MINIREVIEW

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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,

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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 be also 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 benzamides, 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 (G_1, intra S and G_2/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, CtIP 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 G_1/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/XRCC4/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 G_2 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 CtIP 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 RAD50 in melanoma cell lines (Munshi *et al*, 2006) 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 radiosensitises many different cancer cell lines, for example, melanoma, NSCLC, prostate, glioma, osteosarcoma and rhabdomyosarcoma; VPA radiosensitises colorectal cancer cells; and similarly for TSA and melanoma and squamous carcinoma cells; NaB, phenylbutyrate and tributyrin and

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

HDAC inhibitor	Drug structure classification	HDAC class inhibition	Malignancy type	DNA repair involvement	Reference
Effect of HDACi on DNA signalling and repair					
NaB	Short-chain fatty acid	I, IIa	Melanoma A375 and MeWo; lung fibroblast MRC-9	Prolonged γ-H2AX foci expression; ↓Ku70, Ku80, DNA-PK protein and mRNA expression; ↓mRNA levels of ligase IV and XRCC4 (no effects on MRC-9 cells)	Munshi *et al*, 2005
PCI-24781[a]	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 expression; ↓phospho-BRCA1 foci formation (post-IR); ↓phospho-ATM foci formation (post-IR); ↓mRNA levels of ATM, BRCA1 and BRCA 2 (all effects only in cancer cells)	Konsoula *et al*, 2011
Effect of HDACi and irradiation on DNA signalling and repair					
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 SAOS2; 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	Kuribayashi *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.
[a]Also known as abexinostat.

melanoma cells; H6CAHA and prostate cancer cells; PCI-24781 and lung adenocarcinoma, large cell lung and colon cancer cells; and scriptaid and squamous carcinoma cells (Chinnaiyan *et al*, 2005; Munshi *et al*, 2005, 2006; Adimoolam *et al*, 2007; Chen *et al*, 2009; Zhang *et al*, 2009; Blattmann *et al*, 2010; Kuribayashi *et al*, 2010; Konsoula *et al*, 2011).

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,

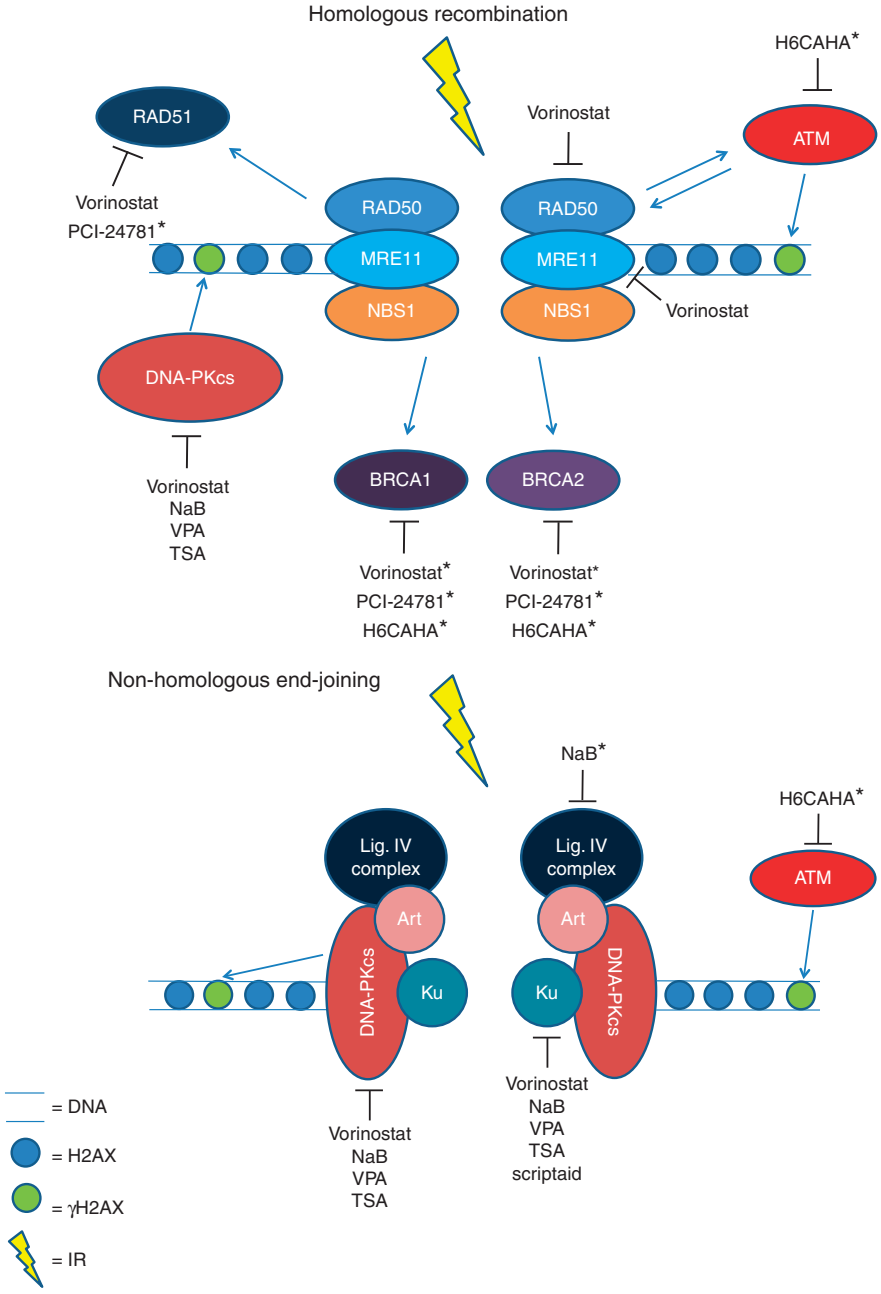

Figure 1. **Schematic overview of the impact of HDACi on DSB signalling, HR and NHEJ DNA repair pathways.** HDACi downregulate the major HR (above) and NHEJ (below) pathway proteins. Art = Artemis; Ku = Ku70/Ku80; Lig. IV complex = ligase IV/XRCC1/XLF; NaB = sodium butyrate; TSA = trichostatin A; VPA = valproic acid. *HDACi downregulate the levels of mRNA.

melanoma, squamous cell, osteosarcoma and rhabdomyosarcoma). 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

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

HDAC inhibitor	Other combination therapy	Malignancy type (no. of patients)	Phase; status	Reference/trial identifier
VPA[a]	Surgery	High-grade sarcoma	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/II (Ongoing recruitment)	NCT00731731
Vorinostat	Stereotactic radiosurgery	Brain metastases in NSCLC	I (Ongoing recruitment)	NCT00946673
LBH589	—	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.
[a]Adjuvant.

carried out using such combination therapy in patients (summarised in Table 2). In this rapidly expanding field, 3 have been published to date, with three terminated early and 12 ongoing or in set-up.

The first published use of HDACi and radiation comes from a case report of anaplastic thyroid carcinoma, an agressive 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 hyponatreamia, 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

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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