The application of histone deacetylases inhibitors in glioblastoma

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Abstract

The epigenetic abnormality is generally accepted as the key to cancer initiation. Epigenetics that ensure the somatic inheritance of differentiated state is defined as a crucial factor influencing malignant phenotype without altering genotype. Histone modification is one such alteration playing an essential role in tumor formation, progression, and resistance to treatment. Notably, changes in histone acetylation have been strongly linked to gene expression, cell cycle, and carcinogenesis. The balance of two types of enzyme, histone acetyltransferases (HATs) and histone deacetylases (HDACs), determines the stage of histone acetylation and then the architecture of chromatin. Changes in chromatin structure result in transcriptional dysregulation of genes that are involved in cell-cycle progression, differentiation, apoptosis, and so on. Recently, HDAC inhibitors (HDACis) are identified as novel agents to keep this balance, leading to numerous researches on it for more effective strategies against cancers, including glioblastoma (GBM). This review elaborated influences on gene expression and tumorigenesis by acetylation and the antitumor mechanism of HDACis. Besdes, we outlined the preclinical and clinical advancement of HDACis in GBM as monotherapies and combination therapies.

Keywords: glioblastoma, epigenetic, histone acetylation, histone deacetylation, histone deacetylases inhibitors, gene expression

Background

Glioblastoma multiforme (GBM) is the most common and malignant intracranial primary tumor in adults, and it has the characteristics of substantial invasion and rapid progress. Although there is a standard combined treatment strategy of surgical plus radiotherapy and chemotherapy, the prognosis is still poor. Thus, the treatment of GBM meets severe challenges. In recent years, the view of epigenetic mechanisms contributing to diverse tumors has drawn people's attention, and histone modification is among the well-understood examples of epigenetics.

Epigenetic changes refer to altering gene expression and cellular phenotype without modifying the DNA sequence itself. Structurally, two copies each of the core histone proteins (H2A, H2B, H3, and H4) whose N-terminal tails extend outward are wrapped around DNA and then package DNA into nucleosomes [1, 2]. Next, regular repeating structure nucleosomes compose chromatin that is the foundation for gene regulation [3]. This architecture changes when amino acid residues on the histone tails are modified by post-translational acetylation, methylation, and phosphorylation [4], leading to alteration of the distance between DNA and histones, which in turn changes the accessibility of transcription factors to gene promoter regions and finally the level of gene expression [5]. These modifications of histone N-terminal tails are determined by two kinds of enzymes. The one includes histone lysine methyltransferases

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(KMTs), histone acetyltransferases (HATs), and DNA methyltransferases (DNMTs). The other contains histone demethylases (KDMs), histone deacetylases (HDACs) and the ten-eleven translocation (TET) family of 5-methylcytosine oxidases [6]. The balance between these two determines chromatin architecture and then influences biological events, such as cell cycle, differentiation, and apoptosis in cancer cells [7].

Among these modifications, DNA hypermethylation and histone deacetylation in GBM has already been discovered for many years [8, 9]. But to date, no drugs that target histone methylation are approved by FDA or under clinical trials. However, another one, histone deacetylase protein is being exploited as therapeutic drug targets in various cancers, making it the focus of attention in GBM researches. Now histone deacetylase protein inhibitors, as the only one of the epigenetic agents, have been investigated in clinical trials in glioblastoma [10]. Thus, this review will firstly provide a brief overview of the effects of acetylation on gene expression and tumor phenotype, elaborate the antitumor mechanism of HDAC inhibitors, and then outline several of these promising HDACis that are in pre-clinical and clinical studies in GBM as monotherapies and combination therapies.

Histone acetylation

There are two groups of proteins that undergo acetylation. The one includes five types of histone proteins(H1, H2A, H2B, H3, and H4) acting as the primary protein components of chromatin [11], which was first observed as early as the 1960s [12, 13]. And every one of histone proteins can be divided into three classes of three-dimensional structural motifs: the histone-fold regions, their diverse extensions, and the histone tails. These tails contain recognition sites of histone post-translational modifications, among which the reversible acetylation of histone tails has brought remarkable advances for the past few decades because of its significant role in gene expression and carcinogenesis [3]. The other is nonhistone proteins, including tumor suppressor protein p53 and the tubulin components of the cytoskeleton. They participate in many critical cellular pathways, including chromatin remodeling, cell cycle, splicing, nuclear transport, actin nucleation, and mitochondrial metabolism [14].

The balance between acetylation and deacetylation of histone is controlled by two groups of enzymes: histone acetyltransferase (HAT) and histone deacetylase (HDAC) [15]. HATs transfer acetyl groups to amino-terminal lysine residues in histones, resulting in an open and accessible chromatin structure. HDACs remove these groups oppositely, contributing to chromatin condensation and transcriptional repression [16, 17]. Herein we separately describe these two molecules as follows for a better understanding of histone acetylation and their relationship to gene expression.

HAT

Histone acetyltransferases are a diverse set of enzymes that can be divided into two groups according to their suspected cellular origin and functions: B-type HATs, in the cytoplasm, likely catalyze acetylation events linked to the transport of newly synthesized histones from the cytoplasm to the nucleus for deposition onto newly replicated DNA [18–20]. A-type HATs, conversely in nuclear, likely catalyze transcription-related acetylation events [21]. Based on several highly conserved structural motifs of the catalytic domains, HATs contain three major families (Table 1): general control nonderepressible 5 (Gcn5)-related N-acetyltransferases (GNATs) that have members of Gcn5, PCAF, Elp3, Hat1, Hpa2, and Hpa2; MYST family comprised by primarily MOZ, Ybf2 (Sas3), Sas2 and Tip60; and p300/CBP family that consist of p300 and CBP [30]. Besides, there are other proteins like Taf1 as well as many nuclear receptor co-activators that do not contain true consensus HAT domains shown to possess intrinsic HAT activity [31].

The completely catalytic role of HAT families requires certain mechanisms. Respectively, GNAT family employs the kinetic mechanism that requires the formation of a ternary complex (enzyme • acetyl-CoA • H3 histone) before catalysis to guide acetyl transferred from acetyl-CoA to the substrate acceptor without the formation of a covalent enzyme intermediate [18, 32–35]. MYST family has been shown to possess a ping-pong catalytic mechanism [36], in which the acetyl group from acetyl-CoA is firstly transferred to the cysteine utilizing base glutamate deprotonates the active site cysteine. Then glutamate protonates the leaving cofactor and deprotonates the substrate lysine so that the cysteine can transfer the acetyl group to the lysine [22]. And p300/CBP family, as a represent of 'orphan class' of HAT enzymes that do not bind directly to DNA but are recruited to particular promoters through interactions with DNA-bound transcription factors [31], appears to hold a Theorell-Chance mechanism, in which the peptide substrate associates only very transiently with the enzyme with no need for a stable ternary complex, thus leaving as soon as the reaction is complete.

Because of HATs' catalytic role in histone proteins and even nonhistone proteins, they may be important for normal cell proliferation, growth, and differentiation. Therefore loss or misregulation of these activities may lead to cancer. And several lines of evidence have indicated that HAT is tied to tumor suppression [37].

Chen *et al. Journal of Experimental & Clinical Cancer Research*   (2020) 39:138

Page 3 of 18

Table 1 Major histone acetyltransferase families

HAT Families	Family members	HAT domain motifs[a]	Function domains[b]	HAT reaction mechanism[c]
GNAT	Gcn5	C-D-A-B	AT domains bromodomains	kinetic mechanism
	PCAF			
	Elp3			
	Hat1			
	Hpa2			
	Nut1			
MYST	MOZ	A	AT domains	ping-pong catalytic mechanism
	Ybf2 (Sas3)		plant homeo domains	
	Sas2		zinc finger domains	
	Tip60		chromodomains	
p300/CBP	p300	E-D-A-B	zinc finger region	Theorell–Chance mechanism
			(cys, ZZ and TAZ domain)	
	CBP		HAT domains	
			Bromodomains	

[a]**HAT domain motifs,** the relative positions of conserved sequence motifs in the three HAT families GNAT, MYST, and p300/CBP [22–24]. Motif A is the most highly conserved motif, which contains an Arg/Gln-X-X-Gly-X-Gly/Ala sequence that is important for acetyl-CoA recognition and binding [18];
[b]**Function domains,** the function domains for the GNAT, MYST and p300/CBP families of HATs. **AT(acetyltransferase) domains,** transfer acetyl groups from acetyl coenzyme A (acetyl-CoA) onto histone acceptors;
Bromodomains; an acetyl-lysine binding domain [21, 25, 26];
Zinc finger domains and chromodomains; protein:protein interaction domains that are often found in heterochromatin-associated proteins [27];
Plant homeo domains, a common structural motif found in all eukaryotic genomes in the nucleus. It is a Zn^{2+}-binding domain involved in nucleosome/histone binding [28, 29];
[c]**HAT reaction mechanism,** see text for details.

HDAC

The discovery of the first HDAC almost accompanied by the first HAT [38]. Afterward, as more HDACs were found, existing 18 genes were subdivided into two types by their dependency on Zn^{2+} and four classes by homology to yeast, resulting in Zinc-dependent enzymes including class I, II and IV and Zinc-independent enzymes composed of class III HDACs (Table 2) [39, 40]. The class I HDAC family consist of HDAC1, 2, 3 and 8, which has similar homology to Rpd-3 yeast transcription factor and generally stay in the nucleus; The class II that shares homology with the yeast Hda1 protein and shuttles between the nucleus and the cytoplasm incorporates class IIa (HDAC4, 5, 7 and 9) and class IIb (HDAC6 and 10); The single HDAC11 belongs to class IV found in the nucleus that has mixed homology between Rpd-3

and Hda1 [41, 42]; And the last class III homologues of the yeast protein Sir2 is comprised of Sirt1-7 and requires NAD^+ for their activities [43]. Among them, the first three-classes are recognized as 'classical HDACs' and common targets for therapy [44].

Acetylation and gene expression

Stable and closed nucleosomes and chromosomal structures generally impede the access to DNA. But researches, over the past few decades, have revealed that covalent modifications of histone proteins and DNA, such as acetylation, methylation, phosphorylation and citrullination of the histone core, can fundamentally alter the organization and function of chromatin, thus regulating all DNA-based processes, like transcription [45]. Among these covalent modifications, histone acetylation

Table 2 Major histone deacetyltransferase families

Class of HDAC	members of each class	homology to yeast	location	HDACs as anticancer targets
class I	HDACS 1, 2, 3 and 8	RPD3 protein	in the nucleus	i) DNA-based process (DNA repair, replication and recombination) ;
class IIa	HDACs 4, 5, 7 and 9	Hda1 protein	shuttle between the nucleus and the cytoplasm	ii) cell-cycle progression (cell proliferation, differentiation, apoptosis) ;
class IIb	HDACs 6 and 10			iii) migration;
class IV	HDAC11	mixed homology between Rpd-3 and Hda1	in the nucleus	iiii) immunity. (See below for more details)
class III	(NAD+-dependent) SIRT1, 2, 3, 4, 5, 6 and 7	Sir2 protein	in the nucleus	

is a major source of dynamic variation in chromatin structure in vivo. Multiple mechanisms of action are involved in the acetylation-dependent disruption of nucleosome array condensation. Two basic connections in chromatin compaction: histone-histone and histone-DNA interactions, are essential to stabilize the condensed chromatin. The octamer of nucleosome core is assembled by a histone (H3-H4)$_2$ tetramer and two H2A-H2B dimers, around which 146 bp of DNA wraps [5]. This process requires histone-histone interactions and incorporates two steps: the first step leads to the formation of the H2A-H2B dimer and the (H3-H4)$_2$ tetramer; the second step of assembly is between two H2A-H2B dimers and one (H3-H4)$_2$ tetramer [46]. Besides, histone-DNA interactions that block hydrophobic histone or charged histone DNA interfaces exist in a side of double-helix DNA facing the core histone octamer and chaperones recognizing specific histone sites [47]. All of these interactions are necessary to assemble into higher-order chromatin structures, which constrain the regulation of DNA.

The packaging of DNA within the tightly folded chromosomes burns a major barrier to transcription. Thus it is important for transcriptional machinery to change the stability and positioning of chromatin structures. Acetylation of the core histone N-termini, as one of the most studied and appreciated modifications, is widely considered to be correlated to the regulation of transcription. Many experiments show that histone acetylation and deacetylation mainly affects gene expression in the following ways (Figure 1).

Firstly, histone acetylation and deacetylation may function by changing the surrounding charge environment of the nucleosome, which will then strengthen or weaken the interactions between proteins related to gene expression and DNA, and in turn lead to altered chromatin architecture [48]. The histone N-terminal tail extending from the nucleosome core is generally rich in hydrophobic amino acids (e.g., lysine, arginine, serine, etc.), among which positively charged lysine residues is most likely to be acetylated by HAT. Then acetylated histone tail through the addition of an acyl group from an acyl-CoA to lysine residues with the positive charge by HATs will be neutralized, thus resulting in a decrease in binding affinity to the DNA backbone and a negatively charged neighboring nucleosome, which subsequently leads to a possibly decreased nucleosome stability, loose chromatin structure and more accessible underlying DNA [49]. On the contrary, HDACs remove the acetyl groups from histones, thereby getting back to the compacted chromatin and transcriptional repression state.

The second way to regulate gene expression by histone acetylation derives from the 'histone code' theory that has been recognized by most researches and incorporates 'writers', 'readers' and 'erasers' [4, 50]. It is just like a variety of different passwords when histone tails form a large number of special signals as a result of acetylation and deacetylation on different sites of it. Multiple HATs, as 'writers', transfer acetyl groups from acyl-CoA to histone N-terminal tails, producing the code identified by 'readers' that almost all contain the bromodomain [51]. Bromodomain, a structurally conserved module present in transcription-associated proteins (or rather more exact, histone chaperones), can recognize and bind to acetylated histones specifically and then changes the remodeling of nucleosomes, subsequently regulating transcription programs [51]. And these 'readers', including Spt6, the FACT (facilitates chromatin
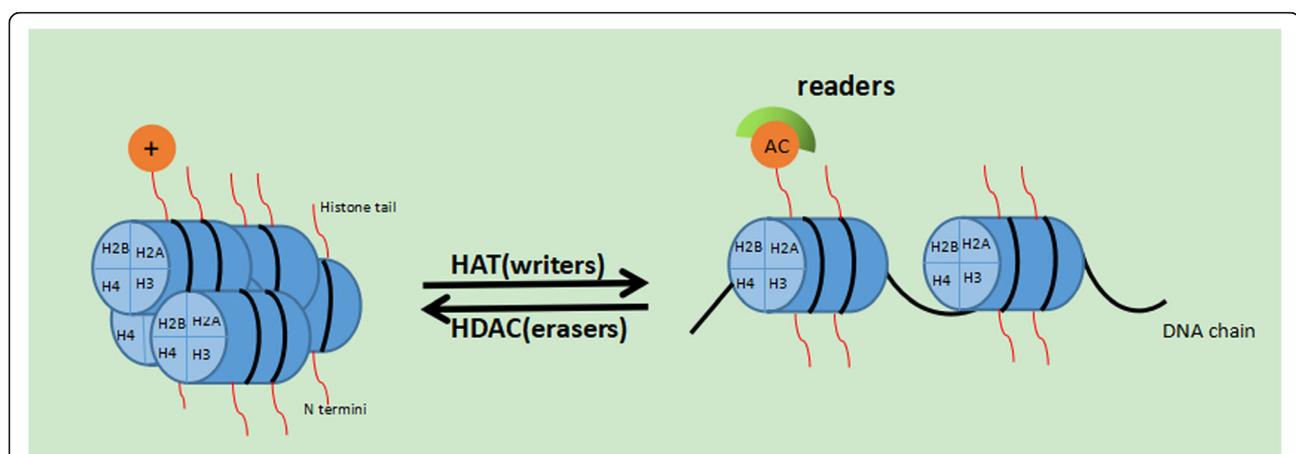

Figure 1 Regulation of chromatin remodeling and gene expression by acetylation. Acetylation(Ac) of the histone tills is the foundation of transcription activation. Acetyl groups on Lys residues transferred by HATs neutralizes the positively charged protruding tails, preventing the interactions with negatively charged one and thus presenting chromatin decondensation. Later this open state allows access to transcription factors and other transcription co-activators termed 'readers' by their contained bromodomain recognizing acetylation site on histone tails

transcription) complex, anti-silencing function 1 (Asf1) and the chromatin assembly factor 1 (Caf1) complex, generally act by invading the nucleosome gradually and/ or interacting with DNA or other proteins to control gene expression indirectly [25]. Once 'readers' bind to particular sites of acetylated lysines, the requisite transcription factors would recruit key regulatory DNA sequences (e.g., promoters and enhancers), leading to initiation of transcription. On the contrary, those enzymes that remove acetylated histone marks are termed as 'erasers', which prevent contacts between those transcription-associated proteins and histones or DNA [52].

Thirdly, there is one kind of protein called ATP-driven nucleosome remodelers, including the SWI/SNF complex and Chd1 or the RSC complex, expected to exert great effects on chromatin structure and mediate transcription factor binding [47]. These proteins use the energy of ATP hydrolysis to rearrange nucleosomal arrays and introduce superhelical torsion into nucleosomal DNA, which causes nucleosome sliding as well as topological and structural changes of chromatin, ultimately yielding a remodeled nucleosome with accessible DNA sites [52, 53]. Up to now, researches have dictated that these complexes, such as SWI/SNF complex, allow recruitment and DNA binding of TFIID to the TATA box, which is responsible for the initiation of transcription involving the crucial steps of sliding of nucleosome to a new position, changes in histone-DNA interactions and an increase in the association with transcription factors [54]. Additionally, another significant function involved in the transcriptional mechanism mediated by ATP-dependent complexes is that histone acetylation creates recognition sites for bromodomains present in these complexes [4]. In contrast, histone deacetylases associate with corepressor complexes to direct gene-specific transcriptional repression. Hence, the state of acetylation on core histone is critical for the recruitment of transcriptional coactivators by ATP-driven nucleosome, which forms another type of 'histone code' similar to chaperones binding to acetylated histone surface. Consequently, it means that chromatin structure and function differ depending on the composition of the histone variants [47].

In addition to these above mechanisms, many ways participate in and assist in transcription regulation by acetylation modification. So it is not just a sample static image of gene regulation, but rather a more dynamic and complex framework for the effects of acetylation on transcription in reality. What's more, not only histone proteins are targeted by HATs and HDACs, but also are non-histone substrates including transcription factors like E2F,p53 and GATA1 activated by HATs [18, 55], and c-Myc, nuclear factor B (NF-B), Stat3, transcription factor IIE (TFIIE), the retinoblastoma protein, hypoxia-inducible factor 1 (HIF-1), as well as estrogen receptor and the androgen receptor complexes repressed by HDACs [10, 56–63]. The activities of these transcription factors can be a major determinant of gene activation or inactivation. Moreover, a wealth of studies uncovered the fact that other post-modifications including methylation, phosphorylation and ubiquitylation may have a tremendous impact on the functional activity of acetylation [11]. Once more than one of them acts on the same site on histones, a cross-talk will occur via multiple mechanisms either competitive or cooperative [64].

HDACs and HDACis in cancer
Given the known function of histone acetylation in transcription, there is a strong need for a balance between histone acetyltransferase and (HAT) and histone deacetylase (HDAC). Shifts in this balance might have dramatic consequences on the cell phenotype [65, 66]. Aberrant recruitment of HDACs, which have been widely studied over the past few years, is tightly associated with malignancies and linked to cancer progression and drug resistance [67]. Accordingly, histone deacetylase inhibitors (HDACis) came into being a few decades ago, which are small moleculors to decrease the high level of HDACs and in turn increase the level of protein acetylation in the cancerous cell, restarting the expression of silenced tumor suppressor genes. Therefore, HDACis are now emerging as novel promising anticancer agents [68].

HDACs in cancer
HDACs, which have various targets of histone and non-histone proteins, are in the focus of cancer researches due to their pleiotropic effects on genome functions including chromatin assembly, DNA repair, replication and recombination [47, 69, 70], as well as on many biological processes, such as cell proliferation, differentiation, apoptosis, and senescence [71]. Numerous evidence have demonstrated the overexpression of HDACs in diverse types of malignancies such as lung cancer [72], breast cancer [73], and hepatocellular carcinoma [74, 75]. Herein we recapitulate several excellent HDAC carcinogenic ways. For example, high level of HDAC1 has been shown to target the oncosuppressor p53 that mediates cell apoptosis [76], to prevent cells from differentiating thus maintaining an undifferentiated phenotype [77], and to enhance cell proliferation through targeting a subset of cyclin dependent kinase (CDK) inhibitors (p21 and p27) [65]. Tip60, a DNA damage-response proteins essential for double-strand break (DSB) repair and apoptosis, might be lack of its activities when it is negatively regulated by SIRT1 and HDAC3 deacetylase activity [78–80], causing accumulation of cell damage and inactivation of the apoptotic

program and finally triggering tumorigenesis [81]. The molecular chaperone heat shock protein 90 (Hsp90) is the target of HDAC6, and the involvement of HDAC6 in the Hsp-mediated regulation of VEGFR can result in an increased fibroblast cell migration [82, 83]. Also, HDAC7 overexpression might induce an increased expression of PDGF-B, which lead to angiogenesis and consequently tumor progression [84]. In addition, growing evidence supports the relation between HDACs and immune system function. Researches suggested that HDACs have a role in not only innate immunity, but also adaptive immune systems [85]. For instance, class I HDACs seem to negatively regulate innate immunity through repressing the production of an inflammatory cytokines such as COX2 [86], NF-κB [87], and IFN-β [88], and also crucially influence adaptive immunity. There is a vast array of other roles in the pathogenesis of cancer as well as other complex biological functions by HDACs. A case in point is the repression of tumour suppressor genes like p21 and the upregulation of oncogenes such as BCL2 by HDAC-mediated deacetylation [89]. Also, many studies over the years indicated that overexpression of individual HDACs correlated with poor cancer patient prognosis independent of other variables such as tumor type and disease progression. For example, elevated HDAC2 levels may be of high relevance to worse prognosis in patients with colorectal cancer [90]. Nevertheless, HDAC overexpression is not always a negative prognostic marker. Indeed, down-regulated expression of HDAC10 in non-small lung carcinoma cells is reported to be related to poor prognosis in lung cancer patients [91].

HDACis

Based on the above introduction, HDACs-mediated deacetylation is responsible for altering a large number of genes implicated in tumorigenesis. For an optimal transcription of these genes, proteins should be in an appropriate acetylated state. Hence, inhibition of histone deacetylases as a therapeutic tool in cancer emerged as a novel class of targeted drugs, which exert an antitumor effect in vitro and in vivo including the induction of the growth arrest, differentiation and apoptosis, and the inhibition of angiogenesis, DNA repair and immunomodulatory activities [92]. For instance, shreds of evidence indicate that suberic bishydroxamate (SBHA), a HDAC inhibitor, induces apoptosis by changing the balance between proapoptotic and antiapoptotic proteins in melanoma cells, which means the overexpression of proapoptotic proteins of Bcl-2 family, such as Bim, Bmf, Bax, Bak and Bik, and the repression of antiapoptotic proteins of Bcl-2 family, such as Bcl-2, Bcl-XL, Bcl-w and Mcl-1 [93]. Also there are some other ways to induce tumour cell death, like autophagy and death receptor pathways. One of the classic HDACis, suberoylanilide hydroxamic acid (SAHA), downregulates AKT-MTOR

signaling (a major suppressive cascade of autophagy) that triggers glioblastoma cell death [94]. In addition, HDACis can downregulate the expression of Nanog, a pluripotency regulator that has been shown to promote cancer progression by regulating CSCs (cancer stem cells) [95]. Table 3 provides examples grouped by pathways of alterations of those genes that play a major role in cancer (see details in Table 3).

HDACis in clinic

Until now, four compounds as HDACis have already been approved by the Food and Drug Administration (FDA) for the treatment of hematological malignancy, especially cutaneous T cell lymphoma (CTCL) and peripheral T cell lymphoma (PTCL). They are vorinostat (also known as suberanilohydroxamic acid, SAHA), romidepsin, belinostat, and panobinostat. Another HDACi, Sodium Phenylbutyrate (4-PB), though not in oncology, is approved by the FDA for the treatment of urea cycle disorders, and is now being investigated for therapy in multiple types of cancer [10]. In addition, CG-745, a new clinical stage histone deacetylase (HDAC) inhibitor produced by CrystalGenomics (a biopharmaceutical company from Korea), has recently been granted as Orphan Drug Designation by FDA for pancreatic cancer (http://www.crystalgenomics.com/), which is currently in Phase II pancreatic cancer trial and the results look promising thus far. All of these encouraging results justified the introduction of more and more HDACis into clinical trials in cancer. The number of these studies that we can search on the web of Clinical-trial.gov so far amount to 622, with more than 350 clinical trials completed or being recruiting, both as single agents and in combination with other therapeutics (https://clinicaltrials.gov/).

HDAC inhibitors that have been found to date are most the pan-HDAC inhibitors targeting multiple HDACs. They either work on Zn2+-dependent HDACs including Class I, II and IV or affect the class III HDACs that rely on NAD as a cofactor [120]. Based on their target and chemical structure, these HDACis are divided into seven categories: short chain fatty acids, benzamides, cyclic peptides, electrophilic ketones, hydro-examines, sirtuin inhibitors and miscellaneous. The common compounds of each category are shown in Table 4. The general pharmacophore essential for the activity of these HDACis includes a hydrophobic capping group for interactions with the surface of the enzyme, a linker essential for interaction with the enzymatic tunnel and connecting the cap by a small connecting unit, and the zinc binding group (ZBG) that chelates the zinc atom in the active site [147]. In these compounds, SAHA is the first and most well-known approved pan-HDACis, which was marketed under the trade name of Zolinza for the treatment of refractory cutaneous

Table 3 Role of HDACis in Cancer

pathway	genes/signalings	tumours affected	representative drug
apoptosis	mutant p53 ↓ [96, 97]	TNBC and pancreatic cancer	SAHA, NaB , VPA and TSA
	proapoptotic proteins of Bcl-2 family, such as Bim, Bmf, Bax, Bak and Bik ↑ antiapoptotic proteins of Bcl-2 family, such as Bcl-2, Bcl-XL, Bcl-w and Mcl-1 ↓ [93]	melanoma	SBHA
	XIAP ↓ [98, 99]	Mesothelioma and leukemia	LBH589 and LAQ824
	TBP2 ↑-Trx ↓-ASK1 signaling ↑ [100]	prostate cancer	SAHA
	ROS ↑ [101]	CLL	MS-275
	TRAIL-DR5 ↑ FASL-FAS (Apo-1 or CD95) ↑ TNF-TNFR-1 ↑ [102, 103]	leukaemia, breast cancer	VPA, SAHA and TSA
	human RAD23 homolog B (HR23B) ↑ [104]	U2OS cells	SAHA
	erbB2 (Her-2) ↓ [105, 106]	breast cancer	TSA and LAQ824
cell death	NF-κβ ↑ [107] AKT-mTOR signaling ↓ [94]	Prostate Cancer glioblastoma	SAHA SAHA
cell arrest	CDKN1A (encoding p21 $^{WAF1/CIP1}$)↑ [108–110]	CML-BC cells, colon cancer and bladder carcinoma	LAQ824, SAHA and Butyrate
	p27 ↑ [111]	leukemia and breast cancer	SAHA and TSA
	GADD45 α and GADD45 β ↑ [112]	colon carcinoma	TSA and Butyrate
	TGF-βRII ↑ - c-MYC ↓ [113]	Ewing's sarcoma (EWS) and neuroblastoma	MS-275
angiogenesis	HIF-1a ↓ [114] VEGF ↓ [115]	Lewis lung carcinoma HepG2 cell	FK228 TSA
DNA repair	Ku86 ↓ Ku70 ↓ [116]	melanoma cells	sodium butyrate (NaB)
	RAD51 ↓ BRCA1&2 ↓ [117]	human squamous carcinoma cells (SQ-20B)	TSA
immunity	MHC class I genes ↑ tumor antigens ↑ PD-1 ligands ↑ [118]	melanoma	LBH589, MS-275 and MGCD0103
	Treg cells ↓ [119]	renal and prostate cancer	entinostat

'↑' or '↓', represent the up-regulated or down-regulated trend of gene expression, respectively.

T-cell lymphoma (CTCL) in 2006. Over the years, Vorinostat has also been found to be a potent agent in the treatment of many types of cancer, such as endometrial cancer [148], lung cancer [149], gastrointestinal (GI) cancer [150] and glioblastoma [151]. And studies have shown that SAHA generally fights against cancer by upregulating the p21 (CDKN1A) cancer suppressor gene, PTEN, p27 and decreasing levels of pro-growth genes CDK2, CDK4, cyclin D1 and cyclin E [152].

Though more and more HDACis have been developed, few have been used in the clinic to fight cancer, the main reasons behind which are their high toxicity and low specificity [122]. And since the toxicity is likely due to broad activity across HDAC isoforms, the development of second-generation HDAC inhibitor has been focused to improve the selectivity of HDACis, resulting in the discovery of series of specific HDACis (see details in Table 4). Until now, most of the agents developed and reported in existing articles have selectivity for HDAC3, HDAC6, HDAC8 and sirt1. For example, there are

several HDAC3-selective inhibitors including RG2833 that increases the frataxin (FXN) gene silenced in Friedreich ataxia and is in a phase I clinical trial in man [153], RGFP966 that inhibits cell growth due to increased apoptosis associated with DNA damage and replication in CTCL cell lines [137], BG45 which induces the death of multiple myeloma cells concomitant with hyperacetylation and hypophosphorylation of STAT3 either singly or in combination with proteasome inhibitors [154], and I-7ab that inhibits cell viability of triple negative breast cancer (TNBC) cells and induces cell apoptosis by promoting hyperacetylation of P53 and its transcriptional activity which in turn induces the expression of p21 and consequently cause cell cycle arrest at G1 phase [155].

Besides these, there are two other special acetylation-modifying agents. The one is a small-molecule inhibitor also termed as BET (bromodomain and extra-terminal family-BRD2, BRD3, BRD4, BRDT, BD1 and BD2) inhibitor including JQ1, I-BET and more recently BY27,

Table 4 The classes of HDACis in clinic

target	chemical classes	compounds	source	Isotype selectivity	study phase	reference
Pan-HDAC	Hydroxamic acid	Vorinostat (SAHA)	Synthetic	class I, II and IV	FDA approval (CTCL)	[121]
		Belinostat(PXD-101)	Synthetic	class I and II	FDA approval (PTCL)	[15]
		Panobinostat (LBH-589)	Synthetic	class I, II and IV	FDA approval (PTCL and multiple myelomas)	[122]
		Trichostatin A (TSA)	Natural	class I and II	Phase I (Relapsed or Refractory hematologic malignancies)	NCT03838926
		Quisinostat (JNJ-16241199)	Synthetic	class I and II	phase II (CTCL)	NCT01486277
		WW437	Synthetic	HDAC 2 and 4	pre-clinical	[123]
	short chain fatty acids	Pivaloyloxmethyl butyrate (AN-9)	Synthetic	class I and IIa	phase II (melanoma) phase I (CLL)	NCT00087477 NCT00083473
		Sodium Butyrate (NaB)	Natural	class I and IIa	phase I (Colorectal cancer)	[124]
		Sodium Phenylbutyrate (4-PB)	Synthetic	class I and IIa	FDA approval (urea cycle disorders)	[10]
		Valproate (valproic acid)	Synthetic	class I and IIa	phase I (Brain and Central Nervous System Tumors)	[125]
	Benzamides	Entinostat (MS-275)	Synthetic	class I	phase II (Hodgkin's Lymphoma)	[126]
		Tacedinaline (CI-994)	Synthetic	class I	phase II (Myeloma)	NCT00005624
		Mocetinostat (MG-0103)	Synthetic	class I and IV	phase I (Hodgkin's Lymphoma)	[127]
	Cyclic peptides	Romidepsin (depsipeptide, FK228)	Natural	class I	FDA approval (CTCL)	[128]
	electrophilic ketones	trapoxins(TPX)	Natural	class I	NA	[129]
		a-ketoamides	Synthetic	NA	NA	[130]
		heterocyclic ketones	Synthetic	NA	NA	[131]
	miscellaneous compounds	Diallyl Trisulfide (DATS)	Natural	NA	pre-clinical (glioblastoma)	[132]
	sirtuin inhibitors	cambinol	Synthetic	SIRT1 and 2	pre-clinical	[133]
		EX-527	Synthetic	SIRT1 and 2	pre-clinical	
		sirtinol	Synthetic	SIRT1 and 2	pre-clinical	
		nicotinamide	Synthetic	class III	phase III (laryngeal cancer)	
specific HDAC	Hydroxamate Derivatives	Azelaic Bishydroxamic Acid (ABHA)	Synthetic	HDAC 3	NA	[134]
		CBHA (m-carboxycinnamic acid bis-hydroxamide)	Synthetic	HDAC 3	pre-clinical	[135]
	NA	I-7ab	Synthetic	HDAC 3	NA	[136]
		RGFP966	Synthetic	HDAC 3	pre-clinical (CTCL)	[137]
		PCI34051	Synthetic	HDAC 8	pre-clinical (T-cell lymphomas or leukemias)	[138]
		C149	Synthetic	HDAC 8	pre-clinical (T-cell lymphoma and neuroblastoma)	[139]
	Benzamides	Ricolinosta(ACY-1215)	Synthetic	HDAC 6	phase II (relapsed/refractory lymphoid malignancies)	NCT02091063
		tubacin	Synthetic	HDAC 6	pre-clinical (ALL)	[140]
	Polyketides	Depudecin	Natural	HDAC 1	NA	[71]
	sirtuin inhibitors	SEN196	Synthetic	SIRT1	NA	[141]

Table 4 The classes of HDACis in clinic *(Continued)*

target	chemical classes	compounds	source	Isotype selectivity	study phase	reference
		COMPOUND 6J	Synthetic	SIRT2	NA	[142]
		JGB1741	Synthetic	SIRT1	NA	[143]
bromodomain	BET inhibitors	JQ1	Synthetic	BRD4	pre-clinical (CAA)	[144]
		I-BET	Synthetic	BET	pre-clinica (Breast and lung cancer)	[145]
		BY27	Synthetic	BD2	NA	[146]
hybrid molecules	chimeric	CUDC907	Synthetic	HDAC /PI3K	phase II (Thyroid Cancer)	NCT03002623
		CUDC101	Synthetic	EGFR/Her-2/HDAC 1	Phase I (head and neck, gastric, breast, liver, and non-small cell lung cancer)	NCT01171924

which disrupt the interaction between the bromodomain and acetyl-Lys. Studies show that I-BET is a type of pan BET inhibitor altering gene transcription mediated by BET proteins, and JQ1, as well as BY27, are selective BET inhibitors that competitively bind BRD4 and BD2 respectively and displaces them from chromatin [146, 156, 157]. Now many clinical trials enrolling patients with hematologic and solid tumors are ongoing, with encouraging preliminary findings [158]. The other is the molecule called a hybrid (chimeric) drug that merges two drug pharmacophores to act on different targets, of which CUDC-101 and CUDC-907 are the most representative two [95]. CUDC-101, a potent EGFR/Her-2/HDAC1 inhibitor, was developed by Cai et al. in 2010 and was found to promote tumor inhibition in various cancer xenograft models including nonsmall cell lung cancer (NSCLC), liver, breast, head and neck, colon, and pancreatic cancer [159]. Furthermore, findings strongly support that CUDC-101 has great potential to combat cancer resistance and tumor metastasis [160].

HDACis in GBM

Virtually, almost all patients with Glioblastoma multiforme (GBM) are at the risk of recurrence, which may be ascribed to limited drug penetration of blood-brain barrier (BBB), intratumor heterogeneity, intrinsic GBM resistance, and toxicity of nonspecific agents [161]. Therefore, more targeted and effective combination strategies are urgently required for GBM treatment. Over the years, a more accurate and detailed gene expression-based molecular classification system has been built in GBM. And TCGA research network has reported three signaling pathways frequently modified in GBM, including receptor tyrosine kinase (RTK)/Ras/phosphoinositide 3-kinase (PI3K), p53, and retinoblastoma (Rb) signaling, with the mutation ratio of 88%, 87%, 78% in adults respectively. Besides, there are also variations in other genes, such as the epidermal growth factor receptor (EGFR), phosphatase and tensin homolog (PTEN) [162]. While, according to the above introduction of HDACis function, it has evoked considerable

interest for the treatment of GBM. Herein, we summarize current knowledge on HDAC inhibitors' clinical studies on GBM as monotherapies and combination therapies.

HDACis monotherapy
Mechanisms of HDACis in GBM

There are several HDACis reported to be able to penetrate into BBB and play an anti-GBM role. Generally, up-regulation of HDAC proteins may be related to the occurrence and development of GBM. For instance, it has shown that the expression of HDAC9 in GBM is significantly upregulated. HDAC9 can promotes GBM proliferation and tumor formation by activating the transcription coactivator with PDZ-binding motif (TAZ), an oncogene and an essential downstream effector of the Hippo pathway [163]. So the depletion of HDAC9 can reduce the expression of TAZ, producing anti-GBM effect. Additionally, silencing of HDAC2 via its specific siRNAs can suppress the in vitro proliferation, migration, and invasion of U87 and A172 cells. Can reckon thereof, inhibitng HDAC proteins may be anti-GBM through a variety of mechanisms. According to the degree of enrichment, the mechanisms reported so far about HDACis in GBM are summarized in the following Table 5.

As shown in the table above, it is clear that most HDACis play an anti-GBM role by upregulating the cell cycle inhibitor p21Waf1/Cip1, thus inducing cell cycle arrest. And the second effect is on proapoptotic genes. For example, HDACis such as romidepsin and DWP0016 induce apoptosis through an increase in Bad and Bax proteins in human glioma cells in vitro [168, 175]. And exposure to belinostat in LN-229 cells leads to induction of apoptosis, associated with an increased expression of proapoptotic genes including Puma, Bim, and Chop [170]. Correspondingly, there is a decrease in anti-apoptotic genes. Researches showed that phenylbutyrate and romidepsin resulted in the reduction of anti-apoptotic proteins Bcl-xL and Bcl-2 in LN-229 cells and U251MG cells, respectively [167, 168]. Thirdly, the angiogenesis of GBM is influenced

Chen *et al. Journal of Experimental & Clinical Cancer Research* (2020) 39:138

Page 10 of 18

Table 5 Mechanisms of HDACis in GBM

alterations	affected part	agents
p21Waf1/Cip1, p27↑	cell cycle arrest	SAHA [151], I-BET151 [164], TSA [165], NaB [166], PB [167], FK228 [168], DATS [169], PXD-101 [170], NBM-HD-3 [171], Scriptaid [172], MS-275 [173]
DR5, TNFα, p53, Bad, Bax, Bim, chop, Puma, m-calpian↑	proapoptotic genes	SAHA [151], TSA [166], NaB [166], VPA [174], FK228 [152], DATS [169], PXD-101 [170], DWP0016 [175]
vasculogenic mimicry, VEGF, EGFR↓	angiogenesis	SAHA , MS-275, MC1568, TSA [176], NaB [177], DATS [169], LBH589 [178]
Bcl2, Bcl-XL↓	antiapoptotic genes	VPA [174], PB [167], FK228 [168], DATS [169]
EZH2, MMP-2↓	invasion	SAHA [179], VPA [174], FK228 [168], W2 [180]
p-PTEN/p-AKT, pFAK/p-STAT3↓	pathways	TSA [181], DATS [169], NBM-HD-3 [171], W2 [180]
CDK2, CDK4, CDK6, cyclins D1, cyclins D2↓	progrowth genes	SAHA [151], TSA [165]
caspase 8, caspase 9, caspase 3	apoptotic cascade activation	SAHA [182], DATS [169]
HOTAIR↓	tumor promoting lncRNA	I-BET151 [183]
Ras, c-myc↓	oncogenes	Scriptaid [172], DATS [169]
CD133, Bmi1↓	GSCs markers	SAHA [182]

'↑' or '↓', represent the up-regulated or down-regulated trend of gene expression, respectively.

by HDACis, either by inhibiting growth factors (VEGF, EGFR) production or by blocking vascular mimicry in GBM . And more strikingly, HDACis have been proved to be efficient in targeting glioblastoma stem cells (GSCs) in the preclinical area. SAHA, TSA and valproic acid have been demonstrated to significantly trigger autophagy in GSCs , reduce proliferation rates of GSCs and stimulate differentiation in GSCs [94, 184]. To sum up, it is enough to see that the application of HDACis in GBM is promising. Here we will list some studies about single HDACis drugs investigated in GBM either in the preclinical or clinical phase as follows.

Single HDACis in GBM

Vorinostat Vorinostat is the first HDAC inhibitor entering the trial for patients with glioma (NCT00238303). This phase II trial is studying how well vorinostat works in patients with progressive or recurrent glioblastoma multiforme who undergo surgery or do not. In general, patients received oral vorinostat (SAHA) twice daily for 14 days every three weeks. Notably, patients who undergo surgical treatment would receive oral vorinostat (SAHA) once or twice daily for a total of six doses before surgery. Finally, the trial met the primary objectives, with nine of the first 52 patients being free of progression at 6 months, and the median duration of the stable disease being 11.2 months, as well as well-tolerated toxicities. In summary, this trial shows that vorinostat has modest single-agent activity and can extend life by a few months in a subpopulation of those with recurrent glioblastoma. Nevertheless, additional testing of vorinostat in combination regimens is warranted [185].

Romidepsin Based on promising preclinical data for romidepsin in glioma, North American Brain Tumor Consortium developed a phase I study to determine the maximum tolerated dose (MTD) and the pharmacokinetics of romidepsin in patients with recurrent glioma on enzyme-inducing antiepileptic drugs (EIAEDs), and a phase II study to evaluate the clinical efficacy of this drug by measuring 6-month progression-free survival and objective tumor response in patients (NCT00085540). Although the reasonably well tolerated characteristic of romidepsin in their study, the trial showed that romidepsin had no significant clinical activity as a single agent in unselected patients with recurrent GBM [186]. It is needed to find a better combination strategy for the treatment of GBM.

Currently, many HDAC inhibitors have shown considerable promise in the GBM pre-clinical phase. Still, only a few of these agents have made it into clinical trials and no one has yet to shown significant efficacy in GBM patients. Nevertheless, during studies into these drugs, researchers note that HDAC inhibitors as part of a combination therapy seem more promising in improving prognosis in this difficult to treat malignancy. So, increasing combined clinical trials in GBM about HDACis now is underway. It is worth summarizing these combination strategies.

HDACis-involved combination therapy
There are limitations in the efficacy of single HDACis for GBM despite the tolerated toxicities that attribute to the poor pharmacokinetic properties and multiple misregulated growth and survival pathways in GBM. Thus it is rational to believe that the combinational treatment modality may represent an attractive approach to

enhance the standard of care in patients with GBM. Many HDAC inhibitors are proved to act synergistically with other chemotherapy drugs, have radiosensitizing effects and enhance immunotherapies. Next, we will give an elaborate list of preclinical and clinical combination studies of HDACis for GBM in Tables 6 and 7 respectively.

Preclinical

In vitro, studies have shown significant promise about HDACis synergizing with other drugs for cancer treatment [223], providing a rationale to apply these synergistic ways to GBM. Several agents have been tested in combination therapy in vitro, either as chemosensitizers or radiosensitizer, or in association with other antitumor treatments (see Table 6 for details).

Generally, HDACis can inhibit DNA repair responses thus leading to increased DNA damage, which may partly contribute to enhanced sensitivity of tumour cells to chemotherapy and radiotherapy [224]. In GBM, studies have found that FK228 can augment temozolomide sensitivity in vivo and in vitro partially by blocking PI3K/AKT/mTOR signal pathways, triggering the cell apoptosis

pathway and finally leading to cell death of glioma cell lines [187]. And histone deacetylase inhibitor RGFP109 has also been proved to be able to enhance TMZ-induced cytotoxicity in four TMZ-resistant GBM cell lines by blocking NF-κB-dependent transcription [191].

Besides, treatment with tubastatin A or ACY-1215 or CAY10603, selective HDAC6 inhibitors, were reported to abrogate temozolomide resistance by decreasing and inactivating EGFR protein, thus reducing glioblastoma clonogenicity and migration capacities, accelerating temozolomide-induced apoptosis, and finally reversing the malignant phenotype [192]. Furthermore, silencing of HDAC2 can also increase the sensitivity of GBM cells to temozolomide (TMZ), which might be due to the significant down-regulation of the multidrug resistance-associated protein 1 (MRP1) [190]. All in all, both of these research results show that HDACis can be an attractive agent to overcome chemoresistance, and combining HDACis with chemotherapy may be a promising approach to GBM.

Except for sensitization to chemotherapy, HDACis are also demonstrated to increase sensitivity to radiotherapy, modulate activities of immunity, and bolster antitumor

Table 6 Combined trials of HDACis in GBM in the preclinical phase

Sensitization	HDACis	synergistic members	reference
chemotherapy	FK228	Temozolomide	[187]
	MS275	Temozolomide, etoposide, and cisplatin	[188]
	trichostatin A	lomustine	[189]
	HDAC2 inhibitor	Temozolomide	[190]
	RGFP109	Temozolomide	[191]
	Tubastatin A	Temozolomide	[192, 193]
Radiotherapy	PCI-24781	Radiation	[194]
	Tinostamustine(EDO-S101)	Radiation	[195]
	trichostatin A	Radiation	[196]
immunotherapy	J22352	PD-L1	[197]
demethylase	vorinostat or PCI-24781	LSD1	[198]
	panobinostat	DZ-Nep	[199]
BRD inhibition	panobinostat	JQ1 or OTX015	[200, 201]
RTKi	4-PB	gefitinib or vandetanib	[202]
	MS275, scriptaid, SAHA, TSA	Erlotinib	[203]
topoisomerase inhibitor	SAHA	SN38	[204]
virotherapy	trichostatin A	dl520	[205]
	Scriptaid, LBH589	Delta24-RGD	[206]
others	valproic acid (VPA)	Fluvastatin	[207]
	sodium butyrate (NaB)	quercetin	[208]
	tubastatin A	celecoxib	[209]
	panobinostat	BEZ235	[210]
	vorinostat	tranylcypromine	[211]
	SAHA	olaparib	[212]

Table 7 Current clinical trials of HDACis as a combination therapy in GBM

HDACis	synergistic members	conditions	phase	trial identifier	reference
Vorinostat (SAHA)	Bevacizumab	Recurrent GBM	phase II	NCT01738646	[213]
	Bevacizumab, Temozolomide	Recurrent Malignant Gliomas	phase I/II	NCT00939991	[214]
	Temozolomide	Malignant Gliomas	phase I	NCT00268385	[215]
	Radiation	Recurrent Glioma	phase I	NCT01378481	-
	Isotretinoin, Temozolomide	Recurrent GBM	phase I/II	NCT00555399	-
	Erlotinib, Temozolomide	Recurrent GBM	phase I/II	NCT01110876	-
	Temozolomide, Radiation	Newly Diagnosed GBM	phase I/II	NCT00731731	[216]
	Pembrolizumab, Temozolomide	Newly Diagnosed GBM	phase I	NCT03426891	-
	Bortezomib	Recurrent GBM	phase II	NCT00641706	[217]
	Bevacizumab, Irinotecan	Recurrent GBM	phase I	NCT00762255	[218]
Panobinostat (LBH-589)	Bevacizumab	Recurrent High Grade Glioma	phase I/II	NCT00859222	[219]
	Radiation	Recurrent Glioma	phase I	NCT01324635	-
Valproate (valproic acid, VPA)	Sorafenib Tosylate, Sildenafil Citrate	Recurrent High-Grade Glioma	phase II	NCT01817751	-
	Temozolomide, Radiation	High Grade Gliomas	phase II	NCT00302159	[220–222]
	Nivolumab, Radiation	Recurrent GBM	phase I	NCT02648633	-
Belinostat (PXD101)	Temozolomide, Radiation	GBM	phase II	NCT02137759	-

effects of many other drugs. For example, a study indicated that TSA, a potent HDACis, could radiosensitize human glioblastoma cells [196]. And the treatment of pan-HDAC inhibitors, LBH589 (panobinostat) and suberoylanilide hydroxamic acid (SAHA, vorinostat), were shown to induce chromatin decondensation and prevent DNA DSBs repair, resulting in increased tumor cell death and radiosensitivity [225]. So clinical trials using HDACis in combination with radiotherapy should be considered useful for glioblastoma patients.

Also, there are some findings provide proof-of-principle evidence in support of a therapeutically relevant immunostimulatory activity of HDACis against GBM. For instance, a high-selective HDAC6 inhibitor, J22352, was reported to increased levels of immune-activating cytokines and the proliferation of CD8^{+} T cells by decreasing negative regulation of PD-L1, which made it possible to combine HDACis with immunotherapy to against GBM [197]. Moreover, TSA can lead GBM cells to release high-mobility group box 1 (HMGB1), an endogenous Toll-like receptor 4 (TLR4) ligand that promotes cytotoxic T-cell mediated antitumor immune responses [226].

Despite successful outcomes from these preclinical studies, only a few combination strategies have entered into clinical trials for GBM patients. Table 7 summarizes these completed or ongoing combination of clinical trials.

Clinical

Vorinostat Vorinostat was the first HDAC inhibitor entering clinical trials in GBM, which was a phase I trial in 2005 using vorinostat together with Temozolomide (TMZ) to treat patients with malignant gliomas (NCT00268385). The primary objective of this trial is to evaluate the safety and tolerability of combining an HDAC inhibitor with TMZ in high-grade glioma (HGG). Based on the information obtained from this phase I clinical trial, a phase II trial of vorinostat with radiotherapy and concomitant TMZ later were underway (NCT00731731). This phase I/II trial demonstrated reasonable tolerability in newly diagnosed GBM. However, the primary efficacy endpoint was not met, with the OS rate at 15 months of 55.1% in the entire cohort and median OS of 16.1 months [216]. And another phase I trial of vorinostat in combination with bevacizumab and irinotecan (a topoisomerase I inhibitor) in recurrent GBM found the cumulative toxicity associated with CPT-11 and its unclear efficacy in glioblastoma, thus providing a more promising strategy for future investigation of combining vorinostat with bevacizumab alone in recurrent glioblastoma (NCT00762255) [218]. Then due to the early success of bevacizumab and subsequent approval of bevacizumab by the FDA for treatment of recurrent GBM, a phase II trial tested the efficacy of vorinostat combination with bevacizumab (NCT01738646). Ultimately, this combined treatment was tolerable, but there was no improvement in progression-free survival at 6 months [213].

Panobinostat There is only two combination strategies about panobinostat in GBM approved into the clinical trial. The first is a phase II trial of panobinostat combination with bevacizumab in recurrent GBM to determine

the efficacy of LBH589 by measuring 6-month progression-free survival (PFS6) (NCT00859222). It finally turned out to be well-tolerated, but it did not significantly improve PFS6 compared with bevacizumab monotherapy in the cohort [219]. The other one is a phase I trial for recurrent glioma combining panobinostat with stereotactic radiation treatment, which was terminated because of the poor accrual (NCT01324635).

Valproic acid A phase II trial investigated the effects of combination treatment of VPA, temozolomide and concurrent radiotherapy for GBM patients, which shown promising results (NCT00302159). This study demonstrated improved outcomes compared to historical data and merits with the median OS of 29.6 months (range: 21–63.8 months), median progression-free survival (PFS) of 10.5 months (range: 6.8–51.2 months) and tolerated toxicities in patients with newly diagnosed GBM [222]. However, another phase I study on patients with recurrent GBM using romidepsin was terminated for the reason that pharmaceutical company (BMS) would no longer provide nivolumab for this study (NCT02648633).

Perspectives and conclusion
Even though our progress in understanding the function of HDACs in tumour pathogenesis and the tumour response to HDACi are fruitful, there is still more hope for the exploitation of this knowledge to develop more effective clinical protocols. The clinical trials about HDACis currently achieve little for GBM treatment, and a better application strategy is urgent required. There are areas that we have not covered and might become relevant in the future. For instance, due to the heterogeneity of GBM tumors, the exist of GSCs, and the sophisticated genetic, epigenetic, and transcriptional profiling in GBM, it is difficult to identify patients who are most likely to respond to HDACis and identify specific biomarkers relative to therapeutic effects. Furthermore, the relationship between the toxicity of HDACis and their pharmacodynamic/pharmacokinetic properties is still mostly unknown, which makes it challenging to convert more viable preclinical studies into clinical trials for the further possible regimens of GBM. And so far, few research had addressed the role of HDACis in GSCs sensitivity, which should be on the agenda to clarify the true potential of HDACi in clinical treatment.

In all, only by fulling understanding the underlying molecular mechanisms can we translate these scientific findings into effective clinical practices to anti-cancer strategies. We should pursue new discoveries to advance GBM treatment.

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