

REVIEW

Panobinostat as Pan-deacetylase Inhibitor for the Treatment of Pancreatic Cancer: Recent Progress and Future Prospects

Avineesh Singh · Vijay K. Patel · Deepak K. Jain · Preeti Patel · Harish Rajak

Published online: June 10, 2016
© The Author(s) 2016. This article is published with open access at Springerlink.com

ABSTRACT

The histone deacetylase (HDAC) inhibitors have been demonstrated as an emerging class of anticancer drugs. HDACs are involved in regulation of gene expression and in chromatin remodeling, thus indicating valid targets for different types of cancer therapeutics. The pan-deacetylase inhibitor panobinostat (Farydac[®], LBH589) was developed by Novartis Pharmaceuticals and has been recently approved by the US Food and Drug Administration (FDA) as a drug to treat multiple myeloma. It is under clinical investigation for a range of haematological and solid tumors worldwide in both oral and intravenous formulations. Panobinostat inhibits tumor cell growth by interacting with acetylation of histones and non-histone proteins as well as various apoptotic, autophagy-mediated targets and

various tumorigenesis pathways involved in development of tumors. The optimal combination regimen for pancreatic cancer remains to be fully elucidated with various combination regimens, and should be investigated in clinical trials. This article summarizes the current preclinical and clinical status of panobinostat in pancreatic cancer. Preclinical data suggests that panobinostat has potential inhibitory activity in pancreatic cancer cells by targeting various pathways and factors involved in the development of cancer. Herein, we reviewed the status of mono and combination therapy and the rationale behind the combination therapy undergoing trials, as well as possible future prospective use in the treatment of pancreatic cancer.

Keywords: HDAC inhibitors; Histone deacetylase inhibitors; Pancreatic cancer; Pan-deacetylase inhibitor; Panobinostat

Enhanced Content To view enhanced content for this article go to www.medengine.com/Redeem/AOD4F060499B6BF7.

A. Singh · V. K. Patel · D. K. Jain · P. Patel · H. Rajak (✉)
Institute of Pharmaceutical Sciences, Guru Ghasidas Vishwavidyalaya, Bilaspur, Chhattisgarh 495 009, India
e-mail: harishdops@yahoo.co.in

INTRODUCTION

Pancreatic cancer is the seventh most common cause of death globally and fourth most

common cause of deaths in the United States among all type of cancer deaths in 2012, contributing to about 3.3 lakhs pancreatic cancer deaths out of 13 million total cancer deaths per year. Pancreatic cancer most often occurs in the developed countries, where about 70% new cases were reported in 2012 [1–3]. There are many types of pancreatic cancer but the most common type is pancreatic adenocarcinoma (accounting for about 85% of cases). Pancreatic cancer is responsible for about 6% of total cancer-related deaths. The global annual rate of occurrence for pancreatic cancer is about 8/100,000 persons [4]. Pancreatic cancer is difficult to diagnose at an early stage, thus 70–80% patients are diagnosed at advanced or metastatic stages of pancreatic cancer [5]. Pancreatic adenocarcinoma has a very poor prognosis, and thus after diagnosis only about 25% of patients survive for 1 year and about 5% patients will live for 5 years. If the cancers have been diagnosed at an early stage, the 5-year survival rate has been seen to increase from 5 to about 20% [1, 6, 7].

Histone deacetylase inhibitors (HDACi) are an emerging class of anticancer drugs showing prominent antitumor activity by modulating transcriptional events incorporated with cell growth arrest, proliferation, and apoptosis [8]. In the last decade, four different HDACi—vorinostat (SAHA; 2006) and romidepsin (FK228; 2009) for cutaneous T-cell lymphoma (CTCL); belinostat (Beliodaq; Spectrum Pharmaceuticals; 2014) for peripheral T-cell lymphoma (PTCL), and panobinostat (Farydak; Novartis; 2015) for multiple myeloma—were approved by the US-FDA [9–11]. Apart from panobinostat there are many hydroxamic acid derivatives recognized as HDACi, such as resminostat (4SC201), givinostat (ITF2357), abexinostat (PCI-24781), SB939 (Pracinostat),

quisinostat (JNJ-26481585), CUDC-101, etc., and that are under clinical trials for various types of cancer [12].

Panobinostat (Farydak, Novartis Pharmaceuticals) is a member of the hydroxamic acid class of HDACi recently approved by US Food and Drug Administration (FDA) for the treatment of multiple myeloma. It is a colorless, clear, slightly viscous liquid and chemically known as (2E)-N-hydroxy-3-[4-[(2-hydroxyethyl)[2-(1H-indol-3-yl)ethyl]amino]methyl]phenyl]-2-propenamide, administered in both oral and intravenous forms [11, 13]. This nonselective hydroxamic acid-based HDACi has been tested for certain types of cancer and is under clinical trials for several, including CTCL, Hodgkin's lymphoma, leukemia, prostate, thyroid, breast and other types of hematologic and solid malignancies [14]. Panobinostat is likely the more potent HDAC inhibitor currently in clinical development, with a longer elimination time than others that have been tested. Panobinostat displays prolonged hyperacetylation of the histone protein, which allows intermittent dosing schedules to reduce the challenging thrombocytopenia observed with all HDAC inhibitors. Furthermore, modulation of gene expression through panobinostat is much more prominent in tumor cells than in healthy cells. These facts warrant further investigation of panobinostat in different types of tumors [15]. In this review, we summarize the current preclinical and clinical status of panobinostat in mono and combination therapy along with the rationale for combination therapy in pancreatic cancer.

This article is based on previously conducted studies and does not involve any new studies of human or animal subjects performed by any of the authors.

HISTONE, HDACS, AND EPIGENETIC MODULATIONS IN PANCREATIC CANCER

Histone deacetylases (HDACs) are a distinct class of enzymes capable of regulating gene expression and epigenetic modifications, and they have played an important role in treating pancreatic cancer. Pancreatic cancer proliferation and survival involves both classes I and II HDACs; class I HDACs play a predominant role in tumorigenesis [16]. Several HDACs such as HDAC1, HDAC2, HDAC3, and HDAC7 were found to be overexpressed in pancreatic cancer cell lines. Overexpression of HDAC1 leads to induction of proliferation and dedifferentiation, poor prognosis, repression of promoters of tumor suppressor genes, development of resistance towards cell cycle arrest, growth inhibition, apoptosis, and autophagy. Overexpression of HDAC2 attenuates the apoptosis via interrupting with TRAIL-mediated apoptosis and death-inducing NOXA gene silencing, while overexpressed HDAC7 interrupts apoptosis initiation and malignant progression, and controls angiogenesis through regulation of angiogenic genes in pancreatic cancer cells [17–22]. Apart from overexpressions of HDACs, the expression of TGFBR2—a tumor suppressor gene responsible for silencing E-cadherin—was found to be reduced alongside increased expressions of SNAIL as well as HDAC1 and HDAC2 (Snail/HDAC1/HDAC2 complex), a combination which promotes the metastatic pancreatic cancer process by suppressing E-cadherin expression via deacetylation of H3 and H4 proteins within the CDH1 promoter [23–26]. The CDH1 further encodes for the E-cadherin, which is downregulated in cancer invasion and metastasis and become an important element in epithelial to mesenchymal transition (EMT) [27].

On the other hand, histone methyl transferases such as EZH2 and SUV39H1 are also involved in tumorigenesis in pancreatic cells. SUV39H1 is involved in trimethylation of H3K9 and subsequent binding by HP1 leading to gene silencing and chromatin repression [28, 29]. Meanwhile, the accumulation of EZH2 is increased, which is associated with acceleration of pancreatic cell proliferation via the downregulation of p27Kip1 (a cell cycle regulator), and thus the depletion of EZH2 may become a target in treating pancreatic cancer [30].

Various studies have demonstrated that expression of miRNAs such as miR-21 is increased, which results induction of proliferation of and invasion by pancreatic cancer cells as compared to control cells [31–33]. The miR-21 has targeted various apoptotic genes such as PDCD4 and PTEN, causing attenuation of apoptosis and induction of tumorigenicity [34]. These observations clearly reveal the importance of histone proteins, HDACs, and epigenetic modulations in pancreatic tumorigenesis.

HDACi AND PANCREATIC CANCER

HDACi increased the acetylation of histone and other non-histone proteins, causing alteration in gene expression, angiogenesis, apoptosis, cell-cycle arrest, and metastasis (Fig. 1) [35].

Role of HDACi in Suppression of Angiogenesis

HDACi play an important role in regulation of vascular endothelial growth factor (VEGF) and hypoxia-inducible factor-1alpha (HIF-1 α). HIF-1 α is able to induce transcription of genes that are involved in cell proliferation and survival. VEGFs are involved in various biological processes including angiogenesis.

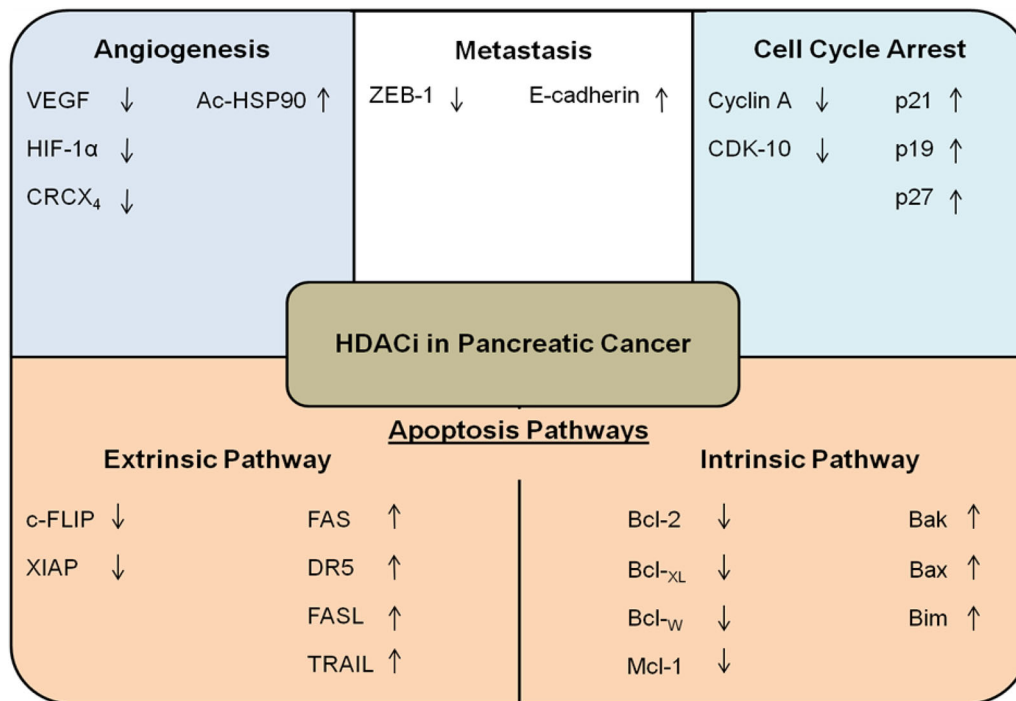


Fig. 1 Role and effects of HDACi in pancreatic cancer with their corresponding targets. *HIF-1α* hypoxia-inducible factor-1 alpha, *TRAIL* tumor necrosis factor-related apoptosis-inducing ligand, *VEGF* vascular endothelial

growth factor, *HSP90* heat shock protein 90, *CDK* cyclin-dependent kinase, *up arrow symbol* upregulation, *down arrow symbol* downregulation

Treatment with HDACi downregulates the expression of VEGF and HIF-1α, resulting in inhibition of transcriptional activity in pancreatic cancer cell lines [36, 37]. HDACi also inhibit the HDAC6 enzyme, leading to acetylation of heat shock protein 90 (HSP90) and inhibition of its chaperon function, resulting in the degradation of HIF-1α [37, 38]. Additionally, HDACi are also able to induce anti-angiogenesis by inhibiting the proliferation of endothelial cells through decreasing the expression of C-X-C chemokine receptor type 4 (CRCX4) [39–41].

Role of HDACi in Apoptosis and Autophagy

HDACi can exert caspase-dependent and -independent apoptosis through both intrinsic

and extrinsic pathways [42, 43]. HDACi mediate with extrinsic apoptosis pathway in pancreatic cancer cells through downregulation of c-FLIP, XIAP, and the induction of FAS, DR5, FASL, and TRAIL (tumor-necrosis factor-related apoptosis-inducing ligand) [44, 45]. As expression of HDAC2 is increased in pancreatic cancer cells, HDACi inhibited HDAC2, leading to pancreatic cancer cell death via TRAIL and death receptor expression [22]. On the other hand, upregulation of anti-apoptotic proteins such as XIAP, Mcl-1, and Bcl-2 makes pancreatic cells insensitive to intrinsic apoptosis [46]. HDACi target the intrinsic apoptosis pathway by downregulating the expression of Bcl-2, Bcl-xl, Bcl-w, and Mcl-1 (anti-apoptotic proteins) and upregulating the expression of Bax, Bak, and Bim (pro-apoptotic protein) [42, 47–51].

HDAC inhibitors are also able to induce caspase-independent autophagic cell death without any nuclear fragmentation. Autophagy is a key mechanism of cell death in apoptotic deficient conditions and plays an important role in cell survival during stress conditions such as hypoxia, unfolded protein response, endoplasmic reticulum (ER) stress, nutrient starvation, and treatment of cancers with chemotherapeutic agents. Studies have also revealed that HDACi are capable of generation and intracellular accumulation of the reactive oxygen species (ROS) responsible for the caspase-independent apoptosis in pancreatic cancer cells [20, 36, 52–54, 106].

Role of HDACi in Cell-cycle Arrest

HDACi can induce cell-cycle arrest in pancreatic cancer cells via a p53-independent manner. In pancreatic cancer cells, the cell-cycle progression was modulated by p21, a cyclin-dependent kinase (CDK) inhibitor leading to histone acetylation around the p21 promoter [55]. Treatment with HDACi causes the upregulation of CDK inhibitors such as p21, p19, and p27, and downregulation of the cyclins and cyclin-dependent kinases (CDKs) such as cyclin A, CDK10 etc., which lead to cell-cycle arrest at the G1/S or/and G2/M phase [36, 47, 56–58]. Inhibition of HDACs with selective class I HDACi causes the cell-cycle arrest at G2/M phase. Meanwhile, only a slight impact on the cell cycle was observed with selective class II HDACi. On the other hand, combination of selective class I and class II HDACi have synergistic effects on cell-cycle arrest via mutual p21 upregulation [59]. These studies provide clear evidence that the pan deacetylase inhibitors can be more efficient than selective HDACi in pancreatic cancer cell lines.

Role of HDACi in Metastasis

Epithelial to mesenchymal transition (EMT) is an important regulatory factor involved in tumor invasion and metastasis in pancreatic cancer by loss of cell polarity and cell–cell adhesion properties of epithelial cells. The cellular polarity and cell–cell adhesion of epithelial cells are maintained by a transmembrane glycoprotein E-cadherin. The expression of E-cadherin is suppressed by EMT, leading to metastasis in pancreatic cancer patients with poor survival. The HDACi play their antimetastatic activity by changing the EMT into MET (mesenchymal to epithelial transition). Studies have shown that treatment with HDACi enhanced the expression of E-cadherin in pancreatic cancer cell lines. It was also reveals that transcriptional factor ZEB1 linked with E-cadherin promoter is responsible for HDAC expression, resulting in histone deacetylation and E-cadherin suppression. Thus, HDACi like vorinostat can downregulate the ZEB1 and upregulate the expression of E-cadherin in pancreatic cancer cell lines [60–65]. On the basis of these facts, it is anticipated that HDACi are able to suppress the metastasis in pancreatic cancer cell lines.

GENERAL PHARMACOKINETIC AND PHARMACOLOGY OF PANOBINOSTAT

Pharmacokinetics of Panobinostat

Absorption

According to Biopharmaceutical Classification System, panobinostat is classified as a class I drug showing high solubility and permeability, but its bioavailability is not affected by food materials [66]. A dose-dependent increase in the

area under curve (AUC) and C_{\max} is observed in oral and intravenous administration of panobinostat with T_{\max} of approximately 1h, but elimination time ($T_{1/2}$) ranged between 8 and 18 h [13, 67–72]. On the other hand, reduced C_{\max} and slightly prolonged T_{\max} are observed upon panobinostat administration after food intake. The rate of absorption is influenced by food material but the extent of absorption has not altered. As the overall absorption of panobinostat is not affected by food, it can be administered with or without food [66]. Co-administration of panobinostat with ketoconazole causes an increased C_{\max} and AUC of panobinostat without any marginal change in T_{\max} or $T_{1/2}$ [73]. Additionally, normal, mild, and moderate hepatic impairments cause the increased systemic level of panobinostat in cancer patients [74].

Metabolism

Panobinostat is metabolized by various pathways such as oxidation, reduction, hydrolysis, and glucuronidation reactions. Oxidative metabolism of panobinostat is mainly carried out by human cytochrome P450 enzymes such as CYP2D6, CYP3A4, and CYP2C19. More than 77 metabolites of panobinostat have been recognized in which about 40 metabolites are detected in circulating plasma. These metabolites are excreted through both feces and urine. About 44–77% metabolites of total oral administered panobinostat are observed in feces and 29–51% are excreted in urine while $\leq 3.5\%$ metabolites are remained unchanged in feces, demonstrating good oral absorption of panobinostat [73, 75, 76]. The pharmacokinetic profile of panobinostat and BJB432 (major metabolite of panobinostat) are not much affected by renal impairment and its severity of diseased conditions [77].

Adverse Effects

The most common toxicities of panobinostat are related to the gastrointestinal, constitutional, and hematological system. Treatment with panobinostat involves dose-dependent grade 1–4 toxicities such as nausea, vomiting, fatigue, diarrhea, and thrombocytopenia. In several cases, dose-dependent cardiac toxicity such as QT prolongation is also reported with panobinostat treatment [78–84]. In addition, normal, mild, and moderate severities of renal and hepatic impairments do not have much influence on the safety profile of panobinostat in advanced cancer patients [74, 77].

General Pharmacology of Panobinostat

Panobinostat is a nonselective histone deacetylase inhibitor having potential HDAC inhibitory activity against all Class I, II, and IV at nanomolar concentrations. Panobinostat interferes with histone and non-histone proteins such as H3, H4, HIF-1 α (hypoxia-inducible factor 1 alpha), β -catenin, α -tubulin, Chaperons (HSP90), estrogen receptor (ER α), androgen receptor (AR), signaling mediators (Stat3, Smad7), DNA repair proteins (Ku70), retinoblastoma protein (pRb), etc., leading to alterations in transcriptional factors (p53, E2F, NF- κ B, c-Myc) [85–87]. Acetylation of α -tubulin is directly related to the inhibition of HDAC6. These effects were cumulatively related to the cell-cycle arrest at the G₂-M phase. A delay in the G₂phase and abnormal mitotic progression is observed with panobinostat treatment, which is mediated by the activation of PLK1 and Cyclin B1 expression and suppression of E2F1 [39, 88]. Further, Hsp90 acetylation induced by panobinostat treatment causes a decrease in the association of Hsp90 with other chaperone proteins (e.g., Akt, EGFR, STAT3, c-Src) and disrupted Hsp90

chaperone function, resulting in oncogenic HSP90 client protein degradation [89, 90]. The antiangiogenesis properties of panobinostat are associated with the inhibition of CXCR4, HIF-1 α , and VEGF-induced signaling in human endothelial cells [39].

Treatment with panobinostat involves the induction of autophagy via a death-associated protein kinase (DAPK)-dependent manner, while apoptosis is associated with a DAPK-independent manner in human colon tumor cells [91]. Panobinostat also interferes with both intrinsic and extrinsic apoptosis pathways by enhancing TRAIL-mediated cytotoxicity via decreasing the expression of Bcl-xl, Bcl-2, and XIAP, and increasing the expression of Bim, BAX, and BAK (pro-apoptotic proteins) [92–95]. Apoptosis was also induced by the generation of ROS with panobinostat alone or in combination with other drugs [96]. Panobinostat selectively induces apoptosis in cancer cells without having a marked effect on normal human epithelial cells, fibroblasts, and peripheral blood mononuclear cells [97]. The antitumor properties of panobinostat and other HDACi are also mediated by the enhanced numbers of activated T cells, systemic cytokine responses, and regulatory T cell ratio [98, 99].

THERAPEUTIC EFFICACY OF PANOBINOSTAT IN PANCREATIC CANCERS

Panobinostat: Mono-therapy in Pancreatic Cancer

Panobinostat induces pancreatic tumor cell death in a dose-dependent manner. It significantly reduces tumor growth in subcutaneous xenograft mouse model at the micromolar range with minimal toxicity toward

normal cells. Panobinostat is found to be as equipotent as gemcitabine in terms of tumor size reduction and more effective than TSA in pancreatic cancer cell lines. Both panobinostat and TSA are able to induce the death of pancreatic tumor cell lines by apoptosis. Panobinostat has been found to induce acetylation of H4 histone protein and promote the expression of p21^{WAF-1/CIP-1}, ultimately leading to cell-cycle arrest at the G₂/M-checkpoint of cancer cell death. It also decreases cellular levels of HDAC1, HDAC2, and HDAC3 as well as SIRT1 in the case of SOJ-6 pancreatic cancer cells compared to control cells, but the level of SIRT2 expression can be up- or downregulated depending upon the cell lines used. Therefore, translational regulation of the HDAC encoding genes are dependent on the cell lines considered during translation [100, 101]. Treatment with panobinostat also induces p $\text{H}2\text{AX}$ (a biomarker of DNA double-strand breaks) and suppresses checkpoint kinase 1 (CHK1) [16]. Furthermore, panobinostat is able to induce apoptosis by potentiating TRAIL-induced apoptosis and cFLIP degradation even in TRAIL-resistant cells. Treatment with panobinostat increases the level of ubiquitinated c-FLIP and increases degradation of c-FLIP mediated by ubiquitin/proteasome, leading to downregulation of c-FLIP. Thus, PAN is able to potentiate TRAIL-induced apoptosis in pancreatic tumor cells [44].

Chien et al. demonstrated the potency of SAHA, belinostat, and panobinostat in pancreatic cancer cell lines and determined the EC₅₀ values, showing that belinostat was more potent than SAHA in Panc0327, Panc0403, and MiaPaCa2 pancreatic cancer cell lines. The EC₅₀ value of belinostat was found to be in the micromolar range while panobinostat was active in the nanomolar range

against 14 different pancreatic cancer cell lines [36]. Preclinical study of panobinostat in a pancreatic cancer xenograft mouse model shows that it is as equipotent as gemcitabine in terms of tumor growth reduction [100]. As among all HDACi, panobinostat is found to be more active and as equipotent as gemcitabine in pancreatic cancer cells, and thus it should be explored in clinical development in combination therapy with other antitumor agents.

Panobinostat: Combination Therapy in Pancreatic Cancer

Panobinostat in Combination with Gemcitabine

The anticancer activity of panobinostat towards pancreatic cancer was first tested by Haefner et al. in 2008. They showed that a combination of panobinostat and gemcitabine significantly reduced tumor mass in vivo in nude mice models, but apoptosis was slightly increased and there was no significant reduction of cell proliferation. They found that panobinostat is more efficient than gemcitabine and increases the potency of gemcitabine in combination therapy [101]. A phase I study was performed to determine the safety and tolerability of panobinostat and gemcitabine in combination therapy. The oral administration of panobinostat 30 mg BIW and gemcitabine 1000 mg/m² IV on days 1, 8, and 15 every 28 days showed grade 4 thrombocytopenia in 2/3 patients. However, panobinostat 10 mg TIW for 2 weeks and gemcitabine 800 mg/m² on days 1 and 8 every 21 days was well tolerated in 5/6 patients with relatively low levels of grade 4 neutropenia and thrombocytopenia toxicities (35% and 18%, respectively). No patients experienced grade 2 or grade 3 QTcF prolongation. The recommended doses for

further study are panobinostat 10 mg TIW orally for 1 week along with gemcitabine 800 mg/m² IV on days 1 and 8 every 21 days. In all instances, dose-limiting toxicities occurred due to myelosuppression during the treatment at all dose levels. Mild to moderate toxicities related to treatment are nausea, vomiting, constipation, diarrhea, anorexia, fatigue, and rashes (Table 1) [14].

Panobinostat in Combination with Bortezomib

Treatment of pancreatic cancer with bortezomib, a proteasome inhibitor, results in the formation of aggresome (aggregates of ubiquitin-conjugated proteins) in both in-vivo and in-vitro models. On the other hand, HDACs are known to regulate the functions of histone and nonhistone proteins through the promotion of ubiquitin-dependent proteasomal degradation, thus, inhibition of aggresome formation via HDACi treatment can potentiate the effect of bortezomib. Panobinostat (20 mg orally once daily on days 1, 3, 5, 8, 10, and 12 followed by nine days of rest) in combination with bortezomib 1.3 mg/m² IV BIW for every 21 days was administered in seven patients in phase II clinical trials. Unfortunately, the study was terminated due to lack of treatment response and grade 4 thrombocytopenia and diarrhea in phase II clinical trials (Table 1) [102].

Panobinostat in Combination with BEZ235

The K-RAS and PI3K/AKT/mTOR signaling pathways play an important role in human pancreatic ductal adenocarcinoma (PDAC). Thus, targeting these pathways or mediators may become an effective means of treatment of PDAC. Therefore, Venkannagari et al. combined panobinostat with dual PI3 K/mTOR inhibitor BEZ235 in a preclinical nude mouse model

Table 1 Preclinical and clinical prospective of panobinostat in pancreatic cancer

Study	Dose	Model(s)	Result	Adverse effects	References
Preclinical	PAN 25 mg/kg IP BIW for 4 weeks	Xenograft mouse	PAN reduced the tumor growth as efficient as gemcitabine	–	[100]
Phase I	Arm I: PAN 20-30 mg O BIW and GEM 800–1000 mg/m ² IV on 1, 8, 15 days (28 days) Arm II: PAN 10 mg O TIW and GEM 1000 mg/m ² IV on 1, 8, 15 days (28 days) Arm III: PAN 10 mg O TIW × 2 weeks and GEM 800–1000 mg/m ² IV on 1, 8 days (21 days)	17 patients (three pancreatic patients)	A dose escalation study. Dose-dependent combination of PAN and gemcitabine was limited by myelosuppression	Dose-dependent grade 1–4 toxicities	[14]
Phase II	PAN 20 mg O TIW × 2 weeks every 21 days + Bortezomib 1.3 mg/m ² IV BIW × 2 weeks every 21 days	Seven patients	Terminated due to lack of treatment responses and unacceptable toxicity	Grade 3 thrombocytopenia (57%) Grade 4 diarrhea (29%)	[102]
Preclinical	PAN 10 mg/kg IP TIW, BEZ235 25 mg/kg po, combination of PAN 10 mg/kg + BEZ235 25 mg/kg	Nude mice model	Significant differences in the mean tumor volumes after three weeks of treatment	–	[103]
Preclinical	PAN 10 mg/kg IP BIW once daily, MK-1775 20 mg/kg O BIW twice daily, Combination PAN 10 mg/kg IP once + MK-1775 20 mg/kg O twice BIW (on day 1 and 3 for 3 weeks)	Xenograft mouse model	Delay in tumor growth 30.9% with MK-1775 and 37.8% with PAN alone therapy on day 20 but 58.7% with combination therapy	–	[104]

BIW twice weekly, *d* days, *GEM* gemcitabine, *IP* intraperitoneal, *IV* intravenous, *PAN* panobinostat, *O* orally, *po* per oral, *TIW* three times weekly

against pancreatic cancer cells. BEZ235 treatment in pancreatic cells inhibited the activity of PI3K, TORC1, and TORC2, causing a reduction in phosphorylated AKT, 4EBP1, and p70S6 K, and leading to the induction of BIM. This led to decreases in cell proliferation and cell-cycle arrest of pancreatic cancer cells. On the other hand, treatment with panobinostat inhibited phosphorylated AKT, leading to nuclear localization of FOXO3A. It also inhibits TORC1 activity leading to reduction in phosphorylated 4EBP1 and p70S6 K, ultimately inducing expression of p21, p27, and pro-apoptotic proteins (BIM and BAK). Co-treatment with BEZ235 and panobinostat caused greater induction of BIM and increased cell death of pancreatic cancer cells. Treatment with panobinostat (10 mg/kg IP TIW), BEZ235 (25 mg/kg, PO) and a combination of panobinostat and BEZ235 for 3 weeks in the preclinical nude mouse model showed marked difference in mean tumor volume in combination therapy as compared to use of a single agent alone. Thus, combination of panobinostat and BEZ235 synergistically potentiate the antitumor activity and induced apoptosis in PDAC cells (Table 1) [103].

Panobinostat in Combination with MK-1775

Wang et al. studied the combination of Wee1 inhibitor, MK-1775, and the pan-deacetylase inhibitor panobinostat in preclinical xenograft mouse model pancreatic cells and demonstrated the rationale behind the combination therapy. MK-1775 induces DNA damage by increasing H2AX phosphorylation and activating CDK1/CDK2. It also activates CDK-dependent CHK1 and ATM/ATR. In addition, MK-1775 increases p-CHK1, indicating activation of the CHK1 pathway. Activation of CHK1 results in inactivation of CDC25s, causing a decrease in active CDK1/2, cell-cycle arrest, DNA repair,

and cell survival. Furthermore, activated CHK1 directly increases the activity of Wee1. These facts demonstrate the mechanism of resistance to treatment with MK-1775. On the other hand, treatment with panobinostat downregulates the CHK1 or inactivates the CHK1 pathway and activates CDK1 and CDK2, thus inducing DNA damage and subsequently apoptosis. Therefore, combining panobinostat with MK-1775 can prevent activation of CHK1 leading to an increase in DNA damage and cell death. Further, panobinostat treatment also increases pH2AX levels, causing the DNA damage. Treatment with panobinostat (10 mg/kg IP BIW once daily), MK-1775 (20 mg/kg O BIW twice daily), and a combination for 3 weeks in a preclinical pancreatic cancer xenograft mouse model showed a significant delay of tumor growth: 58.7% with combination therapy as compared to 30.9% with MK-1775 and 37.8% with panobinostat as a single agent alone [104]. These facts clearly provide the evidence for using a combination of panobinostat with MK-1775 in pancreatic tumor cells in preclinical models, and supported the fact that combination therapy should be promoted in the clinical development for this deadly disease.

Panobinostat in Combination with IMC-RON8

The expression of Ron (Recepteur d'origine nantais) rarely takes place in normal pancreatic cells, but it is overexpressed in cancerous pancreatic cells. The activation and overexpression of Ron is mediated by the macrophage stimulating protein (MSP), resulting in the activation of tumorigenesis pathways. Thus, Ron may become an important therapeutic target for the treatment of pancreatic cancer. On the basis of this assumption, the first Ron monoclonal antibody, IMC-RON8, has been entered into clinical trials for targeting overexpression of

Ron. IMC-RON8 downregulates the expression of Ron, and inhibits MSP-stimulated Ron activation, leading to attenuated phosphorylation of Akt and ERK and expression of survivin mRNA. These effects simultaneously reduce MSP-induced pancreatic cell proliferation. On the other hand, panobinostat decreases Ron expression, pAkt, survivin, and XIAP in pancreatic cancer cells leading to enhanced cell apoptosis. Interestingly, treatment with panobinostat causes reduction in colony formation in cells having low Ron levels to a greater extent than cells having higher levels of Ron. Thus, combination therapy of IMC-RON8 with panobinostat sensitizes the pancreatic cancer cell lines to reduce the numbers and size of colonies and produce more prominent effects than mono therapy in a preclinical study. The combination therapy further reduces the expression of Ron, phosphorylated Akt, and increases the cleavage of PARP as compared to a single treatment. These results suggest a novel and potential combination approach in the treatment of pancreatic cancer and should be explored in clinical developments [105]. The current clinical status of panobinostat and its combination therapy is undergoing trials (Table 1) and should be further explored in the future for the treatment of pancreatic cancer.

CONCLUDING REMARKS AND FUTURE PROSPECTIVE

In conclusion, we have gone through comprehensive studies supporting the role of HDACs in pancreatic carcinoma and shown the aberrant overexpression of different HDACs such as HDAC1, HDAC2, HDAC3, and HDAC7. This aberrant overexpression may contribute to carcinogenesis by unbalancing the histone acetylation and deacetylation,

leading to uncontrolled cell growth and proliferation in pancreatic cancer cells. Thus, inhibiting histone deacetylase enzymes may become an important therapeutic target to treat pancreatic cancer patients.

Panobinostat is a new anticancer agent of histone deacetylase inhibitor that inhibits tumor cell growth, proliferation, and differentiation, ultimately leading to cell-cycle arrest. As histone acetylation is a fundamental function of panobinostat, it mediates its biological effect through the regulation of gene expression via direct histone hyperacetylation. Moreover, it also mediates the antitumor activity by acetylation of non-histone proteins, indicating that it could have a much broader effect on cellular physiology. Apart from hyperacetylation, as a pan-deacetylase inhibitor panobinostat can activate the death-receptors, extrinsic-intrinsic apoptotic pathways, and autophagy-mediated cell death, concomitant with upregulation of transcriptional pro-apoptotic genes and downregulation of pro-survival genes.

As panobinostat is a nonselective histone deacetylase inhibitor, it can target different classes of HDACs. During the exhaustive study we have found that treatment of pancreatic cancer with selective HDAC class inhibitors have less potency as compared to a combination of selective class inhibitors indicating that non-selective inhibitors can be more potent than selective ones. In this review, we have also shown that treatment of pancreatic cancer with panobinostat alone or in combination with other drugs such as gemcitabin, bortezomib, BEZ235, MK-1775, and IMC-RON8 are highly efficient in preclinical models. The phase I study of panobinostat and gemcitabin showed dose-limiting toxicities in pancreatic cancer patients while a combination of bortezomib

was terminated due to lack of treatment response. As many signaling pathways are involved in pancreatic tumorigenesis and interlinked to each other by several mediators, it may be more beneficial to combine the inhibitors of these signaling pathways. On the basis of this assumption, various preclinical studies have been performed in combination of panobinostat with BEZ235 (a RAS and PI3 K/AKT/mTOR inhibitor), IMC-RON8 (Ron monoclonal antibody), and MK-1775 (Wee1 Inhibitor), indicating pronounced antitumor activity in pancreatic cancer preclinical models, and should be further explored in clinical trials for development of new effective combinations.

ACKNOWLEDGMENTS

No funding or sponsorship was received for this study or publication of this article. One of the authors, Avineesh Singh, is thankful to Council of Scientific and Industrial Research (CSIR), New Delhi, India, for awarding senior research fellowship. All authors had full access to all of the data in this study and take complete responsibility for the integrity of the data and accuracy of the data analysis. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this manuscript, take responsibility for the integrity of the work as a whole, and have given final approval for the version to be published.

Disclosures. A. Singh, V. K. Patel, D. K. Jain, P. Patel and H. Rajak declare no conflicts of interest.

Compliance with Ethics Guidelines. This article is based on previously conducted studies and does not involve any new studies

of human or animal subjects performed by any of the authors.

Open Access. This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits any noncommercial use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

REFERENCES

1. Stewart BW, Wild CP. World cancer report 2014. In: World Health Organization. 2014. Chapter 5.7 (ISBN 92-832-0429-8).
2. The medical subject Headings indexing system refers to “islet cell carcinoma”, which is subdivided into gastrinoma, glucagonoma, somatostatinoma and VIPoma. In: MeSH tree at pancreatic neoplasms. 2014. [C04: 588: 322.475]. Accessed 16 Oct 2014.
3. Farrell JJ, Fernandez del Castillo C. Pancreatic cystic neoplasms: management and unanswered questions. *Gastroenterology*. 2013;144(6):1303–15.
4. Raimondi S, Maisonneuve P, Lowenfels AB. Epidemiology of pancreatic cancer: an overview. *Nat Rev Gastroenterol Hepatol*. 2009;6(12):699–708.
5. Foundation for Promotion of Cancer Research. Cancer statistics in Japan-2011. http://ganjoho.jp/public/statistics/backnumber/2011_jp.html. Accessed 20 Oct 2012.
6. Caldas C, Hahn SA, da Costa LT, et al. Frequent somatic mutations and homozygous deletions of the p16 (MTS1) gene in pancreatic adenocarcinoma. *Nat Genet*. 1994;8(1):27–32.
7. Delper Y, Hanoun N, Lulka H, et al. Genetic and epigenetic alterations in pancreatic carcinogenesis. *Curr Genom*. 2011;12(1):15–24.
8. Bolden JE, Peart MJ, Johnstone RW. Anticancer activities of histone deacetylase inhibitors. *Nat Rev Drug Discov*. 2006;5(9):769–84.

9. Gryder BE, Sodji QH, Oyelere AK. Targeted cancer therapy: giving histone deacetylase inhibitors all they need to succeed. *Future Med Chem.* 2012;4(4):505–24.
10. US FDA. FDA approves Beleodaq to treat rare, aggressive form of non-Hodgkin lymphoma. July 3, 2014. <http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm403929.htm>; Accessed 6 Sept 2015.
11. US FDA. FDA approves Farydak for treatment of multiple myeloma. Feb 23, 2015. <http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm435296.htm>. Accessed 6 Sept 2015.
12. Rajak H, Singh A, Raghuwanshi K, et al. A structural insight into hydroxamic acid based histone deacetylase inhibitors for the presence of anticancer activity. *Curr Med Chem.* 2014;21(23):2642–64.
13. Giles F, Fischer T, Cortes J, et al. A phase I study of intravenous LBH589, a novel cinnamic hydroxamic acid analogue histone deacetylase inhibitor, in patients with refractory hematologic malignancies. *Clin Cancer Res.* 2006;12(15):4628–35.
14. Jones SF, Bendell JC, Infante JR, et al. A phase I study of panobinostat in combination with gemcitabine in the treatment of solid tumors. *Clin Adv Hematol Oncol.* 2011;9(3):225–30.
15. Rasmussen TA, Sogaard OS, Brinkmann C, et al. Comparison of HDAC inhibitors in clinical development Effect on HIV production in latently infected cells and T-cell activation. *Human Vaccines Immunother.* 2013;9(5):993–1001.
16. Wang G, He J, Taub JW, et al. Both class I and class II histone deacetylases are required for proliferation and survival of human pancreatic cancer cells (abstract no 1830). *Cancer Res.* 2012;72:1830.
17. Schneider G, Kramer OH, Schmid RM, et al. Acetylation as a transcriptional control mechanism-HDACs and HATs in pancreatic ductal adenocarcinoma. *J Gastrointest Cancer.* 2011;42(2):85–92.
18. Ouaisi M, Sielezneff I, Silvestre R, et al. High histone deacetylase 7 (HDAC7) expression is significantly associated with adenocarcinomas of the pancreas. *Ann Surg Oncol.* 2008;15(8):2318–28.
19. Truty MJ, Lomber G, Fernandez-Zapico ME, et al. Silencing of the transforming growth factor-beta (TGFbeta) receptor II by Kruppel-like factor 14 underscores the importance of a negative feedback mechanism in TGFbeta signaling. *J Biol Chem.* 2009;284(10):6291–300.
20. Rikiishi H. Possible role of autophagy in the treatment of pancreatic cancer with histone deacetylase inhibitors. *Cancer Res.* 2003;63(10):2624–30.
21. Fritsche P, Seidler B, Schuler S, et al. HDAC2 mediates therapeutic resistance of pancreatic cancer cells via the BH3-only protein NOXA. *Gut.* 2009;58(10):1399–409.
22. Schuler S, Fritsche P, Diersch S, et al. HDAC2 attenuates TRAIL-induced apoptosis of pancreatic cancer cells. *Mol Cancer.* 2010;9:80.
23. Goggins M, Shekher M, Turnacioglu K, et al. Genetic alterations of the transforming growth factor beta receptor genes in pancreatic and biliary adenocarcinomas. *Cancer Res.* 1998;58(23):5329–32.
24. Birnbaum DJ, Mamessier E, Birnbaum D. The emerging role of the TGF-beta tumor suppressor pathway in pancreatic cancer. *Cell Cycle.* 2012;11:683–6.
25. Katsuno Y, Lamouille S, Derynck R. TGF-beta signaling and epithelial-mesenchymal transition in cancer progression. *Curr Opin Oncol.* 2013;25:76–84.
26. von Burstin J, Eser S, Paul MC, et al. E-cadherin regulates metastasis of pancreatic cancer in vivo and is suppressed by a SNAIL/HDAC1/HDAC2 repressor complex. *Gastroenterology.* 2009;137(1):361–71, 371.e1–5.
27. van Roy F, Berx G. The cell-cell adhesion molecule E-cadherin. *Cell Mol Life Sci.* 2008;65(23):3756–88.
28. Schuettengruber B, Chourrout D, Vervoort M, et al. Genome regulation by polycomb and trithorax proteins. *Cell.* 2007;128(4):735–45.
29. Baumgart S, Glesel E, Singh G, et al. Restricted heterochromatin formation links NFATc2 repressor activity with growth promotion in pancreatic cancer. *Gastroenterology.* 2012;142(2):388–98.e1–7.
30. Ougolkov AV, Bilim VN, Billadeau DD. Regulation of pancreatic tumor cell proliferation and chemoresistance by the histone methyltransferase enhancer of zeste homologue 2. *Clin Cancer Res.* 2008;14(21):6790–6.
31. Dillhoff M, Liu J, Frankel W, et al. MicroRNA-21 is overexpressed in pancreatic cancer and a potential predictor of survival. *J Gastrointest Surg.* 2008;12(12):2171–6.
32. Moriyama T, Ohuchida K, Mizumoto K, et al. MicroRNA-21 modulates biological functions of pancreatic cancer cells including their

- proliferation, invasion, and chemoresistance. *Mol Cancer Ther.* 2009;8(5):1067–74.
33. Du Y, Liu M, Gao J, et al. Aberrant microRNAs expression patterns in pancreatic cancer and their clinical translation. *Cancer Biother Radiopharm.* 2013;28(5):361–9.
34. Buscaglia LE, Li Y. Apoptosis and the target genes of microRNA-21. *Chin J Cancer.* 2011;30(6):371–80.
35. Ma X, Ezzeldin HH, Diasio RB. Histone deacetylase inhibitors current status and overview of recent clinical trials. *Drugs.* 2009;69(14):1911–34.
36. Chien W, Lee DH, Zheng Y, et al. Growth inhibition of pancreatic cancer cells by histone deacetylase inhibitor belinostat through suppression of multiple pathways including HIF, NFkB, and mTOR signaling in vitro and in vivo. *Mol Carcinog.* 2014;53(9):722–35.
37. Ellis L, Hammers H, Pili R. Targeting tumor angiogenesis with histone deacetylase inhibitors. *Cancer Lett.* 2009;280(2):145–53.
38. Hideshima T. Histone deacetylase inhibitors in multiple myeloma. In: Lonial S, editor. *Myeloma therapy.* Springer: Berlin; 2009. p. 379–92.
39. Qian DZ, Kato Y, Shabbeer S, et al. Targeting tumor angiogenesis with histone deacetylase inhibitors: the hydroxamic acid derivative LBH589. *Clin Cancer Res.* 2006;12(2):634–42.
40. Liu T, Kuljaca S, Tee A, et al. Histone deacetylase inhibitors: multifunctional anticancer agents. *Cancer Treat Rev.* 2006;32(3):157–65.
41. Wang S, Li X, Parra M, et al. Control of endothelial cell proliferation and migration by VEGF signaling to histone deacetylase 7. *Proc. Nat. Acad. Sci.* 2008;105(22):7738–43.
42. Garcia-Morales P, Gomez-Martinez A, Carrato A, et al. Histone deacetylase inhibitors induced caspase independent apoptosis in human pancreatic adenocarcinoma cell lines. *Mol Cancer Ther.* 2005;4(8):1222–30.
43. Mitsiades N, Mitsiades CS, Richardson PG, et al. Molecular sequelae of histone deacetylase inhibition in human malignant B cells. *Blood.* 2003;101(10):4055–62.
44. Kauh J, Fan S, Xia M, et al. C-FLIP degradation mediates sensitization of pancreatic cancer cells to TRAIL-induced apoptosis by the histone deacetylase inhibitor LBH589. *PLoS One.* 2010;5(4):e10376.
45. Natoni F, Diolordi L, Santoni C, et al. Sodium butyrate sensitises human pancreatic cancer cells to both the intrinsic and the extrinsic apoptotic pathways. *Biochim Biophys Acta.* 2005;1745(3):318–29.
46. Arlt A, Muerkoster SS, Schafer H. Targeting apoptosis pathways in pancreatic cancer. *Cancer Lett.* 2013;332(2):346–58.
47. Moore PS, Barbi S, Donadelli M, et al. Gene expression profiling after treatment with the histone deacetylase inhibitor trichostatin A reveals altered expression of both pro- and antiapoptotic genes in pancreatic adenocarcinoma cells. *Biochim Biophys Acta.* 2004;1693(3):167–76.
48. Donadelli M, Costanzo C, Beghelli S, et al. Synergistic inhibition of pancreatic adenocarcinoma cell growth by trichostatin A and gemcitabine. *Biochim Biophys Acta.* 2007;1773(7):1095–106.
49. Newbold A, Lindemann RK, Cluse LA, et al. Characterisation of the novel apoptotic and therapeutic activities of the histone deacetylase inhibitor romidepsin. *Mol Cancer Ther.* 2008;7(5):1066–79.
50. Marks P, Xu WS. Histone deacetylase inhibitors: potential in cancer therapy. *J Cell Biochem.* 2009;107(4):600–8.
51. Dell'Aversana C, Lepore I, Altucci L. HDAC modulation and cell death in the clinic. *Exp Cell Res.* 2012;318(11):1229–44.
52. Ungerstedt J, Sowa Y, Xu WS, et al. Role of thioredoxin in the response of normal and transformed cells to histone deacetylase inhibitors. *Proc Nat Acad Sci USA.* 2005;102(3):673–8.
53. Ungerstedt J, Du Y, Zhang H, et al. In vivo redox state of human thioredoxin and redox shift by the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA). *Free Radical Biol Med.* 2012;53(11):2002–7.
54. Wang B, Wang XB, Chen LY, et al. Belinostat-induced apoptosis and growth inhibition in pancreatic cancer cells involve activation of TAK1-AMPK signaling axis. *Biochem. Biophys. Res. Commun.* 2013;437(1):1–6.
55. Ocker M, Schneider-Stock R. Histone deacetylase inhibitors: signaling towards p21 cip1/waf1. *Int J Biochem Cell Biol.* 2007;39(7–8):1367–74.
56. Ryu JK, Lee WJ, Lee KH, et al. SK-7041, a new histone deacetylase inhibitor, induces G2-M cell cycle arrest and apoptosis in pancreatic cancer cell lines. *Cancer Lett.* 2006;237(1):143–54.
57. Sung V, Richard N, Brady H, et al. Histone deacetylase inhibitor MGCD0103 synergizes with

- gemcitabine in human pancreatic cells. *Cancer Sci.* 2011;102(6):1201–7.
58. Qiao Z, Ren S, Li W, et al. Chidamide, a novel histone deacetylase inhibitor, synergistically enhances gemcitabine cytotoxicity in pancreatic cancer cells. *Biochem. Biophys. Res. Commun.* 2013;434(1):95–101.
59. Wang G, He J, Zhao J, et al. Class I and class II histone deacetylases are potential therapeutic targets for treating pancreatic cancer. *PLoS One.* 2012;7(12):e52095.
60. Arumugam T, Ramachandran V, Fournier KF, et al. Epithelial to mesenchymal transition contributes to drug resistance in pancreatic cancer. *Cancer Res.* 2009;69(14):5820–8.
61. Maier HJ, Wirth T, Beug H. Epithelial–mesenchymal transition in pancreatic carcinoma. *Cancers (Basel).* 2010;2(4):2058–83.
62. Iwatsuki M, Mimori K, Yokobori T, et al. Epithelial–mesenchymal transition in cancer development and its clinical significance. *Cancer Sci.* 2010;101(2):293–9.
63. Aghdassi A, Sendler M, Guenther A, et al. Recruitment of histone deacetylases HDAC1 and HDAC2 by the transcriptional repressor ZEB1 downregulates E-cadherin expression in pancreatic cancer. *Gut.* 2012;61(3):439–48.
64. Jiang JH, Liu C, Cheng H, et al. Epithelial-mesenchymal transition in pancreatic cancer: is it a clinically significant factor? *Biochim Biophys Acta.* 2015;1855(1):43–9.
65. Kumagai T, Wakimoto N, Yin D, et al. Histone deacetylase inhibitor, suberoylanilide hydroxamic acid (Vorinostat, SAHA) profoundly inhibits the growth of human pancreatic cancer cells. *Int J Cancer.* 2007;121(3):656–65.
66. Shapiro GI, Frank R, Dandamudi UB, et al. The effect of food on the bioavailability of panobinostat, an orally active pan-histone deacetylase inhibitor, in patients with advanced cancer. *Cancer Chemother Pharmacol.* 2012;69(2):555–62.
67. Prince HM, George D, Patnaik A, et al. Phase I study of oral LBH589, a novel deacetylase (DAC) inhibitor in advanced solid tumors and non-Hodgkin's lymphoma (abstract no 3500). *J Clin Oncol.* 2007;25(18suppl).
68. Ottmann OG, Spencer A, Prince HM, et al. Phase IA/II study of oral panobinostat (LBH589), a novel pan-deacetylase inhibitor (DACi) demonstrating efficacy in patients with advanced hematologic malignancies (abstract no 958). *Blood.* 2008;112.
69. Konsoula Z, Cao H, Velena A, et al. Pharmacokinetics-pharmacodynamics and antitumor activity of mercaptoacetamide-based histone deacetylase inhibitors. *Mol Cancer Ther.* 2009;8(10):2844–51.
70. Morita S, Oizumi S, Minami H, et al. Phase I dose-escalating study of panobinostat (LBH589) administered intravenously to Japanese patients with advanced solid tumors. *Invest New Drugs.* 2012;30(5):1950–7.
71. Fukutomi A, Hatake K, Matsui K, et al. A phase I study of oral panobinostat (LBH589) in Japanese patients with advanced solid tumors. *Invest New Drugs.* 2012;30(3):1096–106.
72. Garnock-Jones KP. Panobinostat: first global approval. *Drugs.* 2015;75(6):695–704.
73. Hamberg P, Woo MM, Chen LC, et al. Effect of ketoconazole-mediated CYP3A4 inhibition on clinical pharmacokinetics of panobinostat (LBH589), an orally active histone deacetylase inhibitor. *Cancer Chemother Pharmacol.* 2011;68(3):805–13.
74. Slingerland M, Hess D, Clive S, et al. A phase I, open-label, multicenter study to evaluate the pharmacokinetics and safety of oral panobinostat in patients with advanced solid tumors and various degrees of hepatic function. *Cancer Chemother Pharmacol.* 2014;74(5):1089–98.
75. Clive S, Woo MM, Stewart M, et al. Elucidation of the metabolic and elimination pathways of panobinostat (LBH589) using [¹⁴C]-panobinostat (abstract no. 2549). *J Clin Oncol.* 2009;27(15 Suppl).
76. Feld R, Woo MM, Leighl N, et al. A clinical investigation of inhibitory effect of panobinostat on CYP2D6 substrate in patients with advanced cancer. *Cancer Chemother Pharmacol.* 2013;72(4):747–55.
77. Sharma S, Witteveen PO, Lolkema MP, et al. A phase I, open-label, multicenter study to evaluate the pharmacokinetics and safety of oral panobinostat in patients with advanced solid tumors and varying degrees of renal function. *Cancer Chemother Pharmacol.* 2015;75(1):87–95.
78. Giver CR, Jaye DL, Waller EK, et al. Rapid recovery from panobinostat (LBH589) induced thrombocytopenia in mice involves a rebound effect of bone marrow megakaryocytes. *Leukemia.* 2011;25(2):362–5.
79. Subramanian S, Bates SE, Wright JJ, et al. Clinical toxicities of histone deacetylase inhibitors. *Pharmaceuticals.* 2010;3(9):2751–67.

80. Zhang L, Lebowitz D, Masson E, et al. Clinically relevant QTc prolongation is not associated with current dose schedules of LBH589 (panobinostat). *J Clin Oncol*. 2008;26(2):332–9.
81. Kitamura T, Inoue D. HDACi-induced thrombocytopenia is caused by its unexpected target. *Exp Hematol*. 2012;40(9):695–7.
82. Bishton MJ, Prince HM, Harrison SJ, et al. Histone deacetylase inhibitor induced thrombocytopenia occurs due to inhibition platelet shedding by megakaryocytes, via increased phosphorylation of myosin light chain (abstract 2613). *Cancer Res*. 2011;71.
83. Bishton MJ, Gardiner EE, Harrison SJ, et al. Histone deacetylase inhibitors reduce glycoprotein VI expression and platelet responses to collagen related peptide. *Thromb Res*. 2013;131(6):514–20.
84. Atadja PW, Bishton MJ, Harrison SJ, et al. Combination of HDAC inhibitors with thrombocytopenia drugs. 2012;(WO 2012030886 A1).
85. Atadja P. Development of the pan-DAC inhibitor panobinostat (LBH589): successes and challenges. *Cancer Lett*. 2009;280(2):233–41.
86. Singh BN, Zhang G, Hwa YL, et al. Nonhistone protein acetylation as cancer therapy targets. *Expert Rev Anticancer Ther*. 2010;10(6):935–54.
87. Kim HJ, Bae SC. Histone deacetylase inhibitors: molecular mechanisms of action and clinical trials as anti-cancer drugs. *Am J Trans Res*. 2011;3(2):166–79.
88. Prystowsky M, Feeney K, Kawachi N, et al. Inhibition of Plk1 and cyclin B1 expression results in panobinostat-induced G2 delay and mitotic defects. *SciRep*. 2013;3:2640.
89. Bali P, Pranpat M, Bradner J, et al. Inhibition of histone deacetylase 6 acetylates and disrupts the chaperone function of heat shock protein 90: a novel basis for antileukemia activity of histone deacetylase inhibitors. *J Biol Chem*. 2005;280(29):26729–34.
90. Edwards A, Li J, Atadja P, et al. Effect of the histone deacetylase inhibitor LBH589 against epidermal growth factor receptor dependent human lung cancer cells. *Mol Cancer Ther*. 2007;6(9):2515–24.
91. Gandesiri M, Chakilam S, Ivanovska J, et al. DAPK plays an important role in panobinostat-induced autophagy and commits cells to apoptosis under autophagy deficient conditions. *Apoptosis*. 2012;17(12):1300–15.
92. Reddy RM, Yeow WS, Chua A, et al. Rapid and profound potentiation of Apo2L/TRAIL-mediated cytotoxicity and apoptosis in thoracic cancer cells by the histone deacetylase inhibitor Trichostatin A: the essential role of the mitochondria-mediated caspase activation cascade. *Apoptosis*. 2007;12(1):55–71.
93. Fulda S. Modulation of TRAIL-induced apoptosis by HDAC inhibitors. *Curr Cancer Drug Targets*. 2008;8(2):132–40.
94. Meng X, Brachova P, Yang S, et al. Knockdown of MTDH sensitizes endometrial cancer cells to cell death induction by death receptor ligand TRAIL and HDAC inhibitor LBH589 co-treatment. *PLoS One*. 2011;6(6):e20920.
95. Bodo J, Zhao X, Sharma A, et al. The phosphatidylinositol 3-kinases (PI3 K) inhibitor GS-1101 synergistically potentiates histone deacetylase inhibitor-induced proliferation inhibition and apoptosis through the inactivation of PI3K and extracellular signal-regulated kinase pathways. *BrJHaematol*. 2013;163(1):72–80.
96. Yu C, Friday BB, Lai JP, et al. Abrogation of MAPK and Akt signaling by AEE788 synergistically potentiates histone deacetylase inhibitor-induced apoptosis through reactive oxygen species generation. *Clin Cancer Res*. 2007;13(4):1140–8.
97. Shao W, Growney J, Feng Y, et al. Potent anticancer activity of a pan-deacetylase inhibitor panobinostat (LBH589) as a single agent in in vitro and in vivo tumor models (abstract no 735). *Cancer Res*. 2008;68.
98. Lisiero DN, Soto H, Everson RG, et al. The histone deacetylase inhibitor, LBH589, promotes the systemic cytokine and effector responses of adoptively transferred CD8+ T cells. *J Immunother Cancer*. 2014;2:8.
99. Cao K, Wang G, Li W, et al. Histone deacetylase inhibitors prevent activation-induced cell death and promote anti-tumor immunity. *Oncogene*. 2015;. doi:10.1038/ncr.2015.46.
100. Mehdi O, Françoise S, Sofia CL, et al. HDAC gene expression in pancreatic tumor cell lines following treatment with the HDAC inhibitors panobinostat (LBH589) and trichostatin (TSA). *Pancreatol*. 2012;12(2):146–55.
101. Haefner M, Bluethner T, Niederhagen M, et al. Experimental treatment of pancreatic cancer with two novel histone deacetylase inhibitors. *World J Gastroenterol*. 2008;14(23):3681–92.
102. Wang H, Cao Q, Dudek AZ. Phase II study of panobinostat and bortezomib in patients with

-
- pancreatic cancer progressing on gemcitabine-based therapy. *Anticancer Res.* 2012;32(3):1027–31.
103. Venkannagari S, Fiskus W, Peth K, et al. Superior efficacy of co-treatment with dual PI3K/mTOR inhibitor NVP-BEZ235 and pan-histone deacetylase inhibitor against human pancreatic cancer. *Oncotarget.* 2012;3(11):1416–27.
104. Wang G, Niu X, Zhang W, et al. Synergistic antitumor interactions between MK-1775 and panobinostat in preclinical models of pancreatic cancer. *Cancer Lett.* 2015;356(2 Pt B):656–68.
105. Zou Y, Howell GM, Humphrey LE, et al. Ron knockdown and ron monoclonal antibody IMCRON8 sensitize pancreatic cancer to histone deacetylase inhibitors (HDACi). *PLoS One.* 2013;8(7):e69992.
106. Rikiishi H. Possible role of autophagy in the treatment of pancreatic cancer with histone deacetylase inhibitors. *Cancers.* 2010;2:2026–43.