Review

Mechanisms and Clinical Significance of Histone Deacetylase Inhibitors: Epigenetic Glioblastoma Therapy

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Abstract. *Glioblastoma is the most common and deadliest of malignant primary brain tumors (Grade IV astrocytoma) in adults. Current standard treatments have been improving but patient prognosis still remains unacceptably devastating. Glioblastoma recurrence is linked to epigenetic mechanisms and cellular pathways. Thus, greater knowledge of the cellular, genetic and epigenetic origin of glioblastoma is the key for advancing glioblastoma treatment. One rapidly growing field of treatment, epigenetic modifiers; histone deacetylase inhibitors (HDACis), has now shown much promise for improving patient outcomes through regulation of the acetylation states of histone proteins (a form of epigenetic modulation) and other non-histone protein targets. HDAC inhibitors have been shown, in a pre-clinical setting, to be effective anticancer agents via multiple mechanisms, by up-regulating expression of tumor suppressor genes, inhibiting oncogenes, inhibiting tumor angiogenesis and up-regulating the immune system. There are many HDAC inhibitors that are currently in pre-clinical and clinical stages of investigation for various types of cancers. This review will explain the theory of epigenetic cancer therapy, identify HDAC inhibitors that are being investigated for glioblastoma therapy, explain the mechanisms of therapeutic effects as demonstrated by pre-clinical and clinical studies and describe the current status of development of these drugs as they pertain to glioblastoma therapy.*

Glioblastoma (GBM) is the most common malignant adult brain tumor. Standard-of-care treatment includes surgery, radiation and temozolomide; however, this still yields poor prognosis for patients (1). Targeting of key epigenetic enzymes, oncogenes and pathways specific to glioblastoma cells by the drugs is very challenging, which has therefore resulted in low potency in clinical trials (2). In addition, limited stability and unacceptable pharmacokinetic properties of most existing drugs or molecules have made the target-based drug discovery very difficult. The Cancer Genome Atlas (TCGA) research network recently analyzed whole-genome sequencing of GBM tumors and found that GBM recurrence is linked to epigenetic mechanisms and pathways (3). This data was strongly supported by the mutational status of *H3F3A* and *IDH1* genes with differences in global methylation patterns in glioblastomas, which correlate with distinct clinical characteristics (4). Recent studies have also identified a Lys 27-to-methionine (K27M) mutation at one allele of *H3F3A*, and one of the two genes encoding histone H3 variant H3.3, in 60% of high-grade pediatric glioma cases (5). Thus, studies detailing on the histone and DNA modifications specific to glioblastoma can be used to expand the current search for epigenetic drivers of gliomagenesis.

Histone deacetylase (HDAC) inhibitors belong to a class of agents that target the aberrant epigenetic characteristics of tumor cells. (3). Epigenetic changes refer to alterations that affect gene expression and cellular phenotype without modifying the DNA sequence itself. Histone modification is

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Table I. *HDAC characterization, distribution and activity in brain malignancies (6, 12-13, 16-21).*

HDAC class	Name location	Intracellular yeast protein	Homologous (AA)	Size location	Chromosome	*Expression increase in brain tumors	Co-factor	Tissue distribution
Class I	1	Nucleus	Rpd-3	483	1p34.1	630%	Zn^{2+}	All tissues, highest in colon and ovaries
	2	Nucleus	Rpd-3	488	6q21	238%	Zn^{2+}	All tissues, highest in kidney
	3	Nucleus> cytoplasm	Rpd-3	423	5q31	313%	Zn^{2+}	All tissues, highest in kidney
	8	Nucleus	Rpd-3	377	xq13	450%	Fe^{2+}, Zn^{2+}	Pancreas and Kidney
Class IIa	4	Nucleus and cytoplasm	HDA1	1084	2q37.2	61000%	Zn^{2+}	low expression in all tissues
	5	Nucleus and cytoplasm	HDA1	1122	17q21	86%	Zn^{2+}	All tissues, highest in heart and brain
	7	Nnucleus and cytoplasm	HDA1	855	12q13	188%	Zn^{2+}	All tissues, highest in ovary
	9	Nucleus and cytoplasm	HDA1	1011	7p21-p15	213%	Zn^{2+}	Brain, heart, and ovary
Class IIb	6	Cytoplasm> nucleus	HDA1	1215	Xp11.22-33	160%	Zn^{2+}	All tissues, highest in Breast
	10	Cytoplasm> nucleus	HDA1	669	22q13.31-33	130%	Zn^{2+}	All tissues, highest in prostate and kidney
Class III	SirT 1	Nucleus	Sir2	747	10	n/a	NAD	n/a
	SirT 2	Cytoplasm> nucleus	Sir2	373	19q13	n/a	NAD	n/a
	SirT 3	Mitochondria	Sir2	399	11p15.5	n/a	NAD	n/a
	SirT 4	Mitochondria	Sir2	314	12q13	n/a	NAD	n/a
	SirT 5	Mitochondria	Sir2	310	6p23	n/a	NAD	n/a
	SirT 6	Nucleus	Sir2	355	19p13.3	n/a	NAD	n/a
	SirT 7	Nucleolus	Sir2	400	17q	n/a	NAD	n/a
Class IV	11	Nucleus and cytoplasm	Rpd-3 and HDA1	347	3p25.2	n/a	Zn^{2+}	Brain, heart, skeletal muscle, and kidney

*(Amount expression in brain tumor/ amount expression in normal brain tissue)×(100%) Abbreviated terms: amino acid (AA), histone deacetylase 1 (HDA1), histone deacetylase complex (HDAC), nicotinamide adenine dinucleotide (NAD), reduced potassium dependency 3 (Rpd-3), sirtuin (Sir).

one such mechanism of alteration playing an important role in tumor formation, progression and resistance to treatment (4, 5). In normal cellular biology, histone proteins help control gene expression by modulating chromatin structure and function. Post-translational modifications of histone tails, including acetylation, methylation, ubiquitination and phosphorylation (the histone code), determine how these histone proteins control chromatin remodeling (2-5). The ultimate goal of HDAC inhibitor glioblastoam therapy in a pre-clinical setting is to re-establish balance of histone acetyl-transferase (HAT) to HDAC activity, thereby enhancing the body's own cancer fighting abilities and sensitizing tumor cells to HDAC inhibitor not only as monotherapeutic agents but also in combination with radiation therapy. HDAC inhibitor therapy is of particular interest in neurologic cancers because of HDAC inhibitors' potential ability to penetrate the blood brain barrier (BBB) (2). HDAC inhibitors have been shown to be effective anticancer agents *via* multiple mechanisms, including the induction of cell-cycle arrest, intrinsic and extrinsic apoptotic mechanisms, mitotic cell death, autophagic cell death, generation of reactive oxygen species (ROS), inhibition of angiogenesis and improvement in natural killer (NK) cell–mediated tumor immunity. It is important to discuss the basic mechanisms of HDAC inhibitor therapy in which HDAC inhibitors have been applied in glioblastoma therapy in pre-clinical and clinical contexts.

Table II. *Histone deacetylase complex (HDAC) inhibitor characteristics and developmental progress (6, 15, 18, 21, 23, 26-46).*

HDAC inhibitor class	Drug	Histone deacetylase specificity	Blood brain barrier penetration	Phase of development for glioblastoma applications	Other approved uses
Short-chain fatty acid	Pivaloyloxymethyl butyrate (Pivanex, AN-9)	Class I and Class IIa	Yes	Pre-clinical	Phase II for non-small cel lung cancer (stopped)
	Sodium Butyrate	Class I and Class IIa	Yes	Pre-clinical	Phase II for endogenous antibiotics in gut
	Buphenyl (Sodium Phenylbutyrate)	Class I and Class IIa	Yes	Phase II	Treatment urea cycle disorders
	Valproate	Class I and Class IIa	Good, protects the blood brain barrier	Phase II	Treatment of epiplepsy, anorexia nervosa, panic attack, anxiety disorders
Benzamide	Entinostat (MS-275)	HDAC 1, 2, 3	Low	Pre-clinical	Phase III for Breast Cancer
Cyclic	Romidepsin (Istodax, FK228, FR901228)	HDAC 1, 2, 3, 8	Low	Phase I/II	Treatment of cutaneous T-cell lymphoma tetra-peptide and phase trials for many other cancers
Hydroxamate derivatives	Azelaic bishydroxamic acid (ABHA)	HDAC 3	N/a	Pre-clinical	None
	m-carboxycinnamic bishydroxamic acid (CBHA)	HDAC 3	Yes	Pre-clinical	None
	Dacinostat (LAQ824)	Class I and II	Good	Pre-clinical	None
	AR-42 (OSU-HDAC-42)	pan-HDAC	Yes	Pre-clinical	Phase I for acute myeloid leukemia
	Panobinostat (LBH-589)	HDAC 1, 2, 3, 6	Good	Phase II	Phase III for several cancers
	Quisinostat	Class I and II	N/a	Pre-clinical	Phase II T-cell lymphoma
	Suberic bishydroxamic acid (SBHA) Scriptaid	HDAC 1,3	N/a	Pre-clinical	None
	Trichostatin A	HDAC 1, 3, 8	N/a	Pre-clinical	None
	Vorinostat (SAHA)	HDAC 1, 2, 3, 4, 6, 7, 10	No	Pre-clinical	None
		HDAC 1, 2, 3, 6	Low	Phase III	Treatment cutaneous T-cell lymphoma and phase trials for many other cancers
Miscellaneous -trisulfide	Diallyl Trisulfide (DATS)	Unknown	N/a	Pre-clinical	None

HAT and HDAC Regulation to Therapy

Acetylation of histone, which is regulated through the opposing actions of HATs and HDACs provides an important level of epigenetic control on gene expression by altering chromatin activity. HATs and HDACs physically associate with modification-specific modules for sequential actions with different modifications. More specifically, histone acetylation HATs transfer acetyl moieties to lysine residues and HDACs remove the acetyl moieties (8). HAT acetylates the lysine residues of histones, while HDAC deacetylates the lysine tails. Ultimately, this means that increased HAT activity will lead to increased gene transcription, while increased HDAC activity leads to decreased gene transcription (9). The functions of HDAC and HAT on chromatin structure are illustrated in Figure 1. HAT activity relaxes chromatin, permitting various transcription factors to interact with DNA, thereby promoting transcription. There are three major families of HATs: general control non-derepressible 5 (Gcn5)-related N-acetyltransferases (GNATs), p300/CBP and MYST proteins (9). Based on the literature, HDACs can regulate the expression of a large number of genes by direct interaction with transcription factors such as E2f, signal transducer and activator of transcription 3 (Stat3), protein 53 (p53), the retinoblastoma protein, nuclear factor kappa B (NF-kB) and transcription factor IIE (TFIIE). Moreover, HDACs are involved in the deacetylation not only of chromatin proteins, which can lead to altered genetranscription regulation, but also of nonhistone proteins, which regulate important functions that, in turn,

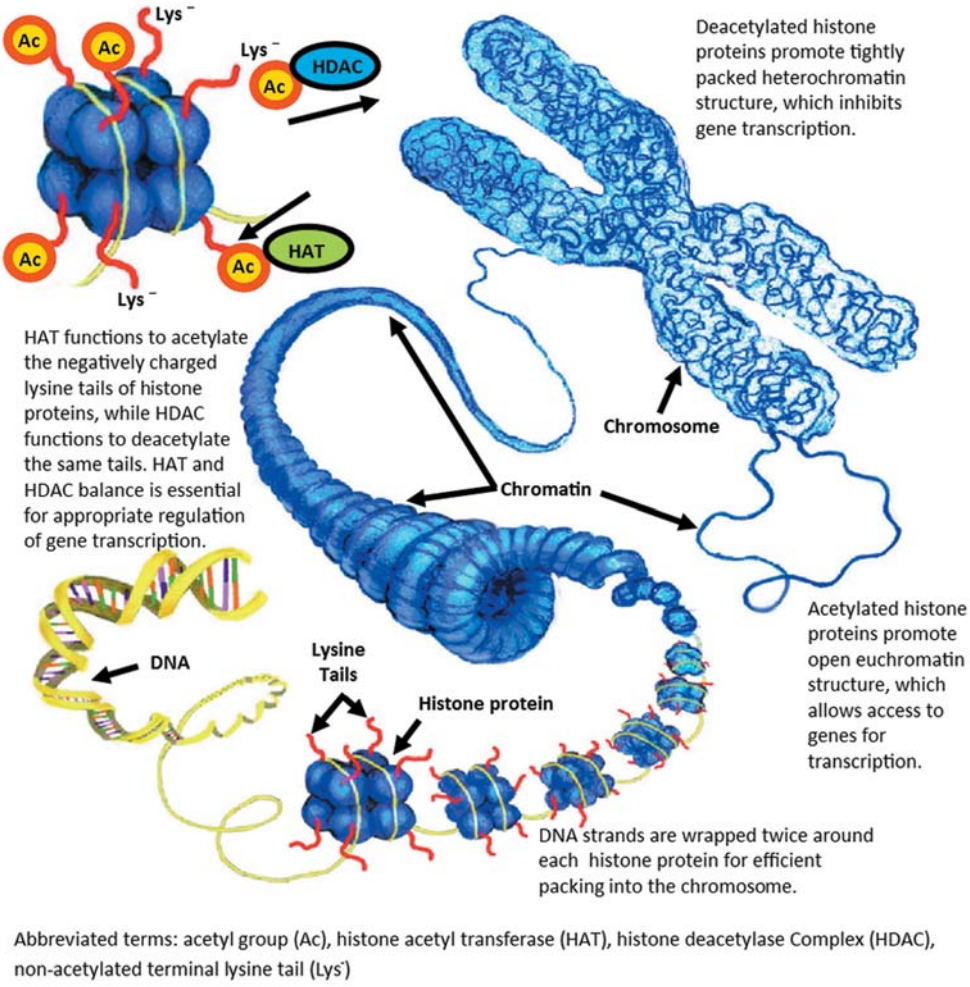

Figure 1. *The functions of HDAC and HAT on chromatin structure.*

regulate cellular homeostasis (cell-cycle progression, differentiation and apoptosis). Many of these pathways are abnormal in tumor cells and consequently can be targeted by HDAC inhibitor therapy.

HDAC in Cancer

Abnormal HDAC activity has been implicated in many types of cancer (3) but the abnormal activities of HDACs in cancer are not well defined (10). The most prominent epigenetic changes in tumor cells include hypermethylation or hypoacetylation of tumor suppressor genes or hypomethylation or hyperacetylation of oncogenes (6). Cancers characteristically up-regulate some specific HDACs (10), while they down-regulate other specific HDACs (11). These effects are not the same in all cancers, for example HDAC 8 is up-regulated and associated with advanced-stage neuroblastoma, while HDAC 2 is associated with early-stage colorectal cancer and HDAC 5 is down-regulated in breast cancer (12). The multiple categories and various activities of HDACs in cancer provide the potential for designing drugs to target epigenetic changes in specific cancers. Before effective treatments are discussed, it is important to understand the classification of HDACs and their specific roles in the origin and progression of glioblastoma.

There are four different classes of HDACs, which are established based on function and similarity to yeast proteins (3). Class I (HDAC 1-3, 8), II (HDAC 4-7, 9-10) and IV (HDAC 11) are recognized as "classical" HDACs and are Zinc-dependent enzymes (12). An exception to this is HDAC 8, which uses Fe^{2+} as its major catalytic activator but it still can be activated by Zn^{2+} (13). Class III (Sirt 1-7) HDACs are composed of nicotinamide adenine dinucleotide-dependent sirtuins (14, 15). Class I has similar homology to Rpd-3 yeast

transcription factor, Class II has similar homology to yeast HDA1, Class III has similar homology to yeast Sir2 and class IV has mixed homology between Rpd-3 and HDA1 (15, 16).

There are few studies, to date, characterizing the activity of HDACs in glioblastoma, so this remains an under-explored step in developing HDAC inhibitors to treat glioblastoma. Lucio-Eterovic *et al*. found that most class II and IV HDACs are expressed at progressively lower levels as astrocytomas progress to glioblastoma, and H3 histones are actually hyperacetylated in glioblastoma compared to normal brain tissue (11). This suggests that there is a relationship between class II and IV HDACs, and astrocytoma progression to glioblastoma (11). This study showed that many of the HDACs expressed at low levels in glioblastoma were expressed at higher levels in grade III astrocytomas, suggesting that the low expression of HDAC II and IV in glioblastoma is more likely a late-stage compensatory response rather than a causative mechanism (11). However, all that can be confirmed with current information is that expression of class II and IV HDACs are inversely proportional to progression from astrocytoma to glioblastoma. A study from the University of Amsterdam found an impressive increase in expression of HDAC 4 in general brain tumors (not glioblastoma specifically), see Table I (17). Recent finding of Mottet *et al*. report that HDAC4 mediates repression of the tumor suppressor gene *p21* (WAF1/Cip1) through Sp1/Sp3 binding. Furthermore, this group found that induction of p21(WAF1/Cip1) *via* silencing of HDAC4 arrested cancer cell growth *in vitro* and inhibited tumor growth in an *in vivo* human glioblastoma model (REF?). This finding also suggests that HDAC 4 plays a role in CNS cancers but there is a strong need for studies further characterizing the specific activities of HDACs in cancer and glioblastoma. See Table I below for summary of HDAC characterization, distribution and activity in brain tumors.

Most HDAC inhibitors that are developed as anti-cancer agents target Class I, II and IV HDACs (22). HDAC inhibitors are pleiotropic molecules, which elicit a wide range of effects on cancer cells, such as cell-cycle arrest, apoptosis, cell differentiation, autophagy and anti-angiogenic effects (15, 22). These effects may be due to changes in histone or non-histone proteins. The main targets in cancer are the intrinsic effects of HDAC inhibitors on cancer cells, such as up-regulation of tumor suppressor genes; but HDAC inhibitors also fight cancer by extrinsic effects on the immune system and inhibiting tumor angiogenesis (23).

HDAC inhibitors can be specific to a particular HDAC or they can be pan-HDAC inhibitors targeting multiple HDACs (24). HDAC inhibitors are divided by structure into seven categories. These categories include short chain fatty acids, benzamides, cyclic peptides, electrophilic ketones, hydroxamines, sirtuin inhibitors and miscellaneous (6). These categories are based on target HDACs and the chemical structure of the HDAC inhibitor (15). Sirtuin inhibitors affect the class III HDACs that rely on NAD as a cofactor. The other HDAC inhibitors work on Zn^{2+} dependent HDACs in Class I, II or IV (6). Aberrant Class III HDACs likely play a role in tumorigenesis and there is potential that inhibiting them may be an effective means of fighting tumors. However, the specific role of class III HDACs is poorly understood and Sirtuin inhibitors have not yet been well explored in glioblastoma therapy.

The inhibitors of Zn^{2+}-dependent HDACs have been established as anticancer drugs, with some of these already on the market for cancer therapy and showing promise in clinical trials for use in treating glioblastoma (6). For this reason, this paper will describe Class I, II and IV HDAC inhibitors that show potential in glioblastoma treatment; the drugs described are summarized below in Table II. It should be noted that the HDAC specificity of the HDAC inhibitors has minor variations between different studies.

Since most HDAC inhibitors being evaluated for glioblastoma therapy have been previously approved for other cancers and diseases, one consideration -important for glioblastoma application- is whether or not these drugs are able to cross the BBB. These drugs may be promising in preclinical studies and clinical studies of other cancers; but if they are not able to cross the BBB, they may not have utility in glioblastoma therapy without special drug targeting techniques. A study in mice showed that gliomas may compromise the BBB, which would increase the ability of HDAC inhibitors to access the tumor tissue, thus giving possible feasibility to HDAC inhibitors unable to cross the BBB (25). BBB penetration data is not available for all drugs examined in this review, but it is noted when available.

Short Chain Fatty Acids

Pivaloyloxmethyl butyrate (Pivanex, AN-9). AN-9 is a class I and IIa HDAC inhibitor in pre-clinical testing for glioblastoma. AN-9 forms the products butyric acid, formaldehyde and pivalic acid from intracellular hydrolytic degradation (47). A glioblastoma xenograft study in mice showed that AN-9 inhibited tumor growth in combination with radiation therapy; AN-9 has been shown effective in combination therapy with radiation and temozolomide (TMZ). A phase II study of pivanex in combination with docetaxel by Titan Pharmaceuticals was halted because of safety concerns but other phase II studies have been completed; a phase II study for heart failure and depressive symptoms is currently recruiting (46). Possible adverse reactions of AN-9 with chemotherapy agents means there is a strong need to evaluate dosing and drug interactions but results of phase I and II trials without serious adverse effects indicate that further investigation of AN-9 as an anticancer agent is warranted (48, 49)

Sodium Butyrate (Butyrate). Sodium butyrate is a class I and IIa HDAC inhibitor that is currently in preclinical testing for glioblastoma therapy and phase II clinical trials as an endogenous antibiotic. Sodium butyrate stabilizes *p21* mRNA, activates p16 and p21, which leads to cell cycle arrest (6). It induces astrocyte growth arrest and differentiation by up-regulating CD81 (50). Butyrate induces apoptosis in glioblastoma cells by up-regulating the Bad protein (51). It also inhibits the formation of tumor vasculature by lowering vascular endothelial growth factor (VEGF) expression in glioblastoma cells (52).

Sodium Phenylbutyrate (Phenylbutyrate, 4-PB, Buphenyl). 4-PB, a class I and IIa HDAC inhibitor, is already approved by the FDA for treatment of urea cycle disorders and is being investigated for therapy in multiple types of cancer. The drug is converted to phenylacetate by mitochondrial beta-oxidation *in vivo* (53). Additionally, it is being explored as a treatment for a wide variety of diseases including insulin resistance, cystic fibrosis and maple syrup urine disease; It is currently in phase II trials for treatment of brain tumors (46). A pharmacological study outlining maximum tolerated dose and pharmacodynamic parameters has been performed and recommended a dose of 27 g/day in heavily pre-treated patients (54). This pharmacological information available should encourage researchers as they plan dosing levels for future clinical trials. Combination studies have shown that 4-PB exerts a synergistic effect with gemcitabine by overcoming drug resistance (55). Some of 4-PB's tumor fighting mechanisms involve inhibition of cellular export, up-regulation of p2 and possibly by increasing the intercellular communication of apoptotic factors through gap junctions (55). A synergistic effect was also found in a combination study with bortezomib, analyzing apoptosis in glioblastoma cells (56). 4-PB also exhibits radiosensitizing in glioblastoma cell lines with mutant p53, but this radiosensitizing effect is not present in cell lines with normal wild type p53 (57). Entin-Meer *et al*. indicated that the HDAC inhibitor AN-113 (butyroyloxymethyl-4-phenylbutyrate), which is made from its 4-PB precursor, may prove to be a more potent anti-neoplastic agent for the treatment of gliomas over 4-PB (58). However no other studies have reported using AN-113.

Valproate (valproic acid). Valproate is a Class I and IIa HDAC inhibitor and an antiepileptic. This provides an interesting dynamic in glioblastoma therapy because, in addition to fighting tumor cells, it can help relieve seizures that a patient may be experiencing because of a tumor. A retrospective study of glioblastoma patients taking valproate to treat seizures showed that patients taking valproate had a longer median survival (23.9 *versus* 15.1 months) than patients not taking valproate, likely due to radiosensitizing effects (59). Valproate is not normally given as a prophylactic antiepileptic in glioblastoma because of more significant side effects compared to antiepileptic drugs; however, it is the only epileptic drug shown to increase survival in glioblastoma patients. In a preclinical combination study, valproate interacted synergistically with bortezomib to induce apoptosis in a study of glioblastoma cells (56). A larger retrospective study of valproate in 102 glioblastoma patients showed that valproate dosed as an antiepileptic level only provided survival benefits to a small subset of patients (37). A retrospective study of high grade glioma in pediatric patients showed that valproate did not increase undesired toxicity but also did not improve outcomes (60). This study should be encouraging to those hoping to safely conduct studies of valproate in pediatric patients (60). Since there were no benefits at anti-epileptic dosing, it also means that any future studies of valproate would need to be justified by treatment changes, such as dosing or combination therapy. Valproate has been shown to be a good penetrator of the BBB (36). Additionally, valproate has been shown to protect the BBB through suppression of NF-kB through HDAC inhibition, MM-9 induction and degradation of tight junctions (37).

Benzamides

Entinostat (MS-275). Entinostat is a class I HDAC inhibitor in pre-clinical testing for glioblastoma and phase III clinical trials for breast cancer. Preclinical testing in combination with temozolomide has shown that entinostat inhibits glioma cell growth by up-regulating p21 and inducing G_0/G_1 cell cycle arrest; and by causing apoptosis (61). Entinostat causes cell death by ROS and mitochondrial damage in other cancer cells lines and may show similar effects in glioblastoma (61). It is able to cross the BBB, but penetration into brain tissue is poor and it has been found that less than 0.001% of an injected dose per cubic cm of entinostat is distributed in the brain tissue (61, 34). This indicates that entinostat therapy may benefit from combination with drug targeting methods.

Cyclic Tetrapeptides

Romidepsin (depsipeptide, FK228, Istodax, FR901228). Romidepsin is a class I HDAC inhibitor and belongs to the depsipeptide group of molecules. It functions by down-regulating the antiapoptotic protein Bcl-xL and up-regulating p21 expression (62). Romidepsin is in phase II trials for glioblastoma and is already approved for use in cutaneous T-cell lymphoma. Berg *et al*. found that romidepsin had a 2% cerebrospinal fluid (CSF) penetration (39). A phase I/II study on gliomas using romidepsin found that the current clinical dose was ineffective for therapy in patients with recurrent glioblastoma (62). Another phase II study on patients with

metastatic neuroendocrine tumors using romidepsin was terminated when it was found that the drug was associated with serious adverse cardiac events and possibly sudden death (63).

Hydroxamate Derivatives

Azelaic Bishydroxamic Acid (ABHA). ABHA is a HDAC 3 inhibitor that functions by up-regulating p21 expression and causing G_1 cell-cycle arrest (64). A cell study found that ABHA inhibits proliferation at low dose as cell cycle is arrested, but is cytotoxic at high doses. Cytotoxic apoptotic effects of ABHA are inhibited by up-regulated p21 (64).

CBHA (m-carboxycinnamic acid bis-hydroxamide). CBHA is an HDAC 3 inhibitor that is in pre-clinical testing for glioblastoma. CBHA induced apoptosis of neuroblastoma cells *in vitro* in combination with retinoids and inhibited neuroblastoma in combination with retinoic acid in severe combined immunodeficiency (SCID) mice (65).

Dacinostat (LAQ824). Dacinostat is a class I and II HDAC inhibitor that is not yet in clinical testing and that has also not yet been tested preclinically in glioblastoma. It has been shown to have antiproliferative effects in leukemia cells in polycomb ring finger oncogene (*BMI1*) and c-MYC proteins (66). Dacinostat is listed because it has been shown to have good BBB permeability, and therefore may prove an effective agent for glioblastoma therapy in future studies (35).

AR-42 (HDAC-42). AR-42 inhibits multiple classical HDACs and is currently in phase I clinical trials for acute myeloid leukemia. A pre-clinical study in acute myeloid leukemia showed that AR-42 effectively induced apoptosis by interfering with mitochondria and cell signaling pathways (67). A study in glioblastoma cell lines found that AR-42 inhibits telomerase activity, which could equate to inhibiting tumorigenesis in an *in vivo* model (68).

Panobinostat (LBH-589). Panobinostat inhibits HDACs 1, 2, 3 and 6. It is currently in phase II trials for glioblastoma therapy, but is in phase III clinical trials for multiple other types of cancers. Panobinostat causes a delay in DNA damage repair after radiation treatment, inhibits migration and invasion of glioma cells and impairs tumor vascular formation (69). A phase I trial for high-grade glioma showed adverse effects, including thrombocytopenia, neutropenia, diarrhea, hypophosphatemia, esophageal hemorrhage and deep venous thrombosis (70). Due to the serious nature of these adverse events (a problem with many HDAC inhibitors) dosing is limited; however, panobinostat was found to be safe and has proceeded to phase II trials (70). Panobinostat has shown potential for future use in combination therapy with chemotherapy and radiation therapy; anti-angiogenic effects demonstrate that it may be effective in combination therapy with a VEGF inhibitor (70).

Quisinostat (JNJ-26481585). Quisinostat is a class I and II HDAC inhibitor that is currently in phase II trials for T-cell lymphoma and in pre-clinical testing for glioblastoma. Xenograft studies of quisinostat in single drug study for glioblastoma showed slowed tumor growth, but the effects *in vivo* in mice were not as strong as what was previously demonstrated in *in vitro* studies (32). Quisinostat is still in early stages of an investigation for glioblastoma and positive preclinical findings certainly indicate the need for further studies of quisinostat as a single agent and in combination therapy. The first phase I clinical trial of quisinostat in humans showed that it is tolerated similar to other HDACs and suggested a phase II intermittent dosing regimen of 12 mg on Monday, Wednesday and Friday to help patients better tolerate quisinostat (71).

SBHA (suberic bishydroxamate). SBHA is a HDAC 1 and 3 inhibitor that is currently in pre-clinical testing. A study in human glioma cells found that SBHA up-regulated p21 and inhibited Cdc-2, which leads to down-regulated survivin and x-linked inhibitor of apoptosis protein (XIAP) resulting in TRAIL-induced apoptosis (69).

Scriptaid. Scriptaid is an HDAC 1, 3 and 8 inhibitor that is in preclinical testing for glioblastoma. A study in glioblastoma cells showed that scriptaid induces apoptosis and reduces cell proliferation by increasing and activating Jun N-terminal kinase (72).

Trichostatin A (TSA). TSA inhibits class I and II HDACs, although HDAC 2 experiences a desensitizing effect after multiple treatments with TSA (16). TSA stabilizes *p21* mRNA and activates p16 and p21 (6). Up-regulation of p53 expression by TSA leads to increased p21 transcription and ultimately results in G_1/S cell cycle arrest (73). TSA also induces astrocyte growth arrest and differentiation by up-regulating CD81 (50). Bajbouj *et al*. found that TSA inhibited proliferation and viability of glioblastoma cells and increased sensitivity to radiation (73). This radiosensitizing effect was reaffirmed in glioblastoma cells receiving TSA 18 h before radiation therapy (18).

Suberanilohydroxamic acid (SAHA, Zolinza). Suberanilohydroxamic acid (vorinostat) is one of the most well-known and best studied HDAC Inhibitors. It has well demonstrated cancer fighting properties including up-regulation of the *p21* (*CDKN1A*) cancer suppressor gene, and thus cell-cycle arrest in G_1 phase (6). It also induces autophagy of tumor cells by increasing LC3 expression and inhibiting mTOR

(74). SAHA is a class I and II HDAC inhibitor and studies show that SAHA induces acetylation of HDACs 3 and 4 near the *p21* promoter region (6). It showed significant synergistic effects with the proteasome inhibitor bortezomib during a pre-clinical study that evaluated apoptosis in glioblastoma cells (56). This synergistic effect is largely due to mitochondrial energy and apoptosis from increased ROS (75). A phase II study of SAHA in combination with bortezomib showed that SAHA, dosed at 400 mg daily intravenously, was well-tolerated with the major toxicities being thrombocytopenia, anemia and fatigue; however, a lack of significant benefits (0 of 34 patients were progression-free at six months) led the investigators to recommend not investigating SAHA further at the dosing level of this study (76). A single-agent phase II study of vorinostat in glioblastoma therapy dosed at 200 mg twice daily by mouth, showed similar toxicity results with the most common toxicities being thrombocytopenia and fatigue; however, this study found that glioblastoma pathways were moderately affected by treatment (5 of 22 patients were progression-free at six months) and the authors did recommend further testing (77). The discrepancy between these studies indicates the need for further study of combination drug interference and drug administration methods. Another explanation of the disappointing results of combination therapy is offered in a TMZ and SAHA combination study in cells. This study found that SAHA actually increased drug resistance to TMZ by increasing acetylation of lysine 9 on histone 3, which up-regulates O6-methylguanine-DNA-methyltransferase (MGMT) expression (MGMT repairs 06-methylguanine lesions and prevents cell death due to collapsed replication forks) (78). Vorinostat appears to be a weak brain HDAC inhibitor likely because of its short half-life and low BBB permeability (42). The BBB permeability-surface area product to free drug of vorinostat is less than 2 magnitudes of that predicted by passive diffusion (33). There is currently an ongoing phase I/II clinical trial for glioblastoma using combination therapy of bevacizumab and vorinostat and another phase I trial for malignant glioma using a combination of temozolomide and vorinostat (46). The results of phase II trials and BBB penetration studies suggest that vorinostat may have some benefit for glioblastoma patients but not nearly as much as predicted by studies in other cancers and *in vitro* glioma cells lines.

Miscellaneous

Diallyl Trisulfide (DATS). DATS is a trisulfide HDAC inhibitor derived from garlic that is currently in pre-clinical testing. It has been shown to fight cancer cells by endoplasmic reticulum stress and mitochondrial damage that leads to increased ROS (37). A study of glioblastoma xenografts in SCID mice showed that DATS up-regulates p21 and p53 expression, causes cell cycle arrest and increases apoptotic factors (79). Perhaps the greater hope for DATS, as a more unique category of HDAC inhibitor derived from garlic, is that it will have less toxicity than other HDAC inhibitors. The xenograft study in SCID mice did not find any hepatotoxicity even at their maximum doses (79). DATS is not currently in any clinical trials but it is often sold as a nutritional supplement.

Challenges and Future Directions

There is still much work to be done in the development of HDAC inhibitors for the treatment of glioblastoma. Current pre-clinical studies have revealed much about the mechanisms of cell death and clinical trials are evaluating how well HDAC inhibitors function in humans; but there are many gaps in our knowledge over the mechanisms that are actually causing cell death, how HDAC inhibitors activate these mechanisms and how the body processes and metabolizes these drugs. One area that has been partially explored is not all HDAC inhibitors, which cross the BBB, are highly effective against this tumor *i.e.*, entinostat (MS-275) and still most types of HDACs show marginal to moderate anti-glioma effects in clinical trials. HDAC inhibitor resistance has been examined *in vitro* to further our understanding over HDAC biology and to suggest strategies for rational combination therapy. A mutation in *HDAC2* was found in cell lines resistant to trichostatin A and the same mutation was found in a subset of primary human tumor samples. Other proposed mechanisms of HDAC inhibitor resistance include up-regulation of cellular antioxidant pathways, increased expression of the anti-apoptotic protein Bcl-2 and the stress-responsive transcription factor NF-κB and use of alternative gene silencing pathways, such as DNA methylation. Finally, the unfolded protein response pathway is implicated in HDAC inhibitor resistance. Now, combination therapy with chemotherapy, radiation therapy or both is becoming a popular topic for study with HDAC inhibitors. Many HDAC inhibitors act synergistically with other chemotherapy drugs or they have radiosensitizing effects. There may be promising results from recently completed studies of combination therapies; however, these drugs do not always act synergistically. It is imperative that future studies explore the possible induction of resistance to radiation and/or chemotherapy agents by HDAC inhibitors in combination trials. Finally, more studies should be performed on glioblastoma tumorigenesis and growth to identify the epigenetic failures that contribute to this malignancy. Identifying specific epigenetic changes and their pathological effects will allow better discrimination of the most effective HDAC inhibitors for specific tumor types based on the HDACs they target and their effects on cellular function. Examples of this type of study would include identifying hypo-acetylated histone and non-

histone proteins, at what stage of malignancy these changes occur and how the body responds to these changes. Although there is still much work to be done, the progress in development of HDAC inhibitors for glioblastoma therapy is highly promising; and, as they become better understood, epigenetic modulators, such as HDAC inhibitors, are sure to play an essential role in cancer therapy.

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Conflicts of Interest

No Author has a conflict of interest.

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