitabine	Acute myelogenous leukemia/ myelodysplastic syndrome	I	MDS n=11 AML n=50	MDS: 2CR, 1PR, 1HI, 7SD AML: 7CR, 4CRi, 2PR, 3HI, 26SD	Kirschbaum et al. (2009)[a]
Rituximab, Ifosphamide, Carboplatin, Etoposide (ICE)	Relapsed/refractory lymphoid malignancies, untreated T-cell/mantle cell lymphoma	I	14	1CR, 2CRu, 9PR, 1SD	Budde et al. (2009)[a]
pelvic palliative radiotherapy	Gastrointestinal carcinoma	I	16	MTD=300 mg once daily	Ree et al. (2010)
5-FU, Leucovorin	Metastatic colorectal cancer	I/II	10	No MTD could be established; 2SD; study was closed	Wilson et al. (2010)
Carboplatin, Paclitaxel	Advanced stage NSCLC	II	94	34% Response with Vorinostat vs 12.5% with Placebo	Ramalingam et al. (2010)
Doxorubicin	Solid tumors	I	24	MTD=800 mg; 2PR (breast, prostate cancer); 2SD (melanoma)	Munster et al. (2009a)
5-FU, Leucovorin, Oxaliplatin	Refractory colorectal cancer	I	21	MTD=300 mg twice daily	Fakih et al. (2009)
Tamoxifen	Advanced breast cancer	II	29	6 Response; 3SD	Munster et al. (2009c)[a]
Bortezomib	Refractory solid tumors	I	29	MTD 300 mg BID	Ninan et al. (2009)[a]
Gemcitabine, Cisplatinum	Advanced NSCLC	I	28	9PR, 8SD	Trédaniel et al. (2009)[a]
Erlotinib	NSCLC	I	13	6SD	Reguart et al. (2009)[a]
Docetaxel	Solid tumors	I	12	No response	Schneider et al. (2009)[a]

[a] Meeting report abstract

approved by the FDA at the end of 2009. Romidepsin was isolated from *Chromobacterium violaceum* and inhibits the activity of HDACs at low nanomolar levels. This natural product is actually a prodrug which is activated by cellular reduction to its active form, a dithiol (Furumai et al. 2002). Romidepsin has been shown to inhibit human and mouse tumor growth in different cancer models. This compound inhibits preferably class I HDACs and is therefore called a class-selective inhibitor in contrast to Vorinostat which also acts strongly, e.g., on HDAC6 (Furumai et al. 2002; see Fig. 1).

The most widely explored class of HDACi are the hydroxamic acids. Besides, Vorinostat, seven additional hydroxamate-based compounds are currently in different stages of clinical development. Belinostat (PXD-101), Panobinostat (LBH589), Dacinostat (LAQ824), and SB939 are all cinnamic acid derivatives. Belinostat is a potent HDACi with an IC_{50} in the low nanomolar region (IC_{50} 27 nM). The cytotoxic effects of this compound correlate with hyperacetylation of histone H4 in tissue culture. A dose-dependent growth reduction in ovarian and colon xenograft models has also been observed (Plumb et al. 2003; www.topotarget.com). Panobinostat is an orally active HDAC inhibitor and has the highest inhibitory potency among the clinically used hydroxamic acids. The compound has been shown to increase the levels of p21 and to induce hyperacetylation of histone H3 and H4. In vitro and in vivo anti-tumor efficacy has been demonstrated in different cell lines and xenograft models (Revill et al. 2007; Atjada 2009). Dacinostat is structurally closely related to Panobinostat and inhibits HDACs in submicromolar concentrations (IC_{50} 0.15 μM). It has been shown to inhibit cell growth and to induce apoptosis. Preclinical activity has been demonstrated in colon, breast and lung cancer xenograft models (www.hdaci.com; Catley et al. 2003). Another cinnamic acid derivative is SB939. This compound has favorable pharmacokinetic properties as it accumulates in tumor tissue and shows a sustained hyperacetylation of histones. In a colon xenograft model, it showed an almost twofold greater effect in inhibition of tumor cell growth compared to Vorinostat (Novoty-Diermayr et al. 2010). Other hydoxamic acid based HDACis in clinical trials are Givinostat (ITF2357), PCI 24781 and R306465 (JNJ-16241199). These compounds are pan-HDACi; the latter ones inhibit the enzyme activity in the low nanomolar range. All three compounds show antiproliferative activities and induction of histone hyperacetylation in different cell lines. Preclinical efficacy has also been demonstrated in tumor xenograft models (Jones 2009; Golay et al. 2007; Arts et al. 2007) (see Fig. 2).

The structurally simplest class of HDACi are the short-chain fatty acids. Despite the low inhibitory potency of these inhibitors, also compounds from this class have been studied in the clinic. Valproic acid, which has been used as an antiepileptic drug for many years and still is used in this indication, has been shown to inhibit preferably class I HDACs in the high micromolar to millimolar range. The compound induces differentiation of transformed cells and causes hyperacetylation of histone proteins (Göttlicher et al. 2001). Due to the profound experiences in antiepileptic therapy with manageable side effects, this compound is investigated as an antileukemic agent in different trials despite its low potency. Butyric acid is another short-chain fatty acid. Because of its short half-life and low plasma levels available, several prodrugs have been designed, of which AN9/Pivanex was tested in the clinic (Rephaeli et al. 2000). Another rather weak HDACi is phenylbutyrate which was reported to have antileukemic activity in a case study (Warrell et al. 1998). Recent combination trials with 5-azacytidine showed only poor response (Lin et al. 2009; see Fig. 3).

The fourth class of HDACi in clinical trials are the benzamides or amino anilides. Their mechanism of inhibition on the molecular level was a subject of controversial debate for several years now. Recently, Bressi et al. (2010) could finally show that the amino anilide group indeed also acts as a zinc-chelating moiety. CI-994 was the first member of this group shown to inhibit HDACs with an

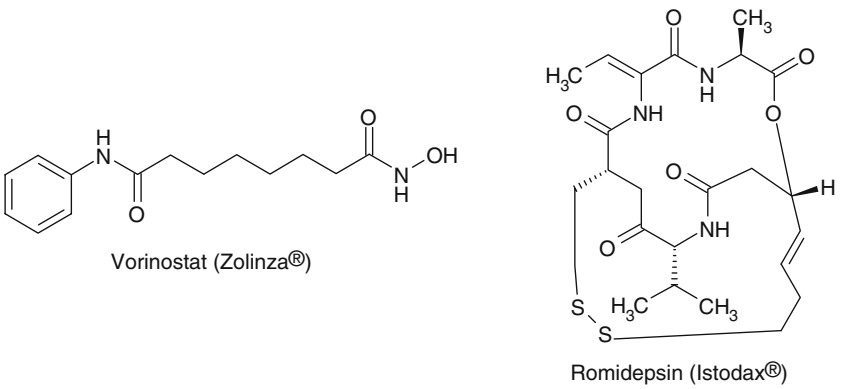

Fig. 1 Approved HDAC inhibitors

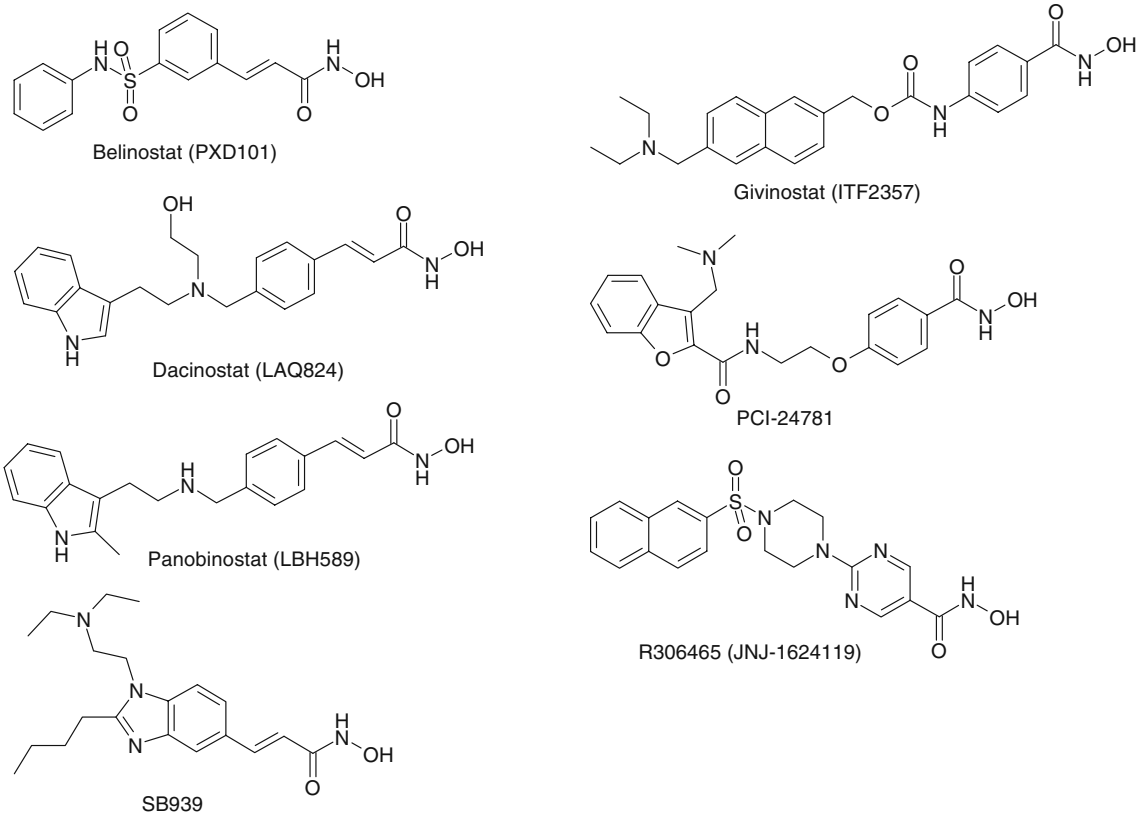

Fig. 2 Other HDAC inhibitors with a hydroxamic acid based structure

IC_{50} between 25 and 50 μM. This compound entered clinical trials but its investigation has been terminated (Jones 2009). Entinostat (SNDX-275/MS-275) is a class-I-selective inhibitor with an IC_{50} of 2 μM (Suzuki et al. 1999). It has also been shown to cause cell cycle arrest and hyperacetylation of histone H4 (Saito et al. 1999). Antitumor activity has been demonstrated in several tumor cell lines and in different xenograft models (Hess-Stump et al. 2007). Due to its relatively long half-life, weekly and biweekly dosing schedules are explored in the clinic (Hauschild et al. 2008). The third compound of this class is Mocetinostat (MGCD0103). It is also a class-selective HDAC inhibitor with IC_{50}s in the submicromolar range. Induction of histone hyperacetylation and apopotosis have been shown as well as antiproliferative activities against a wide panel of tumor cell lines and tumor growth inhibition in multiple xenograft models (Fournel et al. 2008; see Fig. 4).

Biomarkers

A lot of different surrogate markers have been investigated due to their capacity to reflect the pharmacodynamic effects of HDACi or to show a correlation with a response in patients. The most extensively studied biomarker to date has been the acetylation of target proteins pre- and post treatment in PBMC or tumor tissue. Changes can be determined via Western blot and flow cytometry analysis or with immunohistochemical methods. This parameter was analyzed in many clinical trials but a correlation between the therapeutic response and a hyperacetylation of histones or other target proteins was not found. Hyperacetylation of target proteins was rather detected in basically all patients treated with an HDACi, but at least a dose- and time-dependent increase in acetylation levels could be observed (Chung et al. 2006).

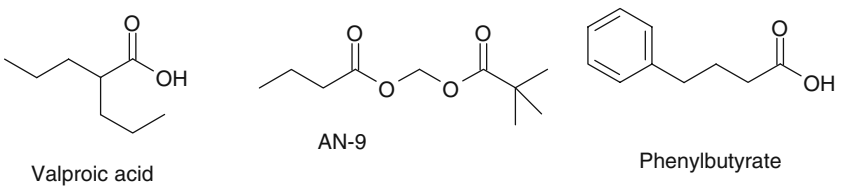

Fig. 3 Short-chain fatty acid HDACi

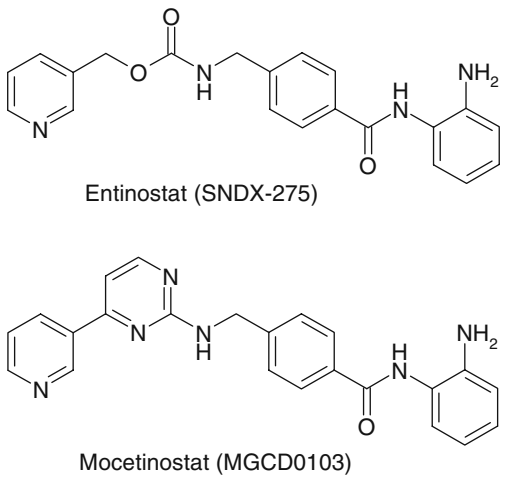

Fig. 4 HDAC inhibitors with an aminoanilide structure

A new assay to determine the pharmacodynamic effects of HDACi was reported by Bonfils et al. (2008; see Fig. 5). The assay is based on the measurement of the HDAC enzyme activity in living cells. The group therefore used a small molecule, cell-permeable substrate that is converted by HDACs (Hoffmann et al. 1999; Heltweg et al. 2003). In a second step, the deacetylated substrate is cleaved into a fluorophor with a longer wavelength shifted emission and a lysine moiety by a protease-like trypsin. The fluorophor can be quantitated by fluorescence intensity measurement. The first results obtained with this assay reveal that the measurement of the enzyme activity seems to be a parameter with a greater dynamic range than the measurement of histone acetylation levels. Thus, this parameter may better reflect the pharmacodynamic effects of HDACi. Whether a correlation between the HDAC enzyme activity and the therapeutic response exists, needs to be determined in future studies.

Furthermore, there are investigations ongoing to determine gene signatures that reflect the response to an HDACi treatment. So far, first studies show that there are indeed distinct changes in gene expression of certain genes (Stimson et al. 2009). A microarray-based study of Belinostat treated cell lines revealed a signature that is selectively induced by HDACi compared to other chemotherapeutic agents (Monks et al. 2009). In another study treatment of two different colon cancer cell lines with Vorinostat and Panobinostat resulted in similar but cell line-dependent changes in gene expression (La Bonte et al. 2009) Due to the multiple roles of the HDAC enzymes in different pathways, it may be questionable whether a defined gene signature can be identified at least for a certain HDAC subtype selectivity profile. It is more likely that this signature will strongly vary with tumor type, drug exposure, and concentration. Another challenging question will be the identification of changes in gene expression that indicate the sensitivity to a treatment with HDACi.

The expression of the HDAC enzymes themselves was suggested to serve as a predictive biomarker. Munster et al. reported data from two clinical trials with HDACi where they found a correlation of pretreatment HDAC2 expression and histone acetylation in tumor tissue. No correlation was found for HDAC6. Based on this data, HDAC2 expression could serve as a predictive parameter to determine patients who can benefit from HDACi treatment (Munster et al. 2009a, b).

Fantin et al. investigated the signal transducer and activator of transcription (STAT) signaling pathway to identify a biomarker predictive for Vorinostat response.

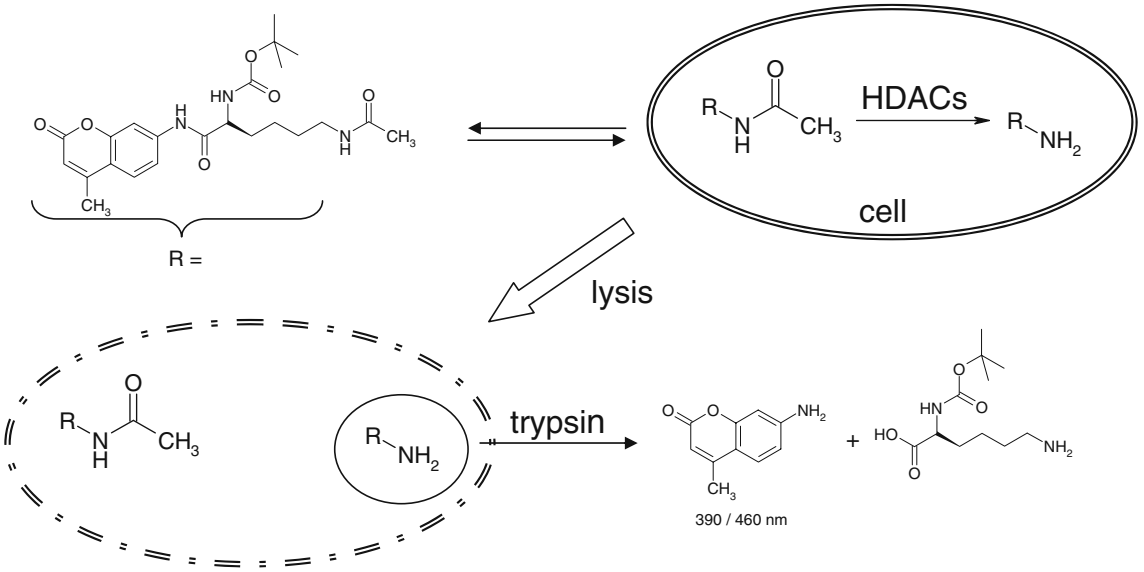

Fig. 5 Whole cell HDAC activity assay

They found higher levels of activated STAT1, STAT3, and STAT5 in lymphoma cell lines that poorly responded to Vorinostat treatment compared to sensitive cell lines. Consistent data came from immunohistochemical analysis of pretreatment skin biopsies collected in a Vorinostat phase IIb trial from nonresponder patients with cutaneous T-cell lymphoma. Accumulation of STAT1 in the nucleus and high nuclear levels of phosphorylated STAT3 correlated with a lack in clinical response (Fantin et al. 2008). This data suggests that deregulation of STAT activity may play a role in Vorinostat resistance. Furthermore, the group could show that coincubation of Vorinostat and a Jak inhibitor, which blocks the STAT pathway, sensitizes cancer cell lines previously resistant to Vorinostat treatment to the HDACi. Cotreatment also leads to a synergistic effect in growth inhibition, thus suggesting the combination of Vorinostat and a Jak inhibitor to be a promising future treatment option for patients resistant to Vorinostat therapy.

In a genome-wide loss-of-function screen, the protein HR23B was identified to sensitize tumor cells to HDAC inhibitors (Fotheringham et al. 2009). HR23B plays a role in shuttling ubiquitinated cargo proteins to the proteasome (Chen et al. 2009, 2002). Upon treatment with HDACi, it is in part responsible for the deregulation of the proteasome activity (Fotheringham et al. 2009). An immunohistochemical analysis of a collection of cutaneous T-cell lymphoma (CTCL) skin biopsies obtained from a Vorinostat phase II trial showed a correlation between HR23B expression and clinical response. When relating the HR23B levels to clinical response, a positive predictive value (PPV) of 71.7% could be determined (Khan et al. 2010). Thus, HR23B expression may serve as a predictive biomarker for HDACi treatment.

Garcia-Manero et al. linked an increased tolerance to oxidative stress to Vorinostat resistance. A cDNA microarray analysis performed during a phase I study of Vorinostat in patients with advanced leukemia, revealed an upregulation of expression of genes mainly coding for antioxidants in non-responder patients (2008). The same group confirmed these results in an HDACi-resistant leukemia cell line (Hu et al. 2010). Furthermore, they found that addition of β-phenylethyl isothiocyanate, a compound that causes a decrease in the cellular glutathione levels, resulted in enhanced toxicity of Vorinostat in leukemia cell lines and primary leukemia cells (Hu et al. 2010). Thus, the combination of an HDACi with an inhibitor of the antioxidant pathway may sensitize non-responder patients to an HDACi therapy.

Clinical trials of Vorinostat

Vorinostat was the first HDACi approved by the FDA for the treatment of refractory cutaneous T-cell lymphoma. Currently, it is investigated for other cancer types including both solid tumors and hematologic malignancies. There were several different trials reported in 2009 and 2010 for Vorinostat as single agent and in combination therapy (see Table 1).

Trials in hematologic malignancies—mono therapy

A phase II study with 37 refractory acute myelogenous leukemia (AML) patients showed only minimal activity of Vorinostat. There was only one patient who responded to therapy. Many others discontinued therapy before the planned four cycles were administered due to failure of Vorinostat to control the leucokyte count or due to patients' or physicians' preference (Schäfer et al. 2009).

In a small phase I study with ten Japanese patients with malignant lymphoma who were treated with 100 or 200 mg Vorinostat twice daily for 14 days followed by a 1 week rest interval.In the cohort that received 200 mg BD, there were two unconfirmed complete responses (CRu) and one partial response (PR) in patients with follicular lymphoma and one CRu in a patient who suffered from mantle cell lymphoma. An increase in the acetylation levels of histone H3 with a maximum effect 8 h after dosing could be seen, but also only in the 200-mg cohort. Acetylation levels decreased to baseline 24 h after dosing. No correlation between response and histone hyperacetylation was found. In this study, Vorinostat showed good activity, thus further investigations in larger patient cohorts with malignant lymphoma are warranted (Watanabe et al. 2010).

Trials in hematologic malignancies—combination therapy

Three trials were conducted with patients who suffered from refractory multiple myeloma (MM). In two of these studies, Vorinostat was administered in combination with either the proteasome inhibitor Bortezomib alone or the combination of Bortezomib and pegylated liposomal Doxorubicine (Badros et al. 2009; Voorhees et al. 2009). In the third trial, patients received Vorinostat in combination with Lenalidomide and Dexamethasone (Siegel et al. 2009). Overall response rates were 42%, 86%, and 84%, respectively, and therapy was well tolerated in general. Even though only small patient populations have been investigated, these combinations demonstrated high clinical activity. Remarkably, patients who received a prior therapy with either Bortezomib or Lenalidomide and who were refractory to their therapy responded again to the combination therapy. Due to these encouraging results, further investigation of these combination therapy regimens with larger patient collectives are warranted.

Two combination trials focussed on the treatment of myelodysplastic syndromes (MDS) and AML (Kirschbaum et al. 2009; Garcia-Manero et al. 2009). In one study, an epigenetic combination therapy consisting of the DNA-

methyltransferase inhibitor Decitabine (Dacogen®) and Vorinostat was administered. The other combination consisted of Idarubicin, Cytarabine, and Vorinostat. Both trials revealed good activity of these combinations with overall response rates of 87% and 80%, respectively. No excess toxicity due to Vorinostat was recognized. Another study investigated Vorinostat in combination therapy for the treatment of refractory or relapsed lymphoid malignancies. Patients were treated with Vorinostat in combination with Rituximab (R), which was only administered in one subgroup, Ifosphamide (I), Carboplatin (C), and Etoposide (E) (V+RICE or V+ICE) (Budde et al. 2009). Twelve of 14 patients responded to therapy, but 57% experienced gastro-intestinal adverse effects.

Taken together, Vorinostat in combination therapy showed encouraging clinical activity in different haematologic maligancies. Further studies should be conducted, investigating the beneficial effect of Vorinostat compared to the other combination partners.

Trials in solid tumor malignacies—mono therapy

Another trial with 18 Japanese patients investigated Vorinostat in solid tumors. In this phase I study, 100 and 200 mg Vorinostat were administered twice daily or 400 and 500 mg were given once daily. The maximum tolerated dose was not achieved even though the pharmacokinetic profile was similar to the one established in non-Japanese patients (Fujiwara et al. 2009). Disappointing results were obtained in a phase II study of Vorinostat in patients with advanced prostate cancer. Vorinostat was associated with significant toxicities and all patients were taken off therapy before 6 months. The best results were stable disease (SD) achieved in two patients, but there was no PSA decline >50% and 44% of the patients experienced grade 3 adverse effects (Bradley et al. 2009). More encouraging results were reported from a phase II trial of patients with recurrent glioblastoma multiforme. Nine of 52 patients were progression-free after 6 months. Immunohistochemistry analysis revealved an increase in histone acetylation post treatment compared to baseline in five patients. Changes in gene expression of genes shown to be regulated by Vorinostat could also be demonstrated (Galanis et al. 2009).

Furthermore, Vorinostat was tested in several combination therapy regimens for different types of cancer. In general, the activity was higher than in the single agent trials, but in most cases it has not been shown that the higher activity results from the addition of Vorinostat to standard therapy.

Trials in solid tumor malignancies—combination therapy

There were also several trials of Vorinostat combination therapy for solid tumors reported in 2009 and 2010. Two trials focussed on the investigation of Vorinostat in refractory colorectal cancer. Preclinical data showed that Vorinostat is able to downregulate the expression of thymidylate synthase (TS) in tumor tissue. As this protein is the target enzyme of 5-fluorouracil, a synergistic effect of this combination could be demonstrated in preclinical experiments. Fakih et al. (2009) reported a phase I trial of Vorinostat in combination with 5-fluorouracil, Leucovorin and Oxaliplatin (Folfox). The maximum tolerated dose (MTD) was determined to be 300 mg twice daily (BID) for 1 week every 2 weeks. However, a downregulation of TS expression could only be detected in two out of six patients. This data is consistent with the results of another phase I study from Wilson et al. (2010). Only one out of ten patients showed a decline in the intratumoral TS levels. The biological activity of Vorinostat was determined by histone H3 hyperacetylation in PBMC.

Another focus is the investigation of Vorinostat in different combination regimes for the treatment of advanced non-small cell lung cancer (NSCLC). Two trials combined Vorinostat with platinum-based chemotherapies as well as Paclitaxel and Gemcitabine (first regimen: Vorinostat, Carboplatin, Paclitaxel; second regimen: Vorinostat, Cisplatin, Gemcitabine) (Ramalingam et al. (2009); Trédaniel et al. 2009). In the first study with the carboplatin-based regimen, an increase in the response rate was seen in the group that received Vorinostat as compared to placebo (34% vs. 12.5%). There was a trend towards improvement in progression-free survival and overall survival. The cisplatinum-based trial was a phase I study, in which the MTD could be determined. In this study, Vorinostat could be administered with standard doses of Gemcitabine and Cisplatinum without additional toxicity. Seventeen from 19 patients responded to therapy.

The combination of Vorinostat and Erlotinib was also tested for the treatment of NSCLC in patients with EGFR mutations after Erlotinib progression. Preclinical experiments suggested a reversal of erlotinib resistance of mutant patients by Vorinostat treatment. The MTD was not reached, but still six out of nine patients experienced stable disease. Thus, this combination was well tolerated and effective (Reguart et al. 2009). There were also combination trials of Vorinostat for patients with solid tumors of different origin. One study investigated the combination of Vorinostat and Docetaxel (Schneider et al. 2009). This trial, however, was closed due to excessive toxicity. No objective responses were seen. In another phase I study of Vorinostat plus Bortezomib, the MTD could be determined at 300 mg twice daily and bortezomib 1.3 mg/m^2. Besides that, there was also evidence of clinical activity of this combination (Ninan et al. 2009). Munster et al. (2009a) conducted a trial of Vorinostat+weekly Doxorubicin. The MTD was determined to be 800 mg for 3 days every week of a 28-day cycle. Two patients with breast and prostate cancer had a

PR, whereas two patients with melanoma experienced SD. In this trial, the authors found a correlation between histone hyperacetylation and pretreatment HDAC2 expression.

Another study from the same group focussed on the combination of Vorinostat and Tamoxifen in breast cancer patients who progressed despite prior hormone therapy (Munster et al. 2009c). Preclinical experiments showed an interference of HDACi with hormone receptor signaling, thus nourishing the hope of reversing resistance to hormone receptor modulators. HDACi have been shown to sensitize ER-negative cell lines to Tamoxifen by inducing the release of HDAC1 from the ERα-promoter and thus restoring expression of ERα (Yang et al. 2001; Zhou et al. 2007) or by activation of ERβ (Hodges-Gallagher et al. 2006). In ER-positive cell lines, HDACi cause a decrease in ERα-expression (Reid et al. 2005; Rocha et al. 2005) and sensitization of cells to Tamoxifen (Hodges-Gallagher et al. 2006; Hirokawa et al. 2005), which involves an upregulation or translocation of ERβ (Duong et al. 2006; Jang et al. 2004). Six patients in this phase II study had an objective response and three had SD that lasted longer than 6 months, suggesting that this drug combination is feasible and that HDACi may restore hormone sensitivity.

The first report of a clinical trial of the combination of an HDACi with radiotherapy was reported by the group of Flatmark (Ree et al. 2010). Vorinostat was combined with pelvic palliative radiotherapy for the treatment of gastrointestinal carcinoma. The MTD was determined to be 300 mg once daily. Most patients had a decrease in tumor volume 6 weeks after completion of treatment. The combination could be safely administered in general, suggesting an investigation of Vorinostat in long-term curative pelvic radiotherapy.

Clinical trials of Romidepsin

Trials that lead to the approval of Romidepsin

The cyclic peptide Romidepsin is the second HDACi approved by the FDA in November 2009 for the treatment of CTCL of patients who had received at least one prior systemic therapy. The approval was based on two single-arm, multicenter, open-label trials in which 167 patients were treated (Kim et al. 2008a, b; Piekarz et al. 2009a, 2009b). Romidepsin can be administered at 14 mg/m^2 intravenously over 4 h on days 1, 8, and 15 of a 28-day cycle. Overall response rates were similar in both studies (34% and 35%). Six resp. four patients had a complete response whereas 27 resp. 20 patients achieved a PR. The duration of response was remarkably long; 14.9 and 13.7 months, respectively. Adverse effects included nausea, fatigue, infections, vomiting, anorexia, anemia, thrombocytopenia, neutropenia, lymphopenia, and ECG T-wave changes. Besides its approval for CTCL, Romidepsin is investigated as a treatment option in other cancer types as monotherapy as well as in combination therapy (see Table 2).

Trials in hematologic malignancies

Encouraging results came from a phase II multicenter trial of patients with relapsed peripheral T-cell lymphoma (PTCL; Piekarz et al. 2009a, b). Forty-six patients were treated; the overall response rate was 33%. Five patients showed a complete response and ten patients a partial remission. Noteworthy, like in CTCL patients, is the long duration of response (9 months). Adverse effects were generally mild. Currently, a phase IIb protocol is ongoing in multiple centers. A combination of Romidepsin and Bortezomib is currently being investigated in a phase II study in patients with refractory MM (Berenson et al. 2009a, b). So far, five patients have been treated with 10 mg/m^2 Romidepsin on days 1, 8, and 15 and 1.0 mg/m^2 Bortezomib on days 1, 4, 8, and 11 of a 28-day cycle. Two of them, who relapsed from Bortezomib-containing regimes in a prior therapy, had minimal responses. Because of two patients, who experienced a grade 3 thrombocytopenia, additional patients are treated with a reduced dose of Romidepsin (8 mg/m^2).

Trials in solid tumor malignancies

Little clinical activity of Romidepsin was noted so far in the treatment of solid tumors. The results of two trials were

Table 2 Romidepsin trials reported in 2009 and 2010

Combination therapy	Type of cancer	Phase	*N*	Outcome (most relevant findings)	Literature
None	Cutaneous T-cell lymphoma	II	71	Response rate 34% 4CR, 20PR	Piekarz et al. (2009a, b)
None	Cutaneous T-cell lymphoma	II	27	2CCR, 13PR	Kim et al. (2008a)[a]
None	Relapsed peripheral T-cell lymphoma	II	46	5CR, 10PR	Piekarz et al. (2009a, b)[a]
None	Metastatic castration-resistant prostate cancer	II	35	2PR	Molife et al. (2010)
None	Advanced colorectal cancer	II	25	4SD; No objective response	Whitehead et al. (2009)
Bortezomib	Refractory multiple myeloma	II	5	2 Minimal responses	Berenson et al. (2009a, b)[a]

[a] Meeting report abstract

reported in 2009. A phase II study of 35 patients with metastatic, castration-resistant prostate cancer revealed minimal clinical activity of Romidepsin (Molife et al. 2010). Two patients had a partial response that lasted longer than 6 months with a PSA decline of over 50%, but there were also 11 patients who had to discontinue the medication due to toxicity. Another phase II study of 25 patients with advanced colorectal cancer was closed due to a lack of activity (Whitehead et al. 2009). No objective responses have been seen; four patients had stable disease as the best result. As for now, Romidepsin has shown promising clinical activity in hematologic malignancies other than CTCL but only weak efficacy in the treatment of solid tumors was observed.

Clinical trials of Panobinostat

Trials in hematologic malignancies—mono therapy

The hydroxamate Panobinostat showed activity in clinical trials with different hematologic malignancies (see Table 3). Younes et al. (2009) reported encouraging data from a phase II trial of oral Panobinostat in patients with Hodgkin lymphoma after high-dose chemotherapy with autologous stem cell transplant. Of 53 patients treated for at least two cycles, one patient achieved a complete response, ten patients had a partial response and 31 patients experienced stable disease. Noteworthy, 77% of the patients experienced thrombocytopenia as a grade 3/4 adverse effect, which, however, was reversible after 7–8 days after discontinuation of therapy. This data indicates good clinical activity of Panobinostat in combination with manageable toxicity in pretreated Hodgkin lymphoma patients.

Two trials focussed on the investigation of Panobinostat in patients with myelofibrosis (DeAngelo et al. 2009; Mascarenhas et al. 2009). Preliminary data indictates that in both studies, some patients received a significant reduction in spleen size as well as an improvement in other disease-related symptoms, e.g., a reduction in transfusion requirements or transfusion independence. One previously untreated patient received a PR. From this first data, Panobinostat appears to be a promising new agent in the therapy of myelofibrosis, thus further investigation is warranted.

Trials in hematologic malignancies—combination therapy

A major focus is the investigation of Panobinostat in different combination regimes for the treatment of refractory MM. Results from three studies in 2009 is available. Berenson et al. (2009b investigated the combination of Panobinostat and Melphalan). In the dose-finding part of the study, several dose adjustments were necessary due to toxicity. Grade 3 neutropenia, thrombocytopenia, and severe fatigue occurred in most patients. Currently, the modified protocol (20 mg Panobinostat on days 1, 3, 5, 8, 10, and 12; 0.05 mg/kg melphalan on days 1, 3, and 5 of a

Table 3 Panobinostat trials reported in 2009 and 2010

Combination therapy	Type of cancer	Phase	*N*	Outcome (most relevant findings)	Literature
None	Relapsed/refractory Hodgkin lymphoma	II	53	1CR, 10PR, 31SD	Younes et al. (2009)[a]
None	Myelofibrosis (MF)	I	176 (thereof 13 with MF)	MF:1PR, 3CI	DeAngelo et al. (2009)[a]
None	Myelofibrosis	I	8	2CI; 4SD	Mascarenhas et al. (2009)[a]
Lenalidomid, Dexamethasone	Multiple myeloma	I	22	5/10 mg Panobinostat: regarded as safe	Spencer et al. (2009)[a]
Bortezomib	Relapsed multiple myeloma	I	28	64% Response: 4CR; 10PR; 4 minor responses	San-Miguel et al. (2009)[a]
Melphalan	Multiple myeloma	I	12	1CR; 3PR; 4SD	Berenson et al. (2009a, b)[a]
Imatinib	Chronic myeloic leukemia	I	5	MTD to be determined	Bhatia et al. (2009)[a]
Docetaxel	Castration-resistant prostate cancer	I	16	no effects as single agents; 5 patients with PSA decline >50%	Rathkopf et al. (2010)
Trastuzumab	HER-2 positive metastatic breast cancer	I	18		Conte et al. (2009)

[a] Meeting report abstract

28-day cycle) is being investigated. Despite toxicity, the combination shows encouraging clinical activity with a disease control rate of 67% including one CR, three PR, and four SD. If the modified protocol results in a manageable toxicity, this drug combination is a promising treatment option for multiple myeloma patients. Similar results came from a combination study of oral Panobinostat and Bortezomib (San-Miguel et al. 2009). The overall response rate in this study was 64% including four CR and a response of patients, refractory to their prior Bortezomib therapy. However, significant thrombocytopenia occurred in many patients, thus warranting a dose adjustment or an alternative dosing schedule in further studies to receive a better safety profile.

A third combination study for the treatment of multiple myeloma focussed on the combination of Panobinostat with Lenalidomide and Dexamethasone (Spencer et al. 2009). So far, dose escalation studies are ongoing with Panobinostat, 5 and 10 mg three times a week, combined with 25 mg Lenalidomide four times daily on days 1–21 and 40 mg Dexamethasone on days 1–4, 9–12, and 17–20 of a 28-day cycle appeared to be safe. Due to these encouraging results from the MM treatment trials, a randomized, double blind, placebo controlled phase 3 study (PANORAMA-1) of Panobinostat in combination with Bortezomib and Dexamethasone for the treatment of relapsed MM has started global enrollment at the end of 2009 (http://www.novartisoncology.com/research-innovation/pipeline/Panobinostat.jsp).

Panobinostat has also been investigated for the treatment of chronic myeloic leukemia in combination with Imatinib (Bhatia et al. 2009). Preliminary results are available so far: two patients showed a reduction in the BCR-Abl levels as detected by qPCR from bone marrow aspirates. The MTD has not been reached yet.

Trials in solid tumor malignancies

Only few data exists for the treatment of solid tumors with Panobinostat. In a combination study of oral Panobinostat versus oral Panobinostat plus Docetaxel in patients with advanced prostate cancer, all patients in the Panobinostat mono-therapy arm progressed under therapy, despite of detectable levels of histone hyperacetylation in PBMCs, whereas five out of eight patients in the combination arm showed a PSA decline >50% (Rathkopf et al. 2010). Further studies need to investigate if there is a clinical benefit of the combination in comparison to a Docetaxel monotherapy. The same group is currently investigating the combination of i.v. Panobinostat and Docetaxel. The combination of either oral or i.v. Panobinostat and Trastuzumab is being investigated in women with metastatic HER2-positive breast cancer (Conte et al. 2009). The dose finding is ongoing. Patients of cohort 1 who received 10 mg Panobinostat three times weekly or 15 mg Panobinostat i.v. on days 1 and 8 of a 21-day cycle in combination with weekly Trastuzumab tolerated the study medication well. So far, two patients showed a reduction in tumor size.

Clinical trials of Belinostat

Trials in hematologic malignancies

Belinostat is another hydroxamate-based HDACi in late stage clinical development (see Table 4). Two trials for the treatment of hematologic malignancies have been reported in 2009. A phase I study aimed to determine the adequate dosing and safety of oral Belinostat in patients with lymphoma (Zain et al. 2009). Belinostat was given in doses

Table 4 Belinostat trials reported in 2009 and 2010

Combination therapy	Type of cancer	Phase	*N*	Outcome (most relevant findings)	Literature
None	Lymphoma	I	9	6SD; tumor shrinkage of 43 and 49% in two patients after cycle 2	Zain et al. (2009)[a]
None	Peripheral/Cutaneous T-cell lymphoma	II	20 (PTCL) 29 (CTCL)	PTCL: 2CR, 3PR, 5SD CTCL: 2CR, 2PR, 17SD	Pohlmann et al. (2009)[a]
None	Platinum resitant epithelial ovarian cancer (EOC)+Micropapillary ovarian tumors (LMP)	II	32	1PRu, 10SD (LMP); 9SD (EOC)	Mackay et al. (2010)
None	Advanced malignant pleural mesothelioma	II	13	2SD; No objective response; 1 death (cardiac arrhythmia)	Ramalingam et al. (2009)
None	Solid tumors	I	92	33SD	Kelly et al. (2009)[a]
None	Thymic malignancies	II	22	2PR; 13SD	Giaccone et al. (2009)[a]

[a] Meeting report abstract

of 750, 1,000, and 1,250 mg a day for 14 days of a 21-day cycle. Nine patients have been enrolled until now, with three per cohort. No dose-limiting toxicity has occurred so far, only one grade 3 and 4 thrombocytopenia has been noticed. The most frequent adverse events were anorexia, fatigue and diarrhea. Five out of six patients evaluable for efficacy showed stable disease. A tumor shrinkage of 43–49% has been observed in three patients after cycle two. Based on this data, Belinostat can be safely administered in patients with myeloma.

Pohlmann et al. (2009) investigated i.v. Belinostat in patients with PTCL, and CTCL. In this phase II study, patients received 1,000 mg/m^2 Belinostat over 30 min on days 1–5 of a 21-day cycle. Out of 20 PTCL patients, two responded with a complete response and two others with a partial remission. Stable disease was observed in five additional patients. Two of the CTCL patients had a CR and PR, respectively. Remarkably, the time to response was only 16 days. Seventeen additional CTCL patients achieved stable disease. Therapy was well tolerated in general; only one grade 3 neutropenia and one thrombocytopenia were noted. These results represent the basis for the conduction of the BELIEF study, a pivotal study of Belinostat in patients with PTCL.

Trials in solid tumor malignancies

The clinical activity of Belinostat is also being investigated in solid tumor malignancies. A phase I study of patients with solid tumors were investigated using different doses and dosing schedules of oral Belinostat (Kelly et al. 2009). Ninety-two patients have been included. The most frequent adverse effects were fatigue, nausea, anorexia, vomiting, and diarrhea. Different therapy regimens were tested. The drug was given either continously, or for the first 2 weeks; respectively for the first 5 days of a 3-week cycle. The recommended dose for continuous dosing is 250 mg once or twice a day and for days 1–14 dosing, 750 mg once a day is recommended. The dose-finding for days 1–5 dosing has not been completed yet. With regard to efficacy, 33 patients had stable disease, thus making Belinostat an interesting option for further investigations in special tumor types.

Promising results also came from a phase II study of Belinostat in patients with thymic malignancies (Giaccone et al. 2009). These tumors are very rare and no second-line therapy for patients with refractory disease exists. A total of 22 patients, 14 with thymoma and eight with thymic carcinoma, have been accrued. Two partial responses were seen in patients with thymoma, while 13 additional patients had stable disease. Nausea was the most common adverse effect, which could be controlled with prophylactic antiemetics. All analyzed patients showed an accumulation of acetylated histones and tubulin in monocytes and lymphocytes analyzed by multiparameter flow cytometry.

Another phase II study with Belinostat for patients with malignant pleural mesothelioma was reported by Ramalingam et al. (2009). Thirteen patients with advanced disease were treated with 1,000 mg/m^2 Belinostat for 5 days every 3 weeks and two cycles were administered. Two patients had stable disease but there were no objective responses. One possibly study-related death occurred, where the patient died of cardiac arrhythmia. In this dosing schedule, Belinostat is not active as monotherapy, suggesting further investigations of different dosing schedules or in combination with other chemotherapeutics.

Kelly et al. reported data from a phase II study of Belinostat in women with platinum-resistant epithelial ovarian cancer (EOC) and micropapillary (LMP) ovarian tumors (Mackay et al. 2010). These tumors are rarely included into clinical trials and have a poor prognosis. Belinostat was administered at 100 mg/m^2 over 30 min on days 1–5 every 3 weeks. Of the LMP patients, one achieved a partial response (unconfirmed) and ten had stable disease whereas nine patients with EOC had stable disease as best result. The progression-free survival was 13.4 months for LMP patients and only 2.3 months for EOC patients. The most common side effect was fatigue. An accumulation of acetylated histones could be observed in tumor tissue and PBMC regardless of response.

Trials of Givinostat

Givinostat is a hydroxamic acid containing HDACi which is currently in early clinical development (see Table 5). Data of a phase II trial in patients with relapsed or progressive multiple myeloma was reported by Galli et al. (2010). The first part of the study focussed on the dose

Table 5 Other hydroxamate and anilide HDACi trials reported in 2009 and 2010

Study medication	Type of cancer	Phase	*N*	Outcome (most relevant findings)	Literature
Givinostat	Relapsed/progressive multiple myeloma	II	19	5SD; MTD 100 mg twice daily	Galli et al. (2010)
Mocetinostat	Advanced chronic lymphocytic leukemia	II	21	No response	Blum et al. (2009)
Entinostat+5-Azacytidine	Myeloid Malignancies	I	38	3 CR, 4 PR, 7 HI	Fandy et al. (2009)

[a] Meeting report abstract

finding. The first six patients received 150 mg twice a day for four consecutive days every week of a 28-day cycle. As two patients experienced dose-limiting toxicities at this dose, the next patients received 100 mg twice daily, which was determined to be the maximum tolerated dose. At the last follow-up, there were five patients with stable disease, but serious adverse effects occurred in four patients (gastrointestinal toxicity, transient electrocardiographic abnormalities) and all but one patient developed a thrombocytopenia. Based on this data, Givinostat showed modest clinical activity with tolerable toxicity.

Trials of PCI-24781

The hydroxamate HDACi PCI-24781 is being developed by Pharmacyclics and is currently in phase I and II studies of clinical development. The compound has been investigated in a phase I/II study for the treatment of lymphoma. Twenty-five patients have been enrolled to date and 15 have been evaluated (http://www.pharmacyclics.com/pdf/PCI-24781_HDAC_Inhibitor_Exec_Overview_Oct09.pdf). Two patients achieved a partial response, nine showed stable disease. In a phase I dose-finding study in patients with solid tumors, five of 25 evaluable patients had stable disease. No QTc prolongation was noticed. Another phase II study of the combination of PCI-24781 with doxorubicin for patients with advanced sarcoma started at the end of 2009 (http://www.pharmacyclics.com/pdf/PCI-24781_HDAC_Inhibitor_Exec_Overview_Oct09.pdf).

Trials of Mocetinostat

The class I-selective benzamide HDACi Mocetinostat has been investigated in different phase 1 and 2 studies for hematologic malignancies and solid tumors (see Table 5). In 2008, the FDA put a partial hold on Mocetinostat due to some cases of pericarditis/pericardial effusion (http://www.methylgene.com/content.asp?node=324). Patients that were currently enrolled in clinical trials and did not show any symptoms of pericardial disease could continue their study. The hold was lifted in 2009, thus patient enrollment could be continued (http://www.ebionews.com/news-center/enterprise-a-industry/34-business-development/8035–methylgene-to-resume-development-of-its-hdac-inhibitor-mgcd0103-mocetinostat-.html).

Data of a phase II trial in patients with advanced chronic lymphocytic leukemia was reported in 2009 (Blum et al. 2009). Patients received 85 mg Mocetinostat a day, three times weekly. A dose escalation to 110 mg or the addition of Rituximab was permitted after two or more cycles without response. No responses were obtained in this study. Three patients received 110 mg and four others additional Rituximab without an improvement in response. Grade 3 and 4 toxicities were infections, thrombocytopenia, anemia, diarrhea, and fatigue. HDAC inhibition was demonstrated in six out of nine patients on day 8. In this clinical setting, Mocetinostat showed limited anti-cancer activity; further investigations should therefore focus on combination therapy.

Trials of Entinostat

Data of one clinical trial with the benzamide Entinostat was reported in 2009 (see Table 5). Fandy et al. (2009) investigated the combination of Entinostat and 5-Azacytidine in patients with myeloid malignancies. Patients were treated for ten consecutive days with 30, 40, or 50 mg/m^2 5-Azacytidine and received 2, 4, 6, or 8 mg/m^2 Entinostat orally on days 3 and 10 of a 28- or 29-day schedule. Among 30 patients who received at least four cycles of therapy, three patients had a CR, four had a PR, and seven patients showed hematologic improvements. This result indicates a greater clinical activity of this epigenetic combination therapy, compared to single agent Entinostat, that showed limited benefit in advanced acute leukemias so far (Gojo et al. 2007). There are also several active trials ongoing (http://www.syndax.com/trials_studies.aspx). Entinostat is being investigated as a single agent in patients with relapsed/refractory Hodgkin's lymphoma. A dose of 10 mg is administered from days 1 to 5 of 28-day cycle. This dose can be expanded to 15 mg if no dose-limiting toxicity occurs. Entinostat in combination with GM-CSF is being explored in patients with myelodysplastic syndromes, acute myeloid leukemia, and chronic myelocytic leukemia. Patients with these malignancies are also treated with Entinostat as monotherapy or in combination with 5-Azacytidine.

Studies in patients with solid tumor malignancies are also ongoing. Non-small cell lung cancer patients are being treated with single agent Erlotinib or the combination of Erlotinib and Entinostat. Another trial for the same type of cancer investigated the combination of Entinostat and 5-Azacytidine. Two trials with women suffering from breast cancer explore the combination of Entinostat and different aromatase inhibitors, respectively. Because of these active trials, an update on the clinical activity of Entinostat can be awaited soon.

Trials of valproic acid

Trials in hematologic malignancies

Even though the carboxylic acid valproate (VPA) is an HDACi with weak inhibitory potential, it is an interesting drug for clinical trials due to its well-characterized

pharmacodynamic and pharmacokinetic profile (see Table 6). Several combination trials of valproate were reported in 2009 and 2010. An epigenetic combination therapy consisting of valproic acid and 5-Azacytidine (Vidaza®) was investigated in patients with intermediate and high risk MDS (Voso et al. 2009). Patients received VPA in doses to achieve a plasma level of 50 μg/ml in combination with 75 mg/m^2 5-Azacytidine for 7 days of a 28-day cycle. From 26 patients who completed eight cycles of therapy, 30.7% achieved a complete or partial remission. Fifteen point four percent showed major hematologic improvements and 38.5% showed SD. The drug-related toxicities were reported to be mild. This epigenetic therapy is active in MDS patients with a poor prognosis and can safely be applied.

Raffoux et al. (2010) treated patients for six cycles with the above-mentioned epigenetic combination therapy of VPA and 5-Azacytidine for 7 days and additional doses of all-trans retinoic acid (ATRA) for 21 days. Among the 65 patients who were enrolled in this study, 14 received a PR and three a CR. Interestingly, overall survival in patients who received the planned six cycles was not dependent on CR or PR; that means that stable disease during treatment correlates with survival. Furthermore, an early platelet response and promoter demethylation of four genes (FZD9, ALOX12, HPN, and CALCA) was associated with clinical response. However, the beneficial effect of ATRA remains questionable as the response rates in this study were not superior to those of earlier studies with the combination of VPA and 5-Azacytidine. Further trials are needed to prove the benefit of adding ATRA to the epigenetic combination therapy.

Trials in solid tumor malignancies

Two trials focussed on the investigation of different VPA combinations in Myeloma treatment. Daud et al. (2009) treated 39 patients who suffered from myeloma stage IV with the combination of VPA and a new topoisomerase-I-inhibitor, Karenitecin. The MTD was 75 mg/kg/day VPA on days 1–5 combined with Karenitecin 1.0 mg/m^2/day in a 28-day cycle. Somnolence occurred as a dose-limiting-toxicity. Of 33 patients evaluable for response, 47% achieved stable disease. The overall survival was 32.8 weeks. Histone hyperacetylation was observed in PBMC.

In another phase I/II study with advanced melanoma patients, valproate was combined with Dacarbazine plus interferon-α (Rocca et al. 2009). As a first part of the study, patients received Valproate monotherapy for 6 weeks. The dose was adjusted by measuring inhibition of HDAC activity in PBMC during therapy with the goal to achieve a measurable inhibition of the target. In the second part, Dacarbazine and interferon-α were added. Twenty-nine patients received VPA monotherapy whereas only 18 received the combination of the three drugs. From the patient treated with the combination regime, one achieved a CR and two a PR. Three additional patients had stable disease, lasting longer than 24 weeks. In this study, valproate did not show results superior to melanoma standard therapy with non-negligible toxicity, thus questioning the clinical benefit of VPA in this clinical setting.

A phase I/II trial of Valproate in combination with Epirubicin or the combination of 5-Fluorouracil, Epirubicin, and Cyclophosphamide (FEC100) for patients with solid tumors was conducted by Munster et al. (2009b). In the first part, 44 patients received escalating doses of valproate with a fixed dose of Epirubicin. The MTD was determined to be 140 mg/kg/day, nine patients achieved a partial response. During the second part of the study, a disease-specific cohort of 15 breast cancer patients were treated with 120 mg/kg/day Valproate and the combination regime FEC100. Nine out of 14 patients responded to therapy. Overall, somnolence was the most noted adverse effect related to Valproate, whereas Epirubicin caused myelosuppression. The biological activity of Valproate was measured via histone acetylation in PBMC. The acetylation levels correlated with VPA serum levels and could be linked to baseline HDAC2—but not HDAC6 expression. This combination shows promising activity in the treatment of solid tumors. A randomized, double-blind study has to reveal if there is a clinical benefit of VPA.

Table 6 Valproate trials reported in 2009 and 2010

Combination therapy	Type of cancer	Phase	*N*	Outcome (most relevant findings)	Literature
5-Azacytidine	High risk MDS	II	62	8CR/PR, 4HI, 10SD	Voso et al. (2009)
5-Azacytidine+ATRA	High risk AML, MDS	II	65	14PR, 3PR	Raffoux et al. (2010)
I Epirubicin II FEC100	Solid tumors	I/II	I 41 II 14	I 9PR II 9 objective response	Munster et al. (2009b)
Karenitecin	Melanoma	I/II	I 33 II 15	I MTD=75 mg/kg/day II 7SD	Daud et al. (2009)
Dacarbazine+IFα	Melanoma	I/II	18	1CR, 2PR, 3SD	Rocca et al. (2009)

Conclusion and further perspectives

HDAC inhibitors are promising new agents in targeted anticancer therapy. Two compounds, Vorinostat and Romidepsin, are already approved by the FDA for the treatment of refractory cutaneous T-cell lymphoma. Several other inhibitors are in late stages of clinical development for cancer therapy. It will be interesting to see whether some of the new compounds will have benefits in terms of increased efficacy or reduced side effects and whether those effects can be correlated with the HDAC subtype selectivity profiles, certain chemical substructures (hydroxamates vs. benzamdes) or might be substance specific. Data of clinical trials indicates a higher activity of HDACi in hematologic malignancies, whereas mostly little or no clinical benefit was observed in solid tumor malignancies.

Remarkably, synergistic effects result from the combination treatment of HDACi with different chemotherapeutics, other epigenetic drugs or target-based agents, as well as radiotherapy. In the majority of the trials reported, the HDACi could be applied in combination with standard doses of other drugs without additional toxicity but synergistic clinical activity, thus suggesting a promising role of HDACi in cancer combination therapy.

Even though there were a lot of concerns regarding toxic side effects of HDACi in the clinical setting due to the roles of HDACs in multiples pathways, until now clinical trials mostly showed manageable side effects. Cardiotoxic effects are regarded as a class effect in the group of HDACi and a hold was put on the clinical development of Mocetinostat in 2008, but only in a few cases, prolongation of the QT interval has been detected in the clinic. This fact may be due to preselection of patients in clinical trials; therefore, the incidence of cardiotoxic effects in unselected patient populations needs to be critically monitored.

In almost every HDACi clinical trial, there were patients resistant to HDACi therapy. Until today, the mechanisms of resistance to HDACi are understood only to a small extent. Deregulated STAT activity and upregulation of certain antioxidant -genes seem to play a role in HDACi resistance. Further work needs to be conducted to fully elucidate the pathways responsible for HDACi resistance so that more patients can benefit from this therapy.

Until now, no differences in the clinical activity and adverse effects could be determined between the pan-HDACi and the class-selective compounds. Thus, the hypothesis that compounds that target selectively one HDAC isoform will reveal a greater clinical benefit combined with a better toxicity profile has yet to be proven. Along with the development of these isoform-selective inhibitors, the biological roles and the roles in cancer of the individual isoforms need to be further elucidated. Experiments with HDAC knockout mice showed a fundamental role of the different HDACs in human development (Haberland et al. 2009). HDAC1, HDAC3, HDAC7 knockout mice are embryonic lethal, HDAC2 and HDAC4 knockouts die shortly after birth. Only HDAC5, HDAC6, and HDAC9 knockout mice are viable. Remarkably, the different HDAC isoforms seem to be involved in quite different processes of embryonic development. Whereas HDAC2 plays a role in heart development, HDAC4 controls the formation of the skeleton and HDAC7 is involved in the formation of the endothelium (Haberland et al. 2009). These findings will contribute to a better understanding of the biological roles of the different HDAC isoforms and thus will finally lead to the development of new and more selective HDACi. Also, the relative roles of deacetylation of histones vs. other substrate proteins with respect to the clinical response have to be dissected further.

In the future, it also needs to be determined if the so called epigenetic drugs really exert their effects through altering misregulated epigenetic states or just through transcriptional regulation processes. Until today, it is unclear what mechanisms cause the clinical effects of HDACi. Only with an improved understanding of the mode of action of HDACi, it will be possible to develop more potent and selective inhibitors.

Another goal for future clinical trials is the search for new and better biomarkers to monitor the effects of HDACi. The detection of target protein hyperacetylation only serves to show that the inhibitors hit their target but there is no correlation with clinical response. The measurement of the enzyme activity in surrogate cells or tumor tissue seems to have a greater dynamic range but future studies have to prove the usefulness of this parameter. Furthermore, in a time of personalized medicine, it is also important to find biomarkers that predict the response resp. resistance to HDACi treatment. Recently, the protein HR23B was suggested to serve as such a predictive parameter. Further analysis of more patient samples need to confirm these initial results.

Besides the clinical development of HDACi for cancer therapy, there is growing evidence that these inhibitors also have a therapeutic potential for the treatment of neurological disorders (Kazantsev et al. 2008). Until today, several HDACi showed positive effects in different preclinical models of Alzheimer's disease, Huntington's disease and other neurologic malignancies. The HDACi 4b has been shown to improve the disease phenotype with low toxic effects in a mouse model of Huntington's disease (Thomas et al. 2008). Kilgore et al. (2010) could show that the HDACi Vorinostat, sodium valproate, and sodium butyrate were able to restore cognitive function in a transgenic mouse model of Alzheimer's disease. Due to these encouraging results from preclinical studies, clinical devel-

opment seems to be plausible in the future, even though there are still a lot of challenges to overcome, e.g., penetration of compounds through the blood–brain barrier.

To summarize, HDACi are a new class of drugs with a therapeutic potential not only in cancer but also in neurologic disorders and there may be other therapeutic indications that are on the horizon but need clinical confirmation.

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Conflict of interest MJ has given talks on histone deacetylase inhibitors at MSD sponsored symposia and has received an honorary for this.

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