Grand rounds at the National Institutes of Health: HDAC inhibitors as radiation modifiers, from bench to clinic

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Abstract

Glioblastoma multiforme (GBM) is the most common and aggressive malignant brain tumour. Patients afflicted with this disease unfortunately have a very poor prognosis, and fewer than 5% of patients survive for 5 years from the time of diagnosis. Therefore, improved therapies to treat this disease are sorely needed. One such class of drugs that have generated great enthusiasm for the treatment of numerous malignancies, including GBM, is histone deacetylase (HDAC) inhibitors. Pre-clinical data have demonstrated the efficacy of various HDAC inhibitors as anticancer agents, with the greatest effects shown when HDAC inhibitors are used in combination with other therapies. As a result of encouraging pre-clinical data, numerous HDAC inhibitors are under investigation in clinical trials, either as monotherapies or in conjunction with other treatments such as chemotherapy, biologic therapy or radiation therapy. In fact, two actively studied HDAC inhibitors, vorinostat and depsipeptide, were recently approved for the treatment of refractory cutaneous T cell lymphoma. In this review, we first present a patient with GBM, and then discuss the pathogenesis, epidemiology and current treatment options of GBM. Finally, we examine the translation of pre-clinical studies that have demonstrated HDAC inhibitors as potent radiosensitizers in *in vitro* and *in vivo* models, to a phase II clinical trial combining the HDAC inhibitor, valproic acid, along with temozolomide and radiation therapy for the treatment of GBM.

Keywords: glioblastoma multiforme ● GBM ● histone deacetylase inhibitor ● HDAC inhibitor ● valproic acid ● VPA ● radiosensitization ● radiosensitizer

Case presentation

A previously healthy 41-year-old women was evaluated by her primary care physician for 6 months of light intolerance and headaches. She was initially diagnosed with migraines and started on Imitrex. However, subsequently she noticed a decrease in the peripheral vision of her right eye. A CT scan was performed and revealed a 2 cm × 2 cm × 3 cm lesion in her left parietal occipital lobe. An MRI confirmed a 3.5 cm × 3.4 cm × 3.4 cm cystic enhancing lesion, typical of a glial neoplasm (Fig. 1A). The patient underwent gross total surgical resection of the mass. The pathology of the resected tumour was reported as a malignant astrocytic neoplasm with anaplastic appearing nuclei, mitotic figures,

microvascular changes and necrosis, consistent with the diagnosis of GBM [World Health Organization (WHO) Grade IV]. Figure 1B is a characteristic histological image of GBM, similar to what was reported for our patient.

Following an uneventful surgical recovery, she presented to the Radiation Oncology Clinic at the National Cancer Institute (NCI) of the National Institutes of Health for consultation regarding postoperative radiation therapy and the possibility of enrolling in a clinical trial. At that time, she continued to complain of impaired right peripheral vision and a mild gait disturbance.

The patient had no significant past medical or surgical history, with no known radiation exposure. Family history was significant for a paternal grandfather with lung cancer and a maternal grandmother with colon cancer, with no family history of intracranial neoplasms. She has never smoked cigarettes. On physical exam, she was well appearing, with a neurological exam significant for a wide-based gait and a right peripheral visual field deficit.

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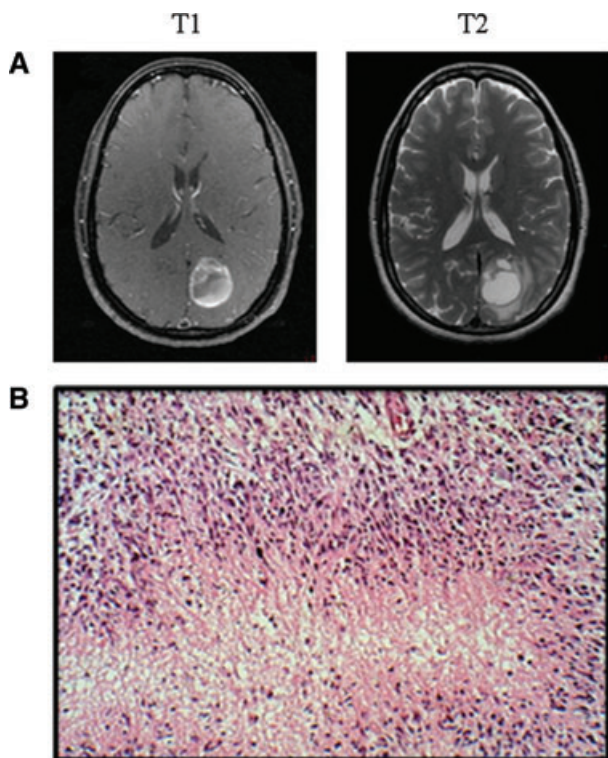E-mail: camphauk@mail.nih.gov

Fig. 1 (**A**) T1 and T2 representative MRI images of the patient at diagnosis. These images display a ring-enhancing lesion in the left parietal occipital lobe, characteristic of GBM. (**B**) Representative pathological image of GBM displaying pseudopalisading formation of malignant cells surrounding areas of necrosis (Frontalcortex.com).

Following a comprehensive discussion regarding the risks and benefits of either enrolling in a Phase II clinical trial (NCT00302159) combining the HDAC inhibitor, valproic acid (VPA), with low-dose temozolomide and radiation, *versus* the standard of care regimen of low-dose temozolomide and radiation, the patient opted to enroll in the clinical trial.

Epidemiology of GBM

The incidence of primary central nervous system malignant tumours is approximately seven cases per 100,000 person years. GBM specifically has an incidence of about three cases per 100,000 person years, and it remains the most common primary malignant brain tumour, comprising 53.8% of such tumours. Disease incidence rises with increasing age, and the majority of cases occur in individuals between 65 and 80 years of age. Unfortunately, survival rates remain poor with approximately only 34% of patients surviving at one year, 12% at 2 years, and less than 5% at 5 years following initial diagnosis

[1]. Older age and incomplete surgical resection typically portend a worse prognosis [2].

Prior radiation therapy is a significant environmental risk factor associated with the development of GBM. Neglia *et al.* examined a cohort of 14,000 survivors from a broad range of paediatric cancers and found that radiation therapy was significantly associated with an increased risk for gliomas (OR = 6.78, 95% CI = 1.54–29.7). The risk increased linearly with increasing doses of radiation. Furthermore, radiation exposure to children under the age of 5 years carried the highest risk of subsequent glioma development, suggesting that the developing brain is more susceptible to radiation-induced carcinogenesis [3]. Interestingly, low-dose ionizing radiation is also a risk factor for the development of malignant glioma, as children treated with 1.5 Gray (Gy) for tinea capitis had an increased incidence of gliomas [4].

Pathogenesis of GBM

GBM tumours are classified by WHO grading system as grade IV astrocytic tumours. All grades of gliomas tend to occur in the white matter of the cerebral hemispheres [5]. GBM is the highest grade glioma and is typically recognized on histology by large areas of necrosis and microvascular proliferation. In addition, GBM tumours characteristically have the appearance of a pseudopalisading formation of malignant cells surrounding areas of necrosis and haemorrhage [5]. Figure 1B demonstrates the typical pathological features of GBM.

Although the histologies of GBM tumours have a common set of features, it is becoming increasingly clear that GBM tumours can be more accurately characterized and distinguished by their genomic and transcriptomic profiles. As such, the NCI, in collaboration with the National Human Genome Research Institute (NHGRI), recently launched a comprehensive research network, The Cancer Genome Atlas (TCGA). TCGA was established to utilize genomic analysis technologies and large-scale sequencing, to better understand the genomic abnormalities that drive tumourigenesis, to help explain resistance to treatment and to ultimately suggest targets of susceptible pathways [6]. GBM was one of the first neoplasms studied under this program, due in large part to its extremely poor prognosis [7]. One of the initial studies using the data generated from TCGA differentiated GBM into four main subgroups, identified as neural, proneural, classical and mesenchymal. These different GBM subtypes were linked with normal neural cell types to provide insight into the possible cell of origin for each of these tumours. More importantly, these different subtypes were correlated with clinical response, which in the future could help guide therapies and inform patients about their appropriate prognoses [8]. TCGA is just one of many efforts attempting to better characterize different subgroups of GBM based on molecular features, to personalize the most effective therapeutic strategies for each individual patient's tumour.

Standard treatment of GBM

The current front line standard of care therapy for GBM includes maximal surgical resection followed by radiotherapy with concurrent temozolomide, followed by adjuvant temozolomide. Unfortunately, surgical resection is often time compromised by the diffusely infiltrative nature of gliomas and the fact that the tumour often invades critical neurological structures [9].

Following surgical resection, radiation therapy has been shown to increase median survival from 14 to 36 weeks [10]. The initial benefits of radiation were established using whole brain radiation therapy, but improvements in technology, such as the use of involved field radiation therapy, have drastically reduced the side effects associated with radiation. For maximal survival benefit, a total dose of 60 Gy of radiation should be delivered to the tumour. Significantly lower doses of radiation are associated with a reduction in survival benefit [11], whereas doses greater than 60 Gy do not offer additional survival benefit [12].

The addition of temozolomide, an alkylating agent, to post-operative radiation is the only chemotherapeutic agent that provides significant further improvements in the survival of patients with GBM. Stupp *et al.* performed the landmark Phase III trial comparing post-operative concurrent temozolomide and radiation to radiation alone. The trial found significant improvement in both median survival (14.6 *versus* 12.1 months) and 2-year overall survival (26.5% *versus* 10.4%), demonstrating the efficacy of temozolomide [13]. Follow-up of this study revealed that the benefit of temozolomide lasted for at least 5 years, with overall survival rates of 10% in the combination group compared to 2% in the group treated initially with only post-operative radiation [14].

Subsequent analysis of tumour tissue samples from patients in this Phase III trial revealed that silencing of the *MGMT* (O^6-methylguanine–DNA methyltransferase) DNA-repair gene, by promoter methylation, was associated with a greater benefit from the temozolomide therapy. Silenced *MGMT* expression in tumour cells impairs their ability to repair DNA damage caused by temozolomide, therefore leading to decreased tumour cell survival. Although less significant, patients with an unmethylated MGMT promoter also had modest benefit from the addition of temozolomide, thus, the combination of temozolomide with radiation remains the standard of care therapy for all patients with GBM [15].

Given that GBM tumours are particularly vascular, and overexpress numerous angiogenic factors, there has been much investigation into the utilization of anti-angiogenic therapies as potential treatment options. Recently, the antibody against vascular endothelial growth factor, bevacizumab (Avastin), was granted accelerated approval by the Food and Drug Administration (FDA) for its use as a single agent in recurrent gliomas [16]. This approval was a result of a successful Phase II trial examining the efficacy of both bevacizumab alone and the combination of bevacizumab with irinotecan (topoisomerase 1 inhibitor). When compared to historical controls, both arms had a greater radiological response, improved progression-free survival and improved median survival. However, because the trial was not designed as a comparative study, it is unclear whether bevacizumab with concurrent irinotecan is superior to bevacizumab monotherapy [17].

Bevacizumab, in combination with other therapies, is also under investigation as front line therapy for GBM. A Phase II trial combining bevacizumab with standard of care radiation and temozolomide has thus far demonstrated encouraging clinical responses [18]. Moreover, Phase III randomized clinical trials are currently underway to assess the efficacy of bevacizumab with current standard of care front line therapy for GBM (NCT00884741 and NCT00943826) [19].

Thus far, only small incremental gains have been made in the therapy of GBM, and the current standard of care therapies are limited in their ability to combat this aggressive and fatal tumour. However, there are a number of pre-clinical and clinical studies examining the utility of novel targeted therapies for the treatment of GBM [20]. HDAC inhibitors comprise one promising class of drugs under investigation for various malignancies, including GBM.

HDAC inhibitors as cancer therapy

HDAC inhibitors belong to a class of agents that target the aberrant epigenetic characteristics of tumour cells. Epigenetic changes refer to alterations that affect gene expression and cellular phenotype without modifying the DNA sequence itself. Histone modification is one such mechanism of alteration, and it plays an important role in tumour formation, progression and resistance to treatment [21]. 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 remodelling [22].

More specifically, histone acetylation is regulated through the opposing actions of histone acetyltransferases (HATs) and histone deacetylases (HDACs). HATs transfer acetyl moieties to lysine residues and HDACs remove the acetyl moieties. HAT activity relaxes chromatin, permitting various transcription factors to interact with DNA, thereby promoting transcription. In contrast, HDAC activity condenses chromatin, preventing access of transcription factors, which leads to transcriptional repression [23]. HAT inactivity and HDAC over activity have been associated with tumourigenesis [24]. Unlike primary genetic mutations, aberrations in an epigenome are reversible and therefore an intriguing potential target for therapy. Because it is pharmacologically much simpler to inhibit an enzyme rather than to induce one, HDAC inhibition has gained enormous clinical interest as an anticancer strategy.

However, it is not only histone modification that enables HAT and HDAC disturbances to lead to tumour development. HATs and HDACs are also able to alter the acetylation status of numerous non-histone targets, including other proteins involved in gene

expression, proliferation, migration, cell death, DNA repair, angiogenesis, inflammation and the immune response [25]. These non-histone targets also likely contribute to tumour progression and resistance to treatments. Correspondingly, HDAC inhibitors have been shown, in a pre-clinical setting, 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, inhibition of angiogenesis [25] and improvement in NK cell mediated tumour immunity [26]. Because of the overlap of these diverse effects on cancer cells and the effects seen after irradiation (discussed later), HDAC inhibitors are attractive not only as monotherapeutic agents, but also in combination with radiation therapy.

HDAC inhibitors as radiosensitizers

The full therapeutic potential of HDAC inhibitors is likely to be achieved when they are combined with other cancer therapies such as radiation. Particularly, there is accumulating evidence from pre-clinical studies, which shows that HDAC inhibitors from diverse chemical classes are effective radiosensitizers in a variety of malignancies [27]. Pre-clinical examples of malignancies for which HDAC inhibitors have exhibited a radiosensitizing effect include GBM [28–30], head and neck squamous cell cancer [31], non–small-cell lung cancer [32], colorectal cancer [33, 34], prostate cancer [29, 35], melanoma [36] and metastatic breast cancer [37].

The exact mechanism by which HDAC inhibitor induced radiosensitization occurs is currently unknown, but it may be due at least in part to the prevention of the DNA damage repair process. HDAC inhibitors prevent DNA double-strand break (DSB) repair, as demonstrated by prolonged expression of phosphorylated H2AX (γH2AX), a marker for DNA DSBs, following radiation [38]. A variety of HDAC inhibitors have been shown to delay the dispersal of radiation-induced γH2AX foci [28–29, 32, 36, 39–40]. Thus, HDAC inhibitors appear to prevent DNA DSB repair leading to enhanced tumour cell death.

The mechanism for this impaired DNA DSB repair process is not entirely clear. Possible explanations include the down-regulation of certain DNA repair molecules, including Ku70, Ku86, Rad50, Ku80 and Rad51 [39, 41]. In addition, the interaction of HDACs with DNA damage response proteins, such as 53BP1 [42], may play an important role in HDAC inhibitor radiosensitization. Furthermore, HDAC inhibitors may affect a tumour cell's response to radiation by altering chromatin condensation patterns. Chromatin exists in a condensed form, known as heterochromatin in which there is decreased gene expression, and a more open form, known as euchromatin in which there is increased gene expression [22]. A recent study reported that tumour cells grown in a three-dimensional matrix exhibit an elevated amount of heterochromatin, similar to tumour xenografts, and they are more

resistant to ionizing radiation when compared to cells grown in two-dimensional monolayer cultures. Treatment with the HDAC inhibitor, LBH589, induced chromatin decondensation in cells grown in a three-dimensional culture, which correlated with an increase in DNA DSBs and radiosensitivity. Therefore, the relaxation of chromatin induced by HDAC inhibition may increase radiation-induced DNA DSBs, and thereby increase killing of tumour cells [43].

A better understanding of the mechanism of HDAC inhibitor induced radiosensitization will help guide the effective and safe use of HDAC inhibitors in combination with radiation. At present, it appears that the pre-clinical protocol for maximal radiosensitization induced by a chemically diverse set of HDAC inhibitors requires drug exposure both pre- and post-irradiation [28, 35, 44]; cells exposed to drug only pre-irradiation typically display a very modest radiosensitization [35]. Interestingly, the HDAC inhibitors MS-275 and VPA have also been studied when administered only post-irradiation, with differing results. MS-275 displays only minimal radiosensitization when given exclusively post-irradiation [35]. However, VPA effectively radiosensitizes cells when administered up to 24 hrs following radiation treatment, although the degree of enhancement is not as robust compared to cells treated both pre- and post-irradiation [45]. Taken together, these studies suggest the importance of maintaining sufficiently high HDAC inhibitor levels both before the tumour cells are irradiated and after irradiation, as the cells attempt to repair the radiation-induced DNA damage. A further understanding of this mechanism of radiosensitization should aid in determining the optimal timing for drug administration relative to the radiation treatment, to maximize the therapeutic effect of this combined modality.

HDAC inhibitors and normal tissue radioresponse

To maximize the therapeutic ratio of a radiosensitizing agent, it is important that the drug not enhance the radiation-induced toxicity of normal cells or tissue. It is hypothesized that the aberrant HDAC activity exhibited in tumour cells makes them more susceptible to HDAC inhibitors, and thus, normal tissue toxicity should be minimized. However, there is conflicting pre-clinical data regarding the specificity of HDAC inhibitors for cancer cells compared to normal cells, which should be further clarified given the important clinical safety implications.

Several *in vitro* studies have reported that exposure to HDAC inhibitors does not increase the radiosensitivity of various normal tissue cell lines, including fibroblasts and osteoblasts [36, 41, 45]. In addition, when examined in normal breast and intestinal epithelial cell lines, HDAC inhibitors did not enhance the cellular toxicity induced by a variety of chemotherapeutic DNA damaging agents [46]. Moreover, *in vitro* data suggest that HDAC inhibitors may actually protect normal tissue from radiation-induced toxicities. For example, the HDAC inhibitor phenylbutyrate has been shown

to improve both DNA repair and cell survival in normal fibroblast cell lines [47].

In contrast, it has also been reported that some HDAC inhibitors do inhibit the repair of radiation-induced DNA DSBs and increase cellular radiosensitivity of normal cells, specifically lymphocytes [48] and primary skin fibroblasts *in vitro* [49]. In the study examining lymphocytes, the HDAC inhibitor sodium butyrate inhibited radiation-induced DNA DSBs. However, there was no mention of cell survival, which makes it difficult to assess for a change in radiation sensitivity as measured by cell death [48]. In the study using fibroblasts, the radiosensitizing effect was demonstrated to vary as a function of the type of HDAC inhibitor used. Of note, fibroblasts treated with VPA did not have an increase in DNA DSBs compared to radiation alone and displayed only a very modest increase in radiation sensitivity [49]. These results indicate that HDAC inhibitors are a complex class of molecules with varied responses to similar assays requiring in-depth study of each drug for its effects on tumour and normal cells.

In addition, there is growing *in vivo* evidence that HDAC inhibitors can actually protect normal tissue from radiation-induced side effects. When applied topically to mice, HDAC inhibitors such as phenylbutyrate, trichostatin A and VPA provide protection against numerous well-defined toxicities of radiation therapy, including cutaneous radiation syndrome, long-term skin fibrosis and radiation-induced malignancies [50]. These observations are correlated at a molecular level with inhibition of pro-inflammatory cytokines such as tumour necrosis factor (TNF)-α and transforming growth factor (TGF)-β, which may be one of the mechanisms by which HDAC inhibitors protect normal tissue [50]. Similarly, an additional study in mice demonstrates that topical phenylbutyrate effectively reduces the severity of radiation-induced oral mucositis, a common and morbid side effect of radiotherapy treatment for head and neck cancers. These findings are similarly associated with a decrease in the expression of TNF-α [47]. Therapies with the potential to mitigate the incidence and severity of radiation-induced normal tissue toxicity would have tremendous clinical benefit, as often times it is the normal tissue toxicity that limits the delivery of the radiation dose necessary for tumour control.

Clinical safety of HDAC inhibitors

With HDAC inhibitors now entering the clinical arena as radiosensitizing agents, there is growing clinical evidence to support the safety of these compounds when delivered in combination with radiotherapy. Initial data from our trial involving VPA, temozolomide and radiation for the treatment of GBM have not shown significantly increased toxicity compared to what is reported in the standard of care treatment regimen of radiation and temozolomide alone. The most significant side effects observed have been drug-related neurological toxicities, which are reversible within 72 hrs after the cessation of VPA [51].

In addition, a retrospective study of paediatric patients with high-grade gliomas who were treated with VPA as an anti-seizure medication, along with standard radiochemotherapy, did not experience an increase in toxicity. Although this was a retrospective study, it offers encouraging results, which suggest that VPA can be safely combined with radiation and chemotherapy [52].

Furthermore, Ree *et al.* recently published the first Phase I trial combining an HDAC inhibitor with radiation. Specifically, they assessed the use of vorinostat combined with pelvic palliative radiation for the treatment of various gastrointestinal carcinomas. Although the study was not designed to determine the additive toxicity of vorinostat to the radiation regimen, the authors determined a safe maximum tolerated dose (MTD) of vorinostat in conjunction with pelvic radiation. The results from this study now pave the way to use the MTD of vorinostat in conjunction with more long-term curative radiation treatment of certain pelvic malignancies [53]. Our understanding of the tolerability and toxicity profiles of HDAC inhibitors combined with radiation will continue to improve as more HDAC inhibitors are introduced into the clinical setting as potential radiosensitizing agents.

Biomarkers and HDAC inhibitors

When investigating a pharmaceutical agent, it is useful to identify a safe and accurate method to measure drug delivery and activity. Because histone acetylation is a direct downstream consequence of HDAC inhibition, one of the most common methods of measuring the activity of HDAC inhibitors is to test for the hyperacetylation of histone H3 and histone H4. Although the most direct method of testing for tumour histone hyperacetylation is *via* tumour biopsy, such a procedure is too invasive to consistently use for determining drug activity in the clinical setting. Therefore, there is interest in utilizing peripheral lymphocytes as surrogate biomarkers for global acetylation after HDAC inhibitor treatment. Although testing peripheral lymphocyte histone hyperacetylation is a potential strategy for optimizing protocols that involve HDAC inhibition and radiation therapies [29], this method does not account for inherent tumour resistance mechanisms, such as drug efflux pumps or the inability of the drug to penetrate bulky tumour masses.

If histone acetylation status proves to be an effective method for measuring drug delivery, it will be additionally important to find susceptibility biomarkers to identify patients who will most likely benefit from HDAC inhibitor therapy. As such, predictive biomarkers are vitally important when testing drugs and tracking patients in clinical trials. For example, if only a small subset of patients benefit from therapy, the success of these responders will likely be lost in the statistics of the larger trial if the subset cannot be adequately identified and analysed.

Interestingly, HR23B, a protein that transports ubiquitinated cargo to proteosomes, was identified as a marker for the sensitivity of HDAC inhibitor induced apoptosis. The proposed mechanism involves disruption of HDACs' normal inhibition of HR23B, and

Table 1 Current clinical trials involving radiation and HDAC inhibitors

HDAC inhibitor	Chemotherapeutic or biological agent(s)	Type of malignancy	Phase	Trial identifier
Panobinostat	None	Prostate, oesophageal and head and neck cancer	I	NCT00670553
Vorinostat	None	Brain metastases	I	NCT00838929
Vorinostat	None	Brain metastases from non-small cell lung cancer	I	NCT00946673
Vorinostat	None	Non-small cell lung cancer	I	NCT00821951
Vorinostat	None	Pelvic cancer	I	NCT00455351
Vorinostat*	None	Resistant/relapsed neuroblastoma	I	NCT01019850
Vorinostat	Capecitabine	Non-metastatic pancreatic cancer	I	NCT00983268
Vorinostat	Cisplatin	Squamous cell carcinoma of the oropharynx	I	NCT01064921
Vorinostat	None	Locally advanced pancreatic cancer	I/II	NCT00831493
Vorinostat	Temozolomide	Glioblastoma multiforme	I/II	NCT00731731
Vorinostat	Paclitaxel	Inoperable stage III non-small cell lung cancer	I/II	NCT00662311
Vorinostat	5-FU	Pancreatic cancer	I/II	NCT00948688
Valproic acid	Hydralazine, Cisplatin	Cervical cancer	II	NCT00404326
Valproic acid	Temozolomide	Glioblastoma multiforme	II	NCT00302159
Valproic acid	Bevacizumab	Paediatric high-grade gliomas	II	NCT00879437

*Radiation form is the radioactive drug, iobenguane I 131.

thus stabilization of proteosome activity. Without inhibition of HR23B, the proteosome becomes overloaded, leading to ER stress and apoptosis [54–55]. In patients with cutaneus T cell lymphoma (CTCL), a disease that typically responds to HDAC inhibitors, there is an association of HR23B expression with the clinical response to vorinostat. Specifically, up-regulated HR23B levels were shown to have a positive predictive value of 71.7% when measuring patient response to vorinostat [55]. Therefore, measuring a tumour's HR23B status could provide an important predictive value for determining which patients with CTCL are likely to respond to HDAC inhibitor based therapy. This study also opens the door for assessing HR23B expression in other neoplasms and measuring how the levels correlate with tumour response to HDAC inhibitors.

Unlike HR23B in CTCL, to date there are no known biomarkers that characterize patients who are more likely to respond to HDAC inhibitors in combination with radiation. However, as we better understand how HDAC inhibitors enhance tumour cell radiosensitivity, we can utilize this knowledge to search for molecular biomarkers to predict this response.

Clinical protocols and HDAC inhibitors

Thus far, vorinostat and depsipeptide are the only HDAC inhibitors that have achieved FDA approval for cancer therapy, specifically for the treatment of refractory CTCL [56–58].

However, there are many ongoing clinical trials assessing the efficacy and safety of other HDAC inhibitors, used alone or in combination therapies, in both solid and haematologic malignancies. Recently, a number of clinical trials have begun to specifically examine the combination of HDAC inhibitors with radiation for various malignancies (Table 1) [19].

Reports from Phase II data on HDAC inhibitors as monotherapies for various solid malignancies demonstrate that they are well tolerated with good toxicity profiles compared to current standard cancer therapies. In general, the side effects of HDAC inhibitors are reversible with drug cessation and primarily include fatigue, nausea, dehydration, diarrhoea, prolonged QT, thrombocytopenia, lymphopenia and neutropenia [59].

Despite the favourable toxicity profile, HDAC inhibitors used as monotherapies in solid cancers do not tend to significantly improve outcomes compared to current standard therapies [60–64]. However, a Phase II study of vorinostat for refractory GBM offers some promise. Although displaying only modest efficacy, this study met its primary endpoint with 9/52 patients progression-free at 6 months. Encouragingly, the patients who met this primary endpoint had a long duration of stable disease, indicating that there may be a subset of patients who benefit from HDAC inhibitor therapy [63].

More encouraging examples of the potential for HDAC inhibitors in cancer therapy are evident from trials combining HDAC inhibitors with chemotherapy. A Phase II randomized, double-blind clinical trial showed that vorinostat enhances the efficacy of carboplatin and paclitaxel for advanced stage
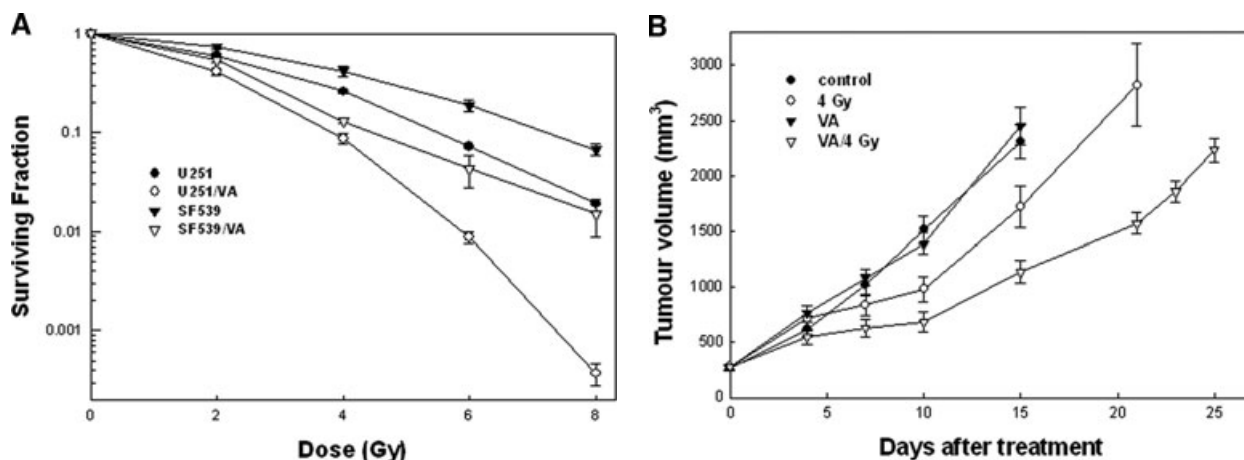
Fig. 2 Excerpt graphs from Camphausen *et al.* showing *in vitro* and *in vivo* radiosensitization of glioma cells by valproic acid treatment (28). (**A**) U251 and SF539 glioma cells were treated with valproic acid both before and following radiation. Clonogenic survival curves reveal an increase in radiosensitivity in valproic acid treated cells. (**B**) The effects of valproic acid and radiation on tumour growth delay. U251 glioma cells were implanted into the hind leg of mice and divided into four treatment groups: (1) control, (2) 4 Gy radiation, (3) valproic acid, (4) valproic acid and 4 Gy radiation. The combination treatment regimen showed the most significant tumour growth delay.

non–small-cell lung cancer (NSCLC). Specifically, upon comparison of chemotherapy plus vorinostat to chemotherapy alone, there was an increase in confirmed response rates (34% *versus* 12.5%, $P = 0.02$). There was also an increase in overall and progression-free survival, but these endpoints did not reach statistical significance. However, these gains were associated with increased toxicity in the vorinostat group, particularly with a significant increase in grade 4 platelet toxicity [65].

In addition, another Phase II clinical trial examined the utility of VPA, hydralazine (a demethylating agent), and different chemotherapies for various solid malignancies refractory to chemotherapy [66]. In this study, the investigators noted an 80% clinical response, defined as either a partial response or stable disease. Again, the toxicity profile was not negligible, consisting of a significant amount of grade 4 haematologic toxicity. Because this study enrolled patients with multiple types of neoplasms (ovarian, cervical, breast, testicular and lung), the results specific to each type of neoplasm are difficult to analyse. Yet, the apparent efficacy in multiple malignancies provides some evidence that combining chemotherapy and HDAC inhibitors may have utility in a large spectrum of cancers [66].

Ongoing NIH/NCI HDAC inhibitor (VPA) trial

VPA is an ideal agent to study as a GBM radiosensitizer for multiple reasons. First, it shows impressive pre-clinical efficacy as a radiosensitizer in glioma cell lines *in vitro* and *in vivo,* at a dose comparable to one that can be achieved in the clinic (Fig. 2) [28]. Furthermore, VPA is FDA approved, and it is safely and commonly

used for the treatment of epilepsy, bipolar disorder and migraine prophylaxis [67]. Finally, VPA effectively crosses the blood–brain barrier, as demonstrated by its anti-seizure, anti-migraine and mood modulatory activity.

Therefore, starting in 2006, we opened a multi-institutional Phase II clinical trial of VPA in combination with temozolomide and radiation therapy for patients with high-grade gliomas. The primary endpoints of this trial are both progression-free survival and overall survival. The secondary endpoint is regimen toxicity. As a surrogate marker for drug activity, peripheral lymphocytes are assessed for histone acetylation. The treatment regimen includes (1) fractionated radiotherapy for 6.5 weeks to a total dose of 60 Gy, (2) oral VPA (25mg/kg/day) twice daily on days 7–49 and (3) oral temozolomide daily throughout the course of radiation. Four weeks following the completion of the radiation regimen, patients receive adjuvant temozolomide. Patients are followed with MRI scans, including 1 month after the completion of the radiation treatment, every 3 months for the first 2 years, and then every 6 months. The total accrual goal for this study is 41 patients and 21 patients have been accrual as of January 2011.

Patient follow-up

The patient presented earlier completed the experimental protocol of 6 weeks of concurrent VPA, temozolomide and radiation to a total dose of 60 Gy, with minimal toxicity. She continued with a total of 20 cycles of adjuvant temozolomide. At the time that this patient was presented at the Grand Rounds lecture, it had been approximately 18 months since her last dose of temozolomide and
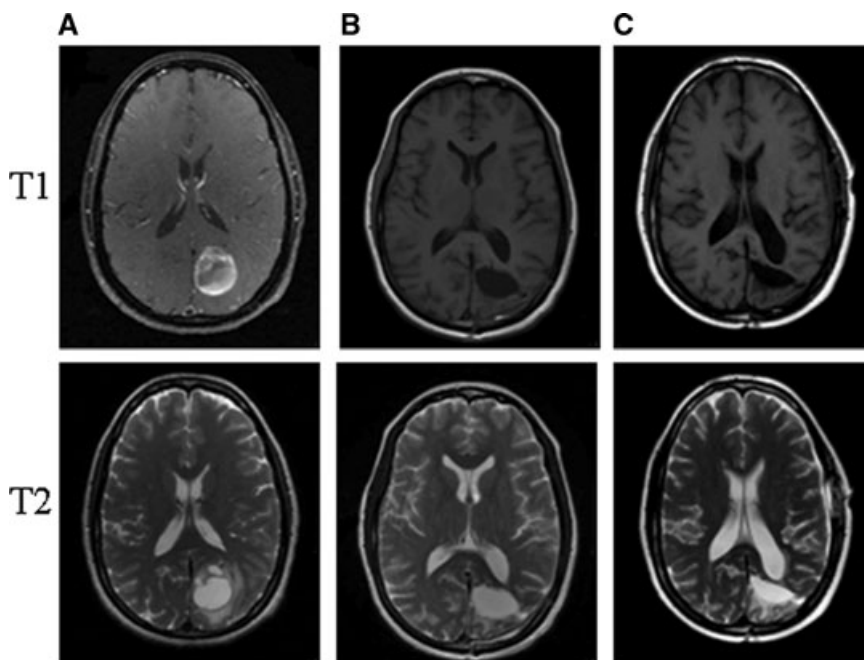
Fig. 3 A series of T1 and T2 weighted Brain MRIs from the presented patient with GBM. (**A**) Represents images from the initial diagnosis. (**B**) Represents images shortly after gross surgical resection, showing typical post-surgical changes. (**C**) Represents the most recent MRI images with no evidence of disease recurrence 3 years after the initial diagnosis of GBM.

3.5 years since her initial diagnosis of GBM. The patient remains clinically and radiologically stable with no evidence of disease progression. Figure 3 shows the patient's most recent MRI image compared to her initial scans at diagnosis and the scans from post-surgical resection. She continues to be followed with regular follow-up visits and MRI scans. Aside from the presenting visual field defect, she remains symptom-free.

Conclusion

Given the number of active clinical trials combining HDAC inhibitors and ionizing radiation, in addition to the trials involving HDAC inhibitors and chemotherapy or biologic agents, the next few years will be revealing regarding the efficacy and safety of HDAC inhibitors as anticancer agents.

Conflict of interest

The authors confirm that there are no conflicts of interest.

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