Histone deacetylase inhibitors as radiosensitisers: effects on DNA damage signalling and repair

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Many cancers display increased expression of histone deacetylases (HDACs) and therefore transcriptionally inactive chromatin, resulting in the downregulation of genes including tumour suppressor and DNA repair genes. Histone deacetylase inhibitors (HDACi) are a heterogeneous group of epigenetic therapeutics, showing promising anticancer effects in both pre-clinical and clinical settings, in particular the effect of radiosensitisation when administered in combination with radiotherapy. Radiotherapy remains one of the most common forms of cancer treatment, leading to cell death through the induction of DNA double-strand breaks (DSBs). Cells have developed mechanisms to repair such DSB through two major pathways: non-homologous end-joining and homologous recombination. Here, we explore the current evidence for the use of HDACi in combination with irradiation, focusing on the effects of HDACi on DNA damage signalling and repair in vitro. In addition, we summarise the clinical evidence for using HDACi with radiotherapy, a growing area of interest with great potential clinical utility.

Epigenetics is an exciting new field of research into heritable changes of gene expression, acting through histone modifications and deoxyribonucleic acid (DNA) methylation, which occur without deoxyribonucleic acid (DNA) sequence alteration. Epigenetic changes are involved in both cancer development and tumour progression. Histone deacetylase inhibitors (HDACi) are epigenetic drugs that can alter histone modifications, and could potentially be used as novel anticancer therapy, either as a single-agent or in combination with other therapy modalities (Wagner et al, 2010); they already show promising anticancer effects in both pre-clinical and clinical settings. In the first part of this minireview, we shall explore the current evidence for the use of HDACi in combination with irradiation, focusing on the effects of HDACi on DNA damage signalling and repair in vitro. In the second part, we summarise the clinical evidence for using HDACi with radiotherapy, a growing area of interest with great potential clinical utility.

HDACs AND HDACi

The basic chromatin unit is the nucleosome, comprising DNA wrapped around an octamer of histones H2A, H2B, H3 and H4. One of the most studied post-translational modifications of nucleosomal histone lysine residues is acetylation, regulated by the opposing action of HDACs and histone acetyltransferases (HATs). Histone deacetylases remove the acetyl moiety from the positively charged histone lysine residues and subsequently the negatively charged DNA can bind to the nucleosome proteins. Histone acetyltransferases mediate the acetylation of lysine residues, the charge is masked and detached DNA is free to interact with transcription factors and enzymes. In cellular oncogenesis, aberrant expression of HDACs is observed (either down- or upregulation) and the activity of HATs is downregulated, which results in repression of antiproliferative genes (Ropero and Esteller, 2007; Sharma et al, 2010). There are 18 different HDACs,
belonging to four classes according to their similarity to yeast analogue proteins. Classes I, II and IV are Zn$^{+2}$-dependent, whereas class III are structurally related to yeast NAD$^{-}$-dependent SIRT proteins and are not inhibited by HDAC pan-inhibitors. Histone deacetylases 1, 2, 3 and 8 belong to class I, HDACs 4, 5, 7, 9 to class IIa, 6 and 10 to IIb and HDAC 11 to class IV (Wagner et al, 2010; Spiegel et al, 2012).

Histone deacetylase inhibitors (HDACi) can also be classified according to their structure and their HDAC class inhibitory specificity: hydroxamic acids, such as suberoylanilide hydroxamic acid (SAHA or vorinostat), panobinostat (LBH589), are pan-HDACi, that is, active against class I and class II HDACs; short-chain fatty acids, including sodium butyrate (NaB) and valproic acid (VPA), inhibit class I and class IIa HDACs; cyclic peptides, such as romidepsin, mainly act against class I HDACs, but can also inhibit class II HDACs at higher concentrations; and benzamidines, including entinostat and mocetinostat, are active against class I HDACs (Wagner et al, 2010; Spiegel et al, 2012).

Histone deacetylase inhibitors have been shown to cause cancer cell death both in vitro and in a variety of pre-clinical models, and there is growing evidence for their anticancer effect in clinical studies, particularly in combination with other chemotherapeutics (Nolan et al, 2008; Wagner et al, 2010; Sharma et al, 2012). Ionising radiation (IR) also kills cancer cells; DNA double-strand breaks (DSBs) are the lethal lesions. These are detected by the DNA damage signalling machinery and the majority are repaired by either non-homologous end-joining (NHEJ) or homologous recombination (HR). The modulation of DNA damage signalling and repair by HDACi may be one underlying mechanism for their radiosensitising effects in cancer cell lines (Nolan et al, 2008; Shabason et al, 2011; Spiegel et al, 2012).

**EFFECTS OF HDACI ON DNA DAMAGE SIGNALLING AND DSB REPAIR**

Cells can repair IR-induced DSBs through a mechanism, which is initiated by a complex of three proteins, MRE11/Rad50/NBS1 (MRN). The MRN complex can both phosphorylate kinase ataxia-telangiectasia-mutated (ATM), and recruit it to the sites of DSB. The ATM has a crucial role in the initiation of cell cycle arrest following DSB induction, through cell cycle checkpoints (G1, S and G2/M), leading to efficient repair of DSBs or cell death (Williams et al, 2010; Thompson, 2012). At sites of DSBs, ATM or DNA-dependent protein phosphokinase (DNA-PK) can phosphorylate the histone H2AX (to form γH2AX). This and the combined activity of MRN and DNA-PK attract other proteins involved in DSB repair, including BRCA1, BRCA2, CTP and 53BP1 (Goodarzi et al, 2010). The HDACi vorinostat has been found to downregulate RAD50 and MRE11 protein levels in prostate cancer and lung adenocarcinoma cells; furthermore, 72-h incubation causes decreased levels of 53BP1 in prostate cancer cells (Lee et al, 2010). Prostate cancer cells treated with a novel adamantyl-hydroxamate HDACi, H6CAHA, show impaired DNA damage signalling with downregulation of ATM gene expression and impaired phospho-ATM foci formation (summarised in Table 1 and Figure 1; Konsoula et al, 2011).

Once detected, attempts are made to repair DSBs through two major mechanisms, NHEJ and HR. NHEJ is used throughout the cell cycle, but predominantly in G1/early S-phase, and it is often error-prone, as the break is not repaired utilising homologous DNA template and therefore small deletions or insertions can be introduced, particularly when DSB ends are modified as is often seen following IR. The Ku heterodimer (Ku70/Ku80) is recruited to the DSB, followed by the DNA-dependent protein kinase catalytic subunit, and the ends are modified by Artemis, before end-ligation by ligase IV/XRC4/XLF (Goodarzi et al, 2010; Rassool and Tomkinson, 2010). In melanoma cells, treatment with vorinostat or NaB downregulates the levels of core NHEJ proteins Ku70, Ku80 and NaB also downregulates DNA-PK (in NaB-treated melanoma cells mRNA levels of Ku70, Ku80, DNA-PK, ligase IV and XRCC4 gene expression were also decreased; Munshi et al, 2005, 2006; Trichostatin A (TSA) causes downregulation of Ku70, Ku80 and DNA-PK in non-small cell lung carcinoma (NSCLC) cells (summarised in Table 1 and Figure 1; Zhang et al, 2009).

Homologous recombination acts in the second half of the cell cycle (late S- and G2 phase) and is an error-free pathway as it uses the undamaged sister chromatid as a template in contrast to the NHEJ pathway discussed before. The MRN complex and the CTP protein facilitate 5′-3′ strand resection at the DSB end, creating a single-stranded DNA (ssDNA) onto which RPA proteins are loaded to protect the ssDNA. Next, RPA proteins are replaced by RAD51 proteins, forming the RAD51 nucleoprotein filament, which initiates the sister chromatid invasion (Adimoolam et al, 2007; Rassool and Tomkinson, 2010). Vorinostat downregulates RAD51 protein expression in osteosarcoma, rhabdomyosarcoma, prostate and PCI-24781 in colon cancer cells (Chinnaiyan et al, 2005; Adimoolam et al, 2007; Blattmann et al, 2010). Furthermore, treating prostate and colon cancer cells with vorinostat or PCI-24781 causes decreased BRCA1, BRCA2 and RAD51 gene expression; in addition, extracts from xenografts of mice treated with PCI-24781 show decreased RAD51 protein expression (Adimoolam et al, 2007; Kachhap et al, 2010). H6CAHA causes downregulation of BRCA1 and BRCA2 gene expression in prostate cancer cells (summarised in Table 1 and Figure 1; Konsoula et al, 2011).

Interestingly, the effects of HDACi on the major DNA-repair signalling, NHEJ and HR proteins to date have not been observed in normal human cells. For example, 72-h treatment of foreskin fibroblasts with vorinostat had no effects on levels of RAD50, MRE11 or 53BP1 proteins (Lee et al, 2010). Furthermore, mRNA and protein levels of Ku70, Ku80 or DNA-PK were unaltered in NaB-treated lung fibroblasts (Munshi et al, 2005).

**HDACI EFFECTS ON DNA DAMAGE SIGNALLING AND REPAIR IN TERMS OF RADIOSENSITISATION**

There is evidence that HDACi lower the cell’s capacity to repair IR-induced DNA damage, both at the level of damage signalling and by affecting the major DNA repair pathways (NHEJ and HR), in many different cell types in vitro (summarised in Table 1).

Tumour cells treated with various HDACi display prolonged resolution of IR-induced γH2AX foci, an indicator of impaired DSB repair, which is due to impaired recruitment of or lower quantities of repair proteins (Munshi et al, 2005; Zhang et al, 2009; Lee et al, 2010). Vorinostat in combination with IR attenuates both the upregulation of IR-induced γH2AX and the upregulation of DNA-PK protein levels in prostate cancer cells (Chinnaiyan et al, 2005; Munshi et al, 2006). Similar effects are seen for IR plus vorinostat or scriptaid, in terms of attenuation of the upregulation of Ku80, in osteosarcoma, rhabdomyosarcoma and squamous carcinoma cells (Blattmann et al, 2010; Kuribayashi et al, 2010). Similarly, VPA and TSA attenuate upregulation of Ku70, Ku80 and DNA-PK in colon cancer cells after IR (Chen et al, 2009; Zhang et al, 2009). In terms of HR, vorinostat attenuates RAD51 upregulation in melanoma and rhabdomyosarcoma cell after IR (Blattmann et al, 2010).

Exposure to vorinostat radiosensitisises many different cancer cell lines, for example, melanoma, NSCLC, prostate, glioma, osteosarcoma and rhabdomyosarcoma; VPA radiosensitisises colorectal cancer cells; and similarly for TSA and melanoma and squamous carcinoma cells; NaB, phenylbutyrate and tributyrin and
HDAC inhibitors and DNA damage signalling and repair

Table 1. Summary of major preclinical studies of HDACi without or in combination with radiation showing effects on DNA damage signalling and repair

| HDAC inhibitor | Drug structure classification | HDAC class inhibition | Malignancy type | DNA repair involvement | Reference |
|----------------|-------------------------------|-----------------------|----------------|-----------------------|-----------|
| NaB            | Short-chain fatty acid        | I, IIA                | Melanoma A375 and MeWo; lung fibroblast MRC-9 | Prolonged γ-H2AX foci expression; | Munshi et al, 2005 |
|                |                               |                       |                | Ku70, Ku80, DNA-PK protein and mRNA expression; mRNA levels of ligase IV and XRCC4 (no effects on MRC-9 cells) |           |
| PCI-24781*     | Hydroxamic acid               | I, II                 | Colon HCT116   | RAD51 foci formation; mRNA levels of BRCA1, BRCA2 and RAD51; RAD51 protein expression | Adimoolam et al, 2007 |
| TSA            | Hydroxamic acid               | I, II                 | NSCLC A549 and H1650 | Prolonged γ-H2AX foci expression; Ku70, Ku80 and DNA-PK protein expression | Zhang et al, 2009 |
| Vorinostat     | Hydroxamic acid               | I, II                 | Fibroblasts HFS; prostate LNCaP; lung adenocarcinoma A549 | Prolonged γ-H2AX foci expression; RAD50 and MRE11 protein expression (in cancer but not normal HFS cells); 53BP1 in prostate cancer cells | Lee et al, 2010 |
| Vorinostat, VPA| Hydroxamic acid/short-chain fatty acid | I, II/I, Iia | Prostate DU145 and LNCaP | mRNA levels of BRCA1, BRCA2 and RAD51; BRCA1, RAD51 and DNA-PK protein expression; of BRCA1 and RAD51 foci | Kachhap et al, 2010 |
| H6CAHA         | Hydroxamic acid               | NA                    | Prostate DU145, PC3 and LNCaP, non-malignant prostate epithelial RWPE1 and 267B1 | Prolonged γ-H2AX and RAD51 foci formation; | Konsoula et al, 2011 |
|                |                               |                       |                | phosho-BRCA1 foci formation (post-IR); |           |
|                |                               |                       |                | phosho-ATM foci formation (post-IR); mRNA levels of ATM, BRCA1 and BRCA 2 (all effects only in cancer cells) |           |

Effect of HDACi and irradiation on DNA signalling and repair

| HDAC inhibitor | Drug structure classification | HDAC class inhibition | Malignancy type | DNA repair involvement | Reference |
|----------------|-------------------------------|-----------------------|----------------|-----------------------|-----------|
| Vorinostat     | Hydroxamic acid               | I, II                 | Prostate DU145; glioma U373 | DNA-PK and RAD51 protein expression | Chinnaiyan et al, 2005 |
|                |                               |                       | Melanoma A375 and MeWo; NSCLC A549 | Prolonged γ-H2AX foci expression; Ku70, Ku80, RAD50 protein expression in A375 cells | Munshi et al, 2006 |
|                |                               |                       | Osteosarcoma KHOS-24OS and SACHOS2, rhabdomyosarcoma A-204 and RD; osteoblasts hFOB 1.19 and fibroblasts NHDFc | Ku80 and RAD51 protein expression | Blattmann et al, 2010 |
| VPA            | Short-chain fatty acid        | I, IIA                | Colon LS174T and HCT116 | Ku70, Ku80 and DNA-PK protein expression | Chen et al, 2009 |
| Scriptaid      | Hydroxamic acid               | I, II                 | Squamous SQ-20B | Prolonged γ-H2AX foci expression; Ku80 protein expression | Kuriyayashi et al, 2010 |

Abbreviations: ATM — ataxia-telangiectasia-mutated; DNA — deoxyribonucleic acid; DNA-PK — DNA-dependent protein phosphokinase; HDAC — histone deacetylase; HDACi — HDAC inhibitors; IR — ionising radiation; NA — not available; NaB — sodium butyrate; NSCLC — non-small cell lung cancer; TSA — trichostatin A; VPA — valproic acid.

*Also known as abexinostat.

Interestingly, in H6CAHA-treated prostate epithelial cells, the surviving fraction of treated normal prostate cells increased after irradiation (Konsoula et al, 2011). Also, NaB did not radiosensitise normal human lung fibroblasts and neither did vorinostat radiosensitise normal human osteoblast nor fibroblast cell lines (Munshi et al, 2005; Blattmann et al, 2010). Further work is required to elucidate the precise pathways and targets through which the various HDACi exert these radiosensitising effects in cancer cells and potential radioprotecting effects in non-cancer cells. However, HDACs have recently been found to participate in the DNA damage response, and downregulation of HDACs has been shown to impair DNA repair pathways. For example, HDAC1 and HDAC2 have been found to be localised to sites of DNA damage and, furthermore, depletion of both HDAC1 and HDAC2 causes hypersensitivity to IR and leads to an impairment of the NHEJ pathway (Miller et al, 2010). In contrast, depletion of HDAC9 or HDAC10 leads to an impairment of the HR pathway, with HDAC9 having a stronger effect (Kotian et al, 2010). We therefore speculate that many of the HDACi radiosensitisation effects could be mediated via DNA damage signalling and repair pathways, and this might be due to acetylation of non-histone proteins involved in these pathways.

As previously discussed and summarised in Table 1, HDACi have been shown to have direct effects (downregulation) on mRNA or protein levels of the key players of the two most important DNA repair pathways in many cancer cell lines (e.g., melanoma, colon, NSCLC and prostate; Figure 1). This suggests that these chemotherapeutics would be well suited to combination treatment with radiotherapy, and indeed their radiosensitising effects have been confirmed in many cancer cell lines (e.g., prostate, glioma,
Of particular importance is their apparent specificity towards cancer cells, with a lack of radiosensitisation observed in many normal cells (e.g., prostate epithelial cells, lung fibroblasts and osteoblasts), suggesting that they might have low toxicity in a clinical setting. It is important to establish the optimum timing of drug relative to radiation to maximise the potential therapeutic benefit. In studies described here, mainly hydroxamic and short-chain fatty acid pan-HDACi were used. In future research, it would be useful to see which are the main HDACs that influence radiosensitivity and if HDAC-specific HDACi are as effective. This would also lead to potential development of biomarkers to select patients for combination HDACi/radiation treatments.

The use of HDACi in pre-clinical cancer models has been reported with several studies demonstrating DNA damage, manifest as DSBs, following HDACi monotherapy, consistent with in vitro data. For example, in a subcutaneous mouse model of acute lymphoblastic leukaemia, treatment with panobinostat resulted in an increase in γ-H2AX levels in the tumour cells (Vilas-Zornoza et al., 2012). In vivo models have also supported the finding of radiosensitisation by HDACi in lung and prostate cancers, with subsequent tumour growth delay (Geng et al., 2006; Konsoula et al., 2011).

**HDACi AND RADIOTHERAPY IN CLINICAL TRIALS**

As pre-clinical studies have demonstrated radiosensitising effects of HDACi in a variety of cancers, several clinical trials have been
Vorinostat was administered 3 h before each radiotherapy session for intrapelvic, pelvic bone or suprapubic abdominal wall metastases, including primary or locally recurrent tumour. Radiotherapy in 10 fractions was delivered over 2 weeks to target lesions, such as stomach carcinoma. Following CT-based planning, 30 Gy of radiotherapy was delivered in 16 patients with gastrointestinal (rectum, colon or oesophagus) tumours.

The pelvic radiation and vorinostat (PRAVO) study was the first published clinical trial combining HDACi and palliative radiotherapy (Ree et al, 2010). This phase I dose-escalation study examined the combination of vorinostat and pelvic palliative radiotherapy in 16 patients with gastrointestinal (rectum, colon or stomach) carcinoma. Following CT-based planning, 30 Gy of radiotherapy was delivered over 2 weeks to target lesions, including primary or locally recurrent tumour and intrapelvic, pelvic bone or suprapubic abdominal wall metastases. Vorinostat was administered 3 h before each radiotherapy treatment, with patients enrolled onto four sequential dose levels of vorinostat with dose escalation in increments of 100 mg (100–400 mg daily). Adverse events up to 6 weeks post-treatment were mostly grade 1 or 2. Seven patients reported grade 3 adverse events; four were considered unrelated to the study treatment, but the remaining three patients reported dose-limiting toxicity (DLT) of (1) anorexia and fatigue, (2) diarrhoea, anorexia and hyponatraemia, and (3) diarrhoea, fatigue and hypokalaemia. Hyperacetylation of histones H3 and H4 was identified two-and-a-half hours after administration of vorinostat, although no data were shown for the duration of this effect. Although designed as a phase I trial, this study also showed considerable post-treatment variation in tumour volume, from 54% reduction to 28% increase, with a mean change of 26% reduction. A recent reanalysis of the PRAVO study toxicity data incorporated details of radiation doses (6–30 Gy) delivered to the small bowel, from treatment-planning CT scans (Bratland et al, 2011), and the authors concluded that the previously reported DLT data might have reflected an adverse radiation dose-volume effect rather than a toxic effect of vorinostat itself. Dose-volume constraints are thus important considerations in planning future clinical trials, as the most common side effects of single-agent HDACi include gastrointestinal toxicities. Acute toxicity data from a phase I clinical trial has also been published for VPA in combination with temozolomide and radiotherapy in

The first published use of HDACi and radiation comes from a case report of anaplastic thyroid carcinoma, an aggressive and frequently deadly carcinoma, as recently as 2009 (Noguchi et al, 2009). The patient successfully received VPA with cisplatin/doxorubicin over three cycles, 40 Gy radiotherapy and surgical resection, followed by 6 months of VPA, with disease-free survival at 2 years. A retrospective study of paediatric patients with high-grade glioma found that those treated with VPA in addition to chemoradiotherapy did not show more frequent adverse effects, but this study lacked prospective follow-up (Masoudi et al, 2008). The pelvic radiation and vorinostat (PRAVO) study was the first published clinical trial combining HDACi and palliative radiotherapy (Ree et al, 2010). This phase I dose-escalation study examined the combination of vorinostat and pelvic palliative radiotherapy in 16 patients with gastrointestinal (rectum, colon or stomach) carcinoma. Following CT-based planning, 30 Gy of radiotherapy in 10 fractions was delivered over 2 weeks to target lesions, including primary or locally recurrent tumour and intrapelvic, pelvic bone or suprapubic abdominal wall metastases. Vorinostat was administered 3 h before each radiotherapy treatment, with patients enrolled onto four sequential dose levels of vorinostat with dose escalation in increments of 100 mg (100–400 mg daily). Adverse events up to 6 weeks post-treatment were mostly grade 1 or 2. Seven patients reported grade 3 adverse events; four were considered unrelated to the study treatment, but the remaining three patients reported dose-limiting toxicity (DLT) of (1) anorexia and fatigue, (2) diarrhoea, anorexia and hyponatraemia, and (3) diarrhoea, fatigue and hypokalaemia. Hyperacetylation of histones H3 and H4 was identified two-and-a-half hours after administration of vorinostat, although no data were shown for the duration of this effect. Although designed as a phase I trial, this study also showed considerable post-treatment variation in tumour volume, from 54% reduction to 28% increase, with a mean change of 26% reduction. A recent reanalysis of the PRAVO study toxicity data incorporated details of radiation doses (6–30 Gy) delivered to the small bowel, from treatment-planning CT scans (Bratland et al, 2011), and the authors concluded that the previously reported DLT data might have reflected an adverse radiation dose-volume effect rather than a toxic effect of vorinostat itself. Dose-volume constraints are thus important considerations in planning future clinical trials, as the most common side effects of single-agent HDACi include gastrointestinal toxicities. Acute toxicity data from a phase I clinical trial has also been published for VPA in combination with temozolomide and radiotherapy in

| HDAC inhibitor | Other combination therapy | Malignancy type (no. of patients) | Phase; status | Reference/trial identifier |
|---------------|--------------------------|----------------------------------|--------------|---------------------------|
| VPA*          | Surgery                  | High-grade sarcoma (17)          | I (Ongoing recruitment) | NCT01010958             |
| VPA           | Cisplatin, doxorubicin, surgery | Anaplastic thyroid (1)           | Case report | Noguchi et al, 2009       |
| VPA           | —/Variety                | Glioma                           | Retrospective (4 out of 66 patients) | Masoudi et al, 2008   |
| VPA           | Cisplatin                | Cervical (18)                    | II; Completed (well-tolerated) | Candelaria et al, 2010 |
| VPA           | Temozolomide             | Brain metastases in solid tumours | I, Terminated (lack of enrolment) | NCT00437957 |
| VPA           | Temozolomide             | Glioblastoma                     | I/II (Ongoing recruitment; well-tolerated) | Kamrava et al, 2008; NCT00302159 |
| Vorinostat    | —                        | Gastrointestinal (16)            | I, Completed (MTD 300 mg daily) | Ree et al, 2010 |
| Vorinostat    | —                        | Pancreatic                       | I/II; Terminated (slow accrual) | NCT00831493 |
| Vorinostat    | —                        | NSCLC                            | I (Ongoing recruitment) | NCT00821951 |
| Vorinostat    | Cisplatin, pemetrexed    | NSCLC                            | I (Ongoing recruitment) | NCT01059552 |
| Vorinostat    | —                        | Brain metastases                 | I (Ongoing recruitment) | NCT00838929 |
| Vorinostat    | 5-FU                     | Pancreas                         | I/II (ongoing) | NCT00948688 |
| Vorinostat    | Paclitaxel               | NSCLC                            | I/II, Terminated (unknown reason) | NCT00662311 |
| Vorinostat    | Cisplatin                | Oropharyngeal                    | I (Ongoing recruitment) | NCT01064921 |
| Vorinostat    | Temozolomide, bevacizumab| Glioma                           | II/III (Ongoing recruitment) | NCT01236560 |
| Vorinostat    | Capecitabine             | Pancreatic                       | I (Ongoing recruitment) | NCT00983268 |
| Vorinostat    | —                        | Glioma                           | I (Ongoing recruitment) | NCT01189266 |
| Vorinostat    | Temozolomide             | Glioblastoma                     | I (Ongoing recruitment) | NCT00731731 |
| Vorinostat    | Stereotactic radiosurgery| Brain metastases in NSCLC        | I (Ongoing recruitment) | NCT00446673 |
| LBHS89        | —                        | Prostate, head and neck, oesophageal (7) | I, Completed, unpublished (II ongoing) | NCT00670553 |
| Vorinostat    | —                        | Glioma                           | Upcoming phase I (including fractionated stereotactic radiation therapy) | NCT01378481 |

Abbreviations: HDAC = histone deacetylase; HDACi = HDAC inhibitors; NSCLC = non-small cell lung cancer; 5-FU = 5-fluorouracil; VPA = valproic acid.

*Adjuvant.

Table 2. Summary of clinical studies of HDACi in combination with radiation, including completed and ongoing trials

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patients with glioblastoma (Kamrava et al, 2008). This study showed no significant increase in haematological or neurological toxicities compared with radiotherapy and temozolomide alone; all neurological toxicities were reversible within 72 h of VPA cessation. The role of epigenetic therapy combined with radiotherapy was further characterised in a study of VPA with hydralazine (an inhibitor of DNA methylation) and cisplatin-based chemoradiotherapy in cervical cancer (Candelaria et al, 2010). Patients were divided into slow and fast acetylators and received 182 or 83 mg of hydralazine daily, respectively, with VPA 30 mg kg⁻¹ thrice daily until completion of either external beam radiotherapy or brachytherapy (to a total of 85 Gy). In all, 18 out of 22 patients completed therapy and although efficacy of epigenetic therapy could not be assessed, the combination of hydralazine, VPA and chemoradiation was found to be well tolerated and safe.

Although some in vitro studies have identified potential biomarkers (e.g., HR23B, (Khan et al, 2010); TYMS, ODC1, STAT1 and SKI (Dejligbjerg et al, 2008)), there are currently no cellular or molecular biomarkers in clinical use, which can accurately predict those patients who would benefit from either HDACi therapy alone or in combination with radiotherapy. Ultimately we need to develop biomarkers, which could identify those patients most likely to respond to treatment and those who will develop toxicities, so that the latter can avoid this treatment and be offered an effective alternative. It may be that the DNA damage signalling and repair pathways will yield such biomarkers. For example, high expression of MRE11, RAD50, Ku70 and/or Ku80 could be predictive of a better response to treatment with HDACi in combination with radiotherapy. Such biomarkers will need to be incorporated into future phase II/III clinical trials to optimise future use of HDACi as radiosensitisers.

CONCLUSIONS

HDACi have shown to be efficient radiosensitisers in many in vitro studies, with potent effects on prostate, glioma, melanoma, NSCLC, colon, squamous, osteosarcoma and lung cancer cell lines among many others. Although only a limited amount of clinical data have been collected so far, HDACi have also shown favourable clinical effects in combination with radiotherapy. In this mini-review, we have focussed on the effects of HDACi on DNA damage signalling and repair pathways after IR DSBs induction. HDACi have been found to downregulate many important DNA damage signalling, NHEJ and HR, proteins, and evidence has recently emerged that some HDACs are directly involved in the cellular DNA damage response. Future implementation of combination therapy comprising HDACi and radiotherapy with require a better understanding of dosing schedules, and there is still insufficient consensus regarding therapy response evaluation. As HDACi have been shown to cause impairment of DNA DSB signalling and repair, there is also an urgent need to develop biomarkers based on these pathways, which could allow clinicians to select patients for this therapeutic combination.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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