

Use of histone deacetylase inhibitors (HDACis) in pancreatic ductal adenocarcinoma (PDAC): A narrative review

Research Article

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Received 6 October 2024; Accepted 23 December 2024

Abstract: This narrative review paper examines the use and potential benefits of histone deacetylase inhibitors (HDACis) in pancreatic ductal adenocarcinoma (PDAC) as a combinational therapy. The paper provides a concise overview of the epigenetic events and their effects on gene expression in PDAC, followed by a summary of selected preclinical and clinical studies, demonstrating the efficacy of HDACis as combinational treatments. The current therapeutic approach in PDAC includes low-efficacy combination therapies, mainly as a palliative treatment, due to delayed diagnosis, rapid disease progression, and development of metastases. The tumor mutational burden and several epigenetic modifications shape tumor characteristics, regulate the gene expression, and alter the cell cycle. Epigenetic modifications at lysine residues of histones, including acetylation, are mediated by histone acetyltransferases and counteracted by HDACs. HDACis are approved as monotherapy in cutaneous T-cell lymphoma and are potentially effective when administered in combination with other therapies in solid tumors. In particular, combinations of HDACis with tyrosine kinases inhibitors, MEK inhibitors, PI3K inhibitors, 13-cis-retinoic acid, EZH2 inhibitors, and BET inhibitors appear to suppress epithelial–mesenchymal transition, promote apoptosis, and increase overall survival in patients with PDAC.

Keywords: HDACis • genetic transcription • epigenetics • acetylation • PDAC

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive form of cancer associated with poor prognosis and 5-year survival rate of 11% in the USA.^[1] Poor outcomes can be attributed to the characteristics of the disease, but late diagnosis, age, tumor location, patient's response to treatment, and comorbidities may also play a role.^[2] Most patients (80%–90%) are diagnosed with unresectable or metastatic PDAC, experience recurrence or metastases after surgery, and as a result require palliative care.^[3] Chemotherapy regimens in metastatic setting include 5-fluorouracil, irinotecan (or nano-liposomal irinotecan), and oxaliplatin (FOLFIRINOX/NALIRIFOX), gemcitabine/nab-paclitaxel (paclitaxel bound to nanoparticles), or gemcitabine monotherapy.^[4] These treatments are often associated

with high toxicity burden, whereas their benefits in terms of overall survival (OS) are low.^[5] PDAC accounts for more than 90% of pancreatic cancers,^[6] whereas 80%–90% of tumors are unresectable due to late diagnosis.^[7] In the early treatment setting, the combination of gemcitabine/nab-paclitaxel, an Food and Drug Administration (FDA)-approved first-line treatment for PDAC, has shown some clinical benefits.^[8,9] However, unmet medical need remains high because 69%–75% of patients receiving adjuvant therapy experience a recurrence within 2 years.^[10,11] Furthermore, response rates remain low due to resistance to treatment, which means that there is a need to focus on tumor's characteristics.^[12] The use of combinational treatments with histone deacetylase inhibitors (HDACis) is a promising intervention which has the potential to improve clinical outcomes.

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This narrative review discusses the use and potential benefits of HDACis in PDAC as a combination therapy. In addition, this paper presents current research on HDACis as combination therapies. Initially, this paper concisely presents the molecular biology background of HDACis and subsequently, it illustrates the current status of research in this area by summarizing a selection of relevant preclinical and clinical studies along with their methods, results, and implications for their potential in clinical practice.

2. Overview of Molecular Biology

Associations between mutations and histological alterations include activation of the oncogene *KRAS*,^[5] inactivation of the tumor suppressor genes *TP53*,^[13] *SMAD4*,^[14] or *CDKN2A*,^[15] and epigenetic modifications that contribute to disease progression.^[16] In PDAC, for example, in 90% of patients, the tumor suppressor gene *CDKN2A* is inactivated due to hypermethylation of its promoter, resulting in the suppression of inhibitor's p16INK4A expression. This leads to activation of cyclin-dependent kinases (CDK4/6) and phosphorylation/inactivation of the retinoblastoma (RB) protein, which then dissociates from the transcription factor E2F, allowing it to activate the transcription of genes that drive the cell from the G1 phase to the S phase, thereby promoting the continuation of the cell cycle and uncontrollable cellular proliferation.^[17] *KRAS* signaling (rapidly accelerated fibrosarcoma-RAF-methyl-ethyl ketone-MEK–extracellular signal-regulated kinases-ERK–mitogen-activated protein kinase-MAPK pathway) activates the transcription factor CCND1 and the expression of cyclin D1, while simultaneously inactivating the GSK3 β protein and the phosphoinositide-3 kinase-PI3K–Akt strain transforming-AKT signaling pathway, which degrades D1 to keep it stable,^[18] which, in turn, allows CDK4/6 to keep the RB protein phosphorylated/inactivated, thereby perpetuating cellular proliferation.^[19]

Although cancer is primarily considered a disease driven by mutations in oncogenes and tumor suppressor genes, there is also a broader strong mechanism that can lead to malignancy without affecting the DNA sequence itself.^[20] The so-called “epigenetic modifications” can alter the three-dimensional structure of chromatin and thus change the regulation of gene expression. Epigenetic modifications include methylation, phosphorylation, sumoylation, deamination, and acetylation.^[20] The N-terminal tails of histones extend from the nucleosomes and can be post-translationally modified, particularly at lysine residues, through the addition and removal of acetyl groups by histone acetyltransferases (HATs)

and HDACs.^[21] The latter remove acetyl groups from the lysine residues of histones, leading to changes in gene expression, specifically the suppression of gene expression.^[22] The acetylation of histones neutralizes the positive charge of lysine residues and weakens their interactions with negatively charged DNA, resulting in an open chromatin structure that is accessible to the transcriptional machinery.^[20]

The HDAC family consists of 18 isoforms numbered according to their discovery, starting with HDAC1 in 1996, and is classified into four classes based on their similarity to yeast protein homologs.^[20] The Zn²⁺-dependent HDACs are classified into class I (HDAC1/2/3/8), class II (HDAC4/5/6/7/9/10), class IV (HDAC11), and the NAD⁺-dependent class III (sirtuins: SIRT1/2/3/4/5/6/7). HDACis preserve the addition of acetyl groups by various HATs, allowing for continued gene expression.

The subcellular localization of HDACs varies. Class I HDACs are primarily located in the nucleus, class IIa HDACs (4/5/7/9) are found in the cytoplasm and are transported into the nucleus, while class IIb HDACs (6/10) are mainly found in the cytoplasm. Class III HDACs have a general impact on cell growth or apoptosis; for example, SIRT1 deacetylates and reduces the expression of the tumor suppressor p53, thus promoting cellular proliferation. The class IIb isoform HDAC6 regulates the Hsp90 protein, tau protein, and the cytoskeleton through its interactions with tubulin and cortactin.^[23, 24]

HDACis have been investigated as therapeutic agents in various types of cancer and have already been used in solid tumors other than PDAC. In some cases, the effect of HDACis on gene expression increases the representation of immune system targets recognized by either CD8⁺ T cells or natural killer cells, making the tumor more susceptible to immunotherapy.^[25–28] For example, HDACis in glioma cells increase the expression of major histocompatibility complex class I (MHC I) genes, enhancing their recognition by CD8⁺ T cells.^[26] Four HDACis (vorinostat, romidepsin, panobinostat, and belinostat),^[29,30] which target Zn²⁺-dependent class I, II, and IV HDACs, are FDA approved and are used in clinical practice for the treatment of PDAC. The use of HDACis shows high efficacy primarily for hematological malignancies, and specifically, they have proven useful in the treatment of cutaneous T-cell lymphoma (CTCL). Contrary to solid tumors, the unique three-dimensional structure of DNA in CTCL plays a more significant role in tumor development compared to mutations in oncogenes/tumor suppressor genes, making monotherapy with HDACis effective. However, monotherapy with HDACis in solid tumors appears to

be ineffective, thus shifting focus toward their use in combination with other therapeutic strategies.^[20]

3. Epigenetic Modifications in PDAC

1) DNA methylation

DNA methylation is induced by DNA methyltransferases (DNMTs).^[31] There are three isoforms: DNMT3A and DNMT3B, which perform *de novo* methylation, and DNMT1, which is responsible for maintaining the methylation pattern in daughter strands. 5-methylcytosine (5mC) can be demethylated through oxidation to 5-hydroxymethylcytosine (5hmC) by 10,11-dioxygenases in the presence of α -ketoglutarate and mediated by isocitrate dehydrogenase.^[32] In general, the pattern of DNA methylation differs between normal and cancer cells.^[33] The most common abnormal methylation is the conversion of cytosine to 5mC, which involves the addition of a methyl group to the 5' carbon of the cytosine ring and primarily occurs at CpG dinucleotides. Scattered approximately 30,000 times throughout the human genome, CpG islands are located in promoter regions, serve as alternative promoters, and their methylation leads to transcriptional silencing^[34,35] due to the recruitment of additional proteins to the methylated-CpG region (methyl-CpG binding domain proteins) and the obstruction of transcriptional machinery binding to the DNA sequence.^[36]

The DNA methylation profile differs among different subtypes of PDAC.^[37] For example, methylation of the promoter of the tumor suppressor gene *ISL2* is associated with poor outcomes and its silencing *in vitro* leads to increased oxidative phosphorylation as a source of cellular energy.^[38] In addition to methylation, the removal of methyl groups from DNA, known as “demethylation,” and the subsequent overexpression of genes (e.g., *PSCA* and *S100A4*) promote tumor progression.^[39] Compared to the classical subtype, the more aggressive squamous/basal subtype of PDAC is characterized by the hypomethylation of repetitive elements and the activation of interferon signaling, which is associated with poor outcomes. The presence or absence of 5hmC impacts gene transcription for the different subtypes,^[40] with its loss leading to squamous/basal PDAC, while its presence restores the phenotype to the classical subtype, which exhibits greater sensitivity to treatment.^[5] PDAC cells constantly interact with the tumor microenvironment (TME) for their growth, tumor progression, and metastasis formation, altering their transcriptional program in response to epigenetic modifications.^[5] Direct contact between PDAC cells and TME fibroblasts (cancer-associated fibroblasts)

induces methylation of suppressor of cytokine- signaling 1 (SOCS1), leading to downstream activation of STAT3 and increased expression of insulin-like growth factor 1.^[41] The altered DNA methylation shapes the phenotypic characteristics of the tumor-associated macrophages.^[42] Studies in macrophage cell lines have linked DNMT1-mediated suppression of SOCS1 or Krüppel-like factor-KLF4 expression with the presence of M1 macrophages.^[43] Moreover, PDAC cells have the ability to differentiate M1 macrophages through DNA methylation and transcriptional downregulation, leading to impaired glucose metabolism and their transformation into M2 macrophages.^[42] This is a critical point in the change of metastatic potential, as demonstrated by *in vivo* models of PDAC that M1 macrophages, unlike M2 macrophages, require DNA methylation for tumor expansion and metastasis.

2) Histone modifications

As for PDAC, HATs act either suppressively or simulatively depending on their action sites, while the role of HDACs is primarily suppressive.^[44] The activity of HDACs is associated with poor outcomes in patients with PDAC.^[45] For example, the suppression of genes encoding proapoptotic proteins, such as NOXA and Nur77, contributes to enhanced cellular proliferation.^[46] This promotes the survival of cancer cells and prevents apoptosis. The acetylated lysine residues are recognized by proteins of the bromodomain and extraterminal domain-BET family (BRD2, BRD3, BRD4, BRDT).^[47] Their binding leads to the formation of super enhancer protein complexes, interaction with the positive transcription elongation factor (P-TEFb), and increased gene expression.^[48] For example, it has been reported that dysregulation of BET proteins leads to carcinogenesis through increased expression of the *c-myc* gene.^[49]

In contrast to the addition of acetyl groups, methyl groups can be added or removed from the lysine residues of histone tails by histone methyltransferases/demethylases (HMTs/HDMs).^[21] The effect of histone lysine methylation on gene expression depends on the specific location of the residue.^[50] For example, trimethylation of lysine 4 on histone 3 (H3K4me3) is generally associated with gene activation, while trimethylation of lysine 27 (H3K27me3) is associated with the repression of gene expression. Aberrant methylation of histones leads to dysregulation of cellular proliferation, dysfunction of the immune response against tumors, and alteration of metabolic pathways in cancer cells.^[51] Increased H3K27me3 and decreased H3K4me3 in genes that determine cellular identity in acinar pancreatic cells lead from dysplasia

to ductal adenocarcinoma, promoting the development of PDAC.^[52] As demonstrated in an *in vivo* PDAC model, the silencing of KDM6A, an HDM that mediates H3K27me3 modification, resulted in an aggressive squamous metastatic phenotype of PDAC. Analysis of pancreatic tissues revealed activated histone H3 lysine 27 acetylation (H3K27ac) enhancers that regulate the transcriptional levels of $\Delta Np63$, *c-myc*, and *RUNX3*.^[53]

Another example is enhancer of Zeste-2-EZH2, the catalytic component of the polycomb repressive complex 2 (PRC2), which mediates H3K27me3 modification.^[54] This is overexpressed in PDAC cells, and its silencing can reduce cell viability by 68%.^[55] Clinical and preclinical studies regarding PDAC have shown an inverse relationship between EZH2 expression and E-cadherin,^[56] with high EZH2 expression being associated with advanced stage and lymph node metastasis,^[57] while the expression of E-cadherin, encoded by the *CDH1* gene, occurs due to the interaction of complexes composed of HDACs and transcription factors (ZEB1, Snail)^[58] and they are suppressive. Conversely, the transcription factors FOXA1 and FOXA2 activate E-cadherin expression,^[59] and therefore, reduction of their expression is associated with induction of epithelial–mesenchymal transition (EMT) and cancer progression, as shown in *in vivo* and *in vitro* models of PDAC. In parallel, ChIP-seq, RNA-seq, and ATAC-seq analyses showed that FOXA1 increases H3K27ac at certain genomic regions in PDAC cells, which activates the expression of foregut growth genes, thus promoting cell growth *in vitro* and metastasis *in vivo*.^[60]

4. HDACis in preclinical and clinical research

The first FDA-approved HDACi for patients with T-lymphoma was vorinostat (suberoylanilide hydroxamic acid [SAHA]), a broad-spectrum HDACi that acts against HDAC1–HDAC11.^[30] However, clinical studies have generated disappointing outcomes in most solid cancers, including PDAC.^[61] Domatinostat, a preclinical class I HDACi, affects the oxidative metabolism of cancer stem cells (CSCs) by reducing the expression levels of the transcription factor FOXM1, which is overexpressed in cancer cells, leading to increased recruitment of β -catenin and Oct-4 complex, located in the Wnt promoter, and contributes to the CSC phenotype, which is resistant to chemotherapy.^[62]

Another HDACi is KH16,^[30] a hydroxamic acid derivative, which has the ability to attack only cancer cells and not healthy cells and causes NOXA protein to

overaccumulate in PDAC cell lines, causing apoptosis.

Further research has been conducted to assess the mechanisms by which HDACis may lead to improved outcomes. A recent study^[63] investigated the effect of acetylation on the expression and localization of PD-L1 encoded by the *CD274* gene, and its presence on the cell membrane suppresses the immune system attack due to its binding to the T-cell PD-1 receptor and downstream activation of Src phosphatases in T lymphocytes.^[64] In addition, PD-L1 also localizes to the cytoplasm, translocates to the nucleus, and can act as a transcription factor inducing the transcription of genes that further suppress immune function, such as *PD-L2*.^[65] PD-L1 can be acetylated at Lys 263 by p300 and deacetylated by HDAC2. This modification affects its import into the nucleus and transcription. Furthermore, the aforementioned study^[63] investigated how valproic acid-VPA, a class I/IIa HDACi that acts specifically on HDAC2, affects PD-L1 expression and localization, and by extension would improve immunotherapy, and whether and how JQ1, a BRD4 inhibitor that recognizes acetylated lysine (BETi) residues, acts synergistically with VPA. The results showed that after 24 h, VPA increased the acetylation of PD-L1 and its expression in the cell membrane more compared to the use of phenylbutyrate-PBA, a nonspecific HDACi. JQ1 counteracted BRD4 binding and RNA polymerase II recruitment, resulting in a decrease in VPA-induced PD-L1 mRNA and protein levels, thus counteracting its effect on increasing PD-L1 surface expression. Nuclear PD-L1 levels, as confirmed by stable PD-L2 target gene expression levels, were not affected by VPA.^[63]

Another recent study^[66] showed that MARK2 modulates chemotherapy response through the HDAC class IIa–YAP axis in pancreatic cancer. MARK2 is phosphorylated by CDK1 in response to chemotherapy and subsequently phosphorylates HDAC4 directly and mainly by anti-tubulin chemotherapies. Phosphorylated HDAC4 enables transcription of the YAP transcription factor, while paclitaxel administration induces the expression of YAP target genes. In organelle cultures and *in vivo* preclinical models of PDAC, combined administration of paclitaxel and an HDAC4i overcomes chemoresistance of PDAC cells. Thus, the MARK2–HDAC4 axis could be a therapeutic target for overcoming PDAC chemoresistance.^[66]

The study by Kuo et al.^[67] showed that the expression of HDACs increases after the silencing of ARID1A, a subunit of the adenosine triphosphate-dependent mammalian SWI/SNF-BAF complex involved in DNA remodeling – an increase that leads to the suppression of gene expression.^[67] The absence of ARID1A in the Langerhans islet cells of the pancreas, acinar, and

ductal cells resulted in a change in insulin production and caused an age-related diabetic phenotype in the experimental animals.^[67] More specifically, there was a reduction in the number of insulin- and glucagon-secreting cells, alterations in lipid metabolism, and increased levels of total bilirubin and triglycerides, with mice over 20 weeks diagnosed with diabetic retinopathy. In cells with a diabetic phenotype induced by ARID1A silencing, increased expression of MHC-I and MHC-II molecules and interleukin-1 β was found.^[68] As a result, the silencing of ARID1A causes dysfunction in β -cells, inflammation, and an immune attack on pancreatic tissues, events that account for the pathological alterations observed in the pancreas of individuals with diabetes. Silencing of ARID1A increases the expression of HDACs 4, 5, and 6 and decreases the expression of HDAC1, as demonstrated by analysis of Langerhans islet cells, resulting in increased abundance of HDAC6 and changes in the expression of 136 genes.^[67]

Studies have also shown that the HDACi M344, a benzamide derivative, is effective as it reduces the proliferation, survival, and migratory abilities of PDAC cancer cells.^[22] Specifically, this inhibitor interrupts the cell cycle at the G1 phase during the first 24 hours of treatment, highlighting that its effect occurs at the early stages of the cell cycle, having a significant impact on the survival of cancer cells.^[22]

Sixto-López et al.^[69] reported that the HDAC6 inhibitor they developed, FH27, reduced cell viability in the HepG2, MCF-7, and MIA PaCa-2 cell lines. In a previous study, it was shown through blind molecular docking that FH27 inhibits HDAC6 more than HDAC1 and HDAC8.^[70,71] Molecular modeling demonstrated that FH27 is positioned in the catalytic site and interacts with Zn²⁺ and residues in the inner cavity, leaving the cover region of the catalytic center DD2-HDAC6 free. This allows bulky groups, such as bulky naphthyl, electron donating groups (-OH), or electron accepting groups (-F, -I, -NO₂), bind to it.^[70,71] Finally, molecular modeling studies report that the DD2-HDAC6 region (the catalytic region 2 of HDAC6) has structural differences in the cover region that make it more flexible compared to the class I HDAC isoforms.^[72,73] It also possesses a larger and shallower region, whose structural data is supported by X-ray crystallography studies. Therefore, it has been proposed that the use of bulky and aromatic compounds would increase the affinity/effectiveness of targeting molecules for HDAC6.^[74,75] Table 1 summarizes the clinical studies evaluating the effectiveness of HDACi therapies in solid tumors and lymphomas.

5. Discussion

The literature shows that epigenetic modifications and regulation of the cell cycle actively participate in the development of PDAC and in the migratory ability of the cells. In other words, the metastatic potential can be influenced by the different gene expression of transcription factors, the regulation of which is largely controlled by epigenetic modifications.^[5] Therefore, it is not surprising that treatment strategies targeting or involving epigenetic regulation are at the core of scientific research.

CDK inhibitors such as CDK4/6 (palbociclib, abemaciclib, and ribociclib) have been approved and used for ER-positive/HER2-negative breast cancers in combination with hormone therapies. Trilaciclib is a myeloprotective agent that is combined with chemotherapy in small cell lung cancer, and lerociclib is under clinical investigation. Palbociclib, ribociclib, and lerociclib target both CDKs (CDK4/6), while abemaciclib simultaneously targets CDK9. Despite the encouraging results generated by preclinical studies of CDK4/6 inhibitors in combination with *KRAS* mutation-specific inhibitors for the treatment of PDAC, evidence from clinical research shows the opposite.^[19] In the Phase II trial (NCT02981342) of abemaciclib monotherapy versus combination therapy with a PI3K inhibitor, no improvement in OS was found. Similarly, in the Phase I study (NCT02985125) which assessed ribociclib in combination with a mammalian target of rapamycin-mTOR inhibitor (everolimus), no significant clinical response was observed.^[19] Hence, there is unmet medical need, necessitating some level of effectiveness form approved therapies.

Other combinations have shown a more promising profile. The combination of JQ1 (BET inhibitor) and vorinostat (HDACi) suppressed tumor growth in *in vivo* models of PDAC.^[76] In pancreatic cell lines, HDACis in combination with other therapeutic agents such as gemcitabine^[77] and proteasome inhibitors^[78] have shown promising anticancer results. In an *in vivo* metastatic model of PDAC, the application of HDACis reduced the immunosuppressive ability of granulocyte myeloid-derived suppressor cells (G-MDSCs) present in TME, leading to sensitization to treatment with immune checkpoint inhibitors.^[79] For this purpose, a Phase II clinical trial (NCT03250273) is being conducted with the aim to determine the effectiveness of entinostat (HDACi) in combination with the PD-1 inhibitor nivolumab in patients with unresectable metastatic PDAC.

Table 1: Clinical studies evaluating the efficacy of HDACis in PDAC.

Drug type	Combination	Comparison	Study phase	Cancer type	Results	Reference
Azacitidine (DNA methyl-transferase inhibitor-DMNTi) and/or romidepsin (HDACi)	nab-Paclitaxel/ gemcitabine	-	I (a)	Advanced PDAC	Not available	NCT04257448
Ivaltinostat iv (HDACi)	Capecitabine	Capecitabine monotherapy	I/II	Metastatic PDAC	Not available	NCT05249101
Belinostat iv (HDACi)	Carboplatin Paclitaxel	-	I (c)	Solid tumors	PDAC: 3 pts: PR $n = 1$	⁹²
Tacedinaline po (HDACi)	Gemcitabine	Gemcitabine (1:1)	II (c)	PDAC (unresectable/ metastatic)	174 pts: ORR 12% versus 14%, OS HR 0.98, median OS 6.5 not available versus 7.1 months	NCT00004861
Vorinostat po (HDACi)	Capecitabine Radiotherapy	-	I (c)	Non-metastatic PDAC	21 pts: median OS 13.2 months	NCT00983268
Vorinostat po (HDACi)	Marizomib	-	I (c)	PDAC NSCLC Melanoma	PDAC: 2 pts	NCT00667082
Panobinostat po (HDACi)	Bortezomib	-	II (c)	Metastatic PDAC	7 pts: PD $n = 7$, median PFS 0.86 not available, median OS 4.01 months	NCT01056601
Vorinostat po (HDACi)	Bortezomib	-	I (c)	Solid tumors	PDAC: 6 pts	NCT00227513
Valproic acid po (HDACi)	S-1	-	I/II (c)	PDAC (unresectable/ metastatic) biliary tract cancer (BTC) - Radiation	PDAC: 7 pts	
Mocetinostat po (HDACi)	Gemcitabine	-	I/II (c)	Solid tumors	PDAC: 13 pts (Ph II): SD $n = 9$, median PFS 5.3 not available, median OS 7.4 months	NCT00372437
Resminostat po (HDACi)	S-1	-	I (c)	PDAC (non-resectable/ metastatic) BTC	PDAC: 7 pts; 3 pts (regimen 3): SD $n = 2$, median PFS 2.3 months, OS 4.7 months	⁹³
Vorinostat	Radiation therapy-XRT and 5-fluoruracil-5-FU	-	I/II	Advanced PDAC	PDAC: 10 pts; median PFS 9.3 months	NCT00948688
Vorinostat po (HDACi)	Gemcitabine Sorafenib Radiotherapy	-	I (a)	PDAC (resectable, marginally resectable, unresectable)	22 pts	NCT02349867
Romidepsin iv (HDACi)	Gemcitabine	-	I (c)	PDAC (unresectable/ metastatic) Others solid tumors	14 pts; SD $n = 9$, PD $n = 3$; 85% $\geq$ G3 AE	NCT00379639
Romidepsin iv (HDACi)	-	-	I (a)	Solid tumors/lymphomas	PDAC/BTC: 5 pts	NCT01638533
MS-275 (HDACi)	-	-	I (c)	Solid tumors/lymphomas	Not available	NCT00020579
Entinostat po	GSK525762C	-	I	Resistant solid tumors/ lymphomas	Not available	NCT03925428
Entinostat po	ZEN003694	-	I/II	Resistant solid tumors/ lymphomas	Not available	NCT05053971
Entinostat po	FOLFOX	-	I	PDAC	Not available	NCT03760614
Entinostat po (HDACi)	Nivolumab	-	II (c)	PDAC (non-resectable/ metastatic) BTC	PDAC: 18 pts: CR/PR $n = 3$, median OS 3.9 months; 63% $\geq$ G3 AE	NCT03250273
Isotretinoin po	Belinostat (HDACi)	-	I (c)	Solid tumors	PDAC: 3 pts: CR/PR/ SD $n = 0$	NCT00334789
SMMART-prime treatment	-	-	I (a)	Breast cancer (IV), lymphomas, advanced/ metastatic/unresectable PDAC, prostate cancer	Not available	NCT03878524

a, active (recruiting); AE, adverse event; c, completed; CR, complete response; DCR, disease control rate (CR + PR + SD); G3, Grade 3 adverse event; GI, gastrointestinal; HCC, hepatocellular carcinoma; HDACi, histone deacetylase inhibitor; iv, intravenous; NSCLC, non-small cell lung cancer; OBS, observation; ORR, overall response rate (CR + PR); OS, overall survival; PD, progression disease; PDAC, pancreatic ductal adenocarcinoma; PFS, progression-free survival; po, per os (oral administration); PR, partial response; pts, patients; r, recruiting; SD, stable disease.

The combination of HDACis with tyrosine kinases inhibitors (TKIs) has also been investigated in a Phase I/II clinical trial, which examined the combination of the HDACi vorinostat and the TKI sorafenib with gemcitabine and radiotherapy.^[80] This study evaluated the safety and preliminary efficacy of a novel treatment regimen for pancreatic cancer that combined neoadjuvant chemotherapy with gemcitabine, sorafenib, and vorinostat. The trial aimed to assess the tolerability and optimal dosing of this combination in patients with resectable or borderline resectable pancreatic cancer, focusing on its safety profile and adverse events. Results indicated that the treatment was feasible, with some patients experiencing significant tumor shrinkage or achieving a pathological complete response, suggesting potential improvements in surgical outcomes.^[80] However, various manageable side effects were observed, including hematological and gastrointestinal toxicities. Overall, the findings supported further investigation into this treatment strategy to refine protocols and confirm its effectiveness in larger trials.^[80]

Moreover, findings from a recent study in hepatocellular carcinoma suggest that resminostat (an HDACi) in combination with sorafenib inhibits the progression of platelet-induced cancer, possibly through the reduction of CD44 expression, suppression of EMT, and MEK/ERK signaling.^[81] In fact, the combination of HDACis with MEK and PI3K inhibitors, which are downstream effectors of *KRAS*, enhanced apoptosis and reduced metastasis, treatment resistance, and cellular proliferation of PDAC cells.^[82,83]

Another interesting development is the approval of the first-generation retinoid all-trans retinoic acid (ATRA or tretinoin) for the treatment of acute promyelocytic leukemia (APL), which increased the effectiveness of the international non-proprietary names-INN – decitabine in patients with acute myeloid leukemia and APL without causing further side effects.^[84] ATRA alters the chromatin configuration^[85, 86] and acts synergistically with INN – decitabine^[85] and HDACis.^[87] The combination of belinostat (an HDACi) with 13-cis-retinoic acid (isotretinoin, a prodrug of ATRA) showed promising results in patients with advanced stage cancers, including three patients with PDAC.^[88] These results are encouraging and generated the need for further evaluation of its effectiveness in PDAC. Considering preclinical studies in PDAC, the anticancer activity of zebularine was enhanced when combined with SAHA in PDAC cell lines; however, this effect was not the same in xenograft models.^[89] However, significant tumor suppression was observed when BET inhibitors were combined with HDACis, HAT inhibitors, or EZH2 inhibitors in both *in vitro* and *in vivo* PDAC models. This evidence suggests that the use of HDACis in combinational therapeutic strategies is worth exploring as a means of enhancing the effectiveness of currently used treatments in an attempt to address unmet medical needs in patients with PDAC.^[90, 91]

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