Review Article

Targeting the epigenome of pancreatic cancer for therapy: challenges and opportunities

Marina Baretti[1], Nita Ahuja[2], Nilofer Saba Azad[1]

[1]Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins Hospital, Baltimore, Maryland, USA; [2]Department of Surgery; Yale-New Haven Health, Yale University, School of Medicine, New Haven, Connecticut, USA

Contributions: (I) Conception and design: All authors; (II) Administrative support: All authors; (III) Provision of study materials or patients: All authors; (IV) Collection and assembly of data: All authors; (V) Data analysis and interpretation: All authors; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Nilofer Saba Azad. Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, 1650 Orleans Street, CRB1, Room 4M10, Baltimore, Maryland 21287, USA. Email: nazad2@jhmi.edu.

Abstract: In 2018, there was an estimated 55,440 new cases of pancreatic adenocarcinoma (PDAC) in the United States. Generally, most new cases are in an advanced stage, usually due to the lack of symptoms during the early stages of the disease. Currently, the vast majority of therapeutic regimens have shown only modest effects in this setting, and median survival ranges from 6 to 11 months. Indeed, better therapies for these patients are urgently needed. Epigenetics refers to the somatically heritable differences in gene expression not attributable to intrinsic alterations in the primary sequence of DNA. Core elements of the epigenetic regulation of gene expression include how DNA is packaged around nucleosomes, how chromatin and nucleosomes are modified by a complex series of enzymes and their subsequent interactions with proteins that recognize these modifications. The recognition of the essential role of epigenetic alterations in the development and progression of PDAC has revolutionized our knowledge of this disease and has immediate translational implications for targeting epigenetic abnormalities in PDAC for therapeutic purposes. Moreover, recent work with epigenetic modulatory drugs (EMDs) has shown that these agents may be capable of altering the immunogenicity of the tumor microenvironment (TME), to reverse immune suppression and to 'prime' tumors for immunotherapy. This review summarizes the current knowledge of epigenetic alterations in PDAC with a focus on the translational application of targeting epigenetic-based events as new therapeutic approach for this disease.

Keywords: Pancreatic adenocarcinoma (PDAC); epigenetics; chromatin dynamics; noncoding RNAs; therapeutics

Received: 10 April 2019; Accepted: 26 September 2019; Published: 23 October 2019.

doi: 10.21037/apc.2019.10.01

View this article at: http://dx.doi.org/10.21037/apc.2019.10.01

Introduction

Pancreatic adenocarcinoma (PDAC) is currently the fourth leading cause of cancer death and is expected to climb to the second leading cause of cancer mortality by 2030, with the highest incidence-to-mortality ratios of any histology (1). At diagnosis, only 20% of patients are surgical candidates with any chance of cure; however, even with adjuvant therapy, the majority will recur and the median survival is under 2 years in this group, despite recent progress (2). Most patients have advanced disease at diagnosis, with a dismal overall prognosis that has remained virtually unchanged for many decades. Patients refractory to first-line therapy have 7% expected 5-year survival (3) and limited therapeutic options (4). Therefore, there is a great need for novel and more effective treatment strategies in PDAC.

The term epigenetics was first used by Waddington in 1942, was defined as "any heritable trait not involving the DNA sequence that influences the phenotype of a developing organism, providing a rapid and dynamic

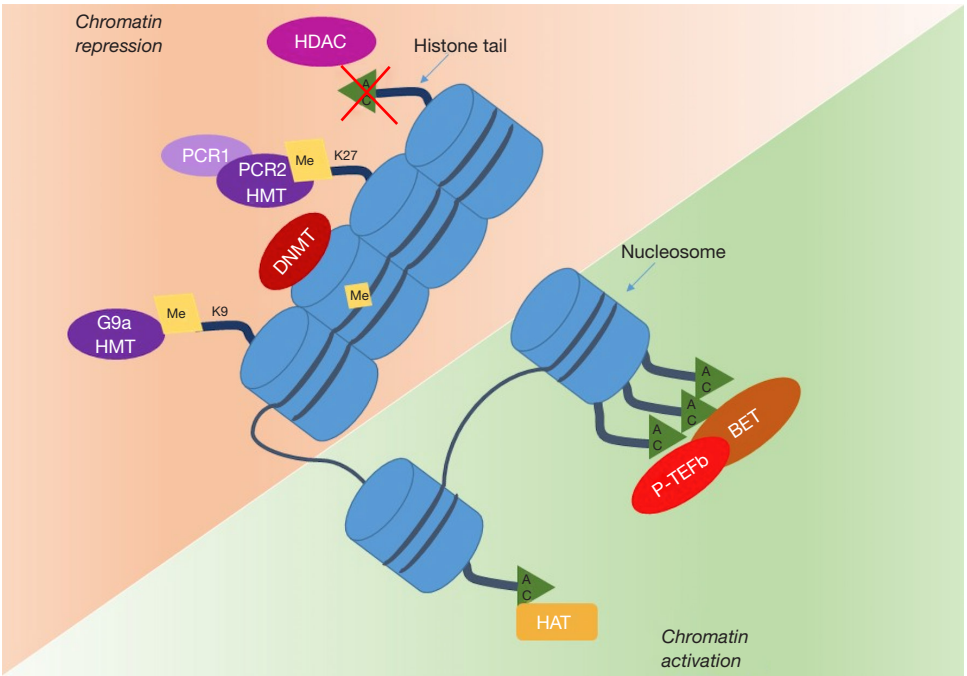

Figure 1 Schematic overview of epigenetic mechanisms. The upper left side of the figure depicts the mechanisms leading to heterochromatin (the repressive status of chromatin) and transcriptional repression, including DNA methylation, histone deacetylation, and histone methylation. The bottom and right side of the figure depicts epigenetic post-translational histone acetylation associated with chromatin activation. Acetylated lysines are subsequently recognized by BET proteins, which further recruit P-TEFb complex, thus initiating gene transcription. For interpretation of figure, the reader is referred to the body of the article. AC, acetylation; BET, bromodomain and Extra-Terminal motif; DNMT, DNA methyltransferase; HAT, histone acetyltransferase; HDAC, histone deacetylase; HMT, histone methyltransferase; K, lysine; Me, methylation; PCR1, polycomb repressive complex 1; PCR2, polycomb repressive complex 2; p-TEFb, positive transcription elongation factor.

response to environmental changes"(5). Epigenetic refers to the somatically heritable differences in gene expression not attributable to intrinsic alterations in the primary sequence of DNA (6,7) (*Figure 1*) but to specific covalent modifications of chromatin components—which include DNA, RNA and proteins (e.g., histones). The vast majority of human cancers harbour both genetic and epigenetic abnormalities, with a fascinating interplay between the two. At present, the most studied epigenetic alterations associated with neoplastic phenotype include DNA methylation, histone modifications, and gene regulation by non-coding RNAs (8-11). In cancer, these epigenetic pathways can lead to silencing of important tumor suppressor genes or cell cycle checkpoints as well as hyperactivation of oncogenes and growth signaling pathways (12,13), contributing to cancer development and propagation (14-16).

Pathogenesis of PDAC has been most studied in the context of DNA mutations, suggesting potential therapeutic approaches targeting the molecular pathways disrupted by these mutations (17,18). However, genetic-based drivers of PDAC do not account for all of the phenotypic and molecular alterations. The identification of aberrant activated epigenetic pathways seen in early PanIN lesions through the development of PDAC strongly suggest that PDAC initiation and progression is the result of epigenetic changes that occurs in parallel to genetic ones, widening the window for therapeutic opportunity in PDAC (19-22).

In this review, we will discuss the epigenetic aberrations in PDAC and will review translational significance for the treatment of PDAC patients, discussing existing challenges and emerging strategies to overcome them.

DNA methylation

DNA methylation commonly occurs on extended regions of cytosine-guanine dinucleotides-called CpG islands- in

the promoter regulatory regions of genes, resulting in the addition of a methyl group to the number 5 carbon of the cytosine pyrimidine ring to form 5-methylcytosine (6,23). Unmethylated CpG islands leads to an open chromatin state that allows gene transcription. The addition of the methyl group induces transcriptional silencing by interfering with transcription factors' binding and recruiting methyl-CpG-binding domain proteins to initiate chromatin compaction (23,24). DNA methylation is catalyzed by a family of enzymes called DNA methyltransferases (DNMTs), responsible for maintenance and addition of methylation patterns, which can be either inherited or *de novo* modifications. DNMT1 functions mainly to maintain methylation patterns from the parent strand of DNA to the newly synthesized strand; DNMT3a and DNMT3b are responsible for *de novo* methylation (24,25). The ten eleven translocation (TET) proteins have been shown to mediate the conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian cells: this modification can be crucial as certain proteins, including DNMT1 do not recognize the 5-hydroxymethylcytosines, resulting in a loss of maintenance of methylation patterns (22).

Global demethylation may increase genomic instability and mutation rate (26); inappropriate methylation of the promoter region of genes can result in inactivation and silencing of genes critical for cell proliferation, DNA damage repair and apoptosis. Both process favor ultimately tumorigenesis (27).

DNMTs are over-expressed in about 80% of PDAC (28) and many promoters of tumor-suppressor genes are hypermethylated in PDAC (29-31). One example is the tumor-suppressor gene p16. The p16 protein inhibits the binding of the D-family cyclins to their cyclin-dependent kinase (CDK) partners, and its loss results in increased phosphorylation of retinoblastoma protein and subsequent progression through G1 phase into S phase of the cell cycle (32). Greater than 95% of PDACs have a loss of p16, with methylation of its promoter being one of the most common mechanisms of inactivation (32).

The use of genome-wide methylation analysis has allowed the identification of other genes affected by aberrant methylation in PDAC *vs.* normal pancreas (33), including genes involved in stem cell pluripotency (BM11, BMP3), WNT signalling (WNT5A, APC2, SOX1), cell adhesions (CDH2, CDH4) (34-36) and in the axonal guidance signalling pathway (SLIT/ROBO), which has been related with tumor neoangiogenesis (37). Additionally, the hypermethylation of the promoter regions of mismatch repair genes (hMLHI), growth inhibitory genes (ARHI), cell cycle control genes (cyclin D2), and proapoptotic genes (TNFRSF10c37, TMS138, and CRABP239) have also been reported in PDAC.

Yet it is important to note that in PDAC the loss of methylation of a normally silenced promoter is important as well. An example is VAV1, the gene encoding the hematopoietic-specific guanine nucleotide exchange factor: its promoter is demethylated in PDACs, leading to the activation of KRAS pathway expression and favoring cellular proliferation (38). Although there is an overall good degree of overlap in genome-wide studies about the set of genes found to be differently methylated in PDAC, some discrepancy exists (39-41). This variance could be explained by the different samples used (cell lines versus human tumor samples) as well as by analysis on varied platforms and how comprehensive the methylation analysis is. Nonetheless, these studies have been crucial to understand the significance of DNA methylation aberrations in driving PDAC, contributing to PDAC development and progression as well as to chemotherapy resistance.

Therapeutic significance of epigenetic alterations

DNMTs have been used as novel cancer therapeutic targets mostly due to their robust responses to inhibitors, credited to the intrinsic reversible nature of the methylation marks. The pyrimidine analogs, 5-azacytidine (AZA) and its deoxy derivative, 5-aza-2'-deoxycytidine (decitabine- DAC), are the most widely used DNMT inhibitors. When used at higher doses, these agents are also cytotoxic, owing to their direct incorporation into both DNA and RNA (AZA) or DNA only (DAC) (42,43). This strategy has already been shown to be effective in hematologic malignancies and AZA and DAC, have been FDA approved for the treatment of myelodysplastic syndromes and acute myeloid leukemia (44,45).

These DNA demethylating agents have also been used in preclinical model of PDAC. Kumari *et al*. showed that treatment of PDAC cell lines with AZA results in the reduction of telomerase activity, a key component of cellular immortality, and re-expression of the myeloid/lymphoid or mixed-lineage leukemia 3 (MLL3) gene, a tumor suppressor gene, usually downregulated in PDAC cell lines, tumor xenografts and archived patient tumors (46). A synergistic effect of AZA with either gemcitabine or docetaxel was also reported (47).

DAC treatment results in global DNA demethylation, tumor suppressor genes re-expression, significant growth inhibition and apoptosis of PDAC cell lines (48). Maitra and colleagues showed that while hypermethylation of the promoter regions of CRABP2 was associated with retinoic acid resistance in PDAC cell lines, treatment of these cells with DAC induced CRABP2 re-expression, increased apoptosis and restaured sensitivity to retinoic acid (49). Pan *et al*. showed a synergistic effect of emodin in combination with DAC in growth inhibition of Panc-1 cells, associated with enhanced demethylation of tumor-suppressor genes, such as p16, RASSF1A (50). Pretreatment with DAC increased the sensitivity of these cells to other chemotherapy agents, including gemcitabine and MEK inhibitors (51) (52). Recently, our group reported the chemosensitization effect of guadecitabine, a DAC prodrug with a favorable pharmacokinetic profile, in PDAC models. In an *in vitro* study, we showed that nanomolar doses of guadecitabine significantly improved the effects of irinotecan on decreasing cell viability, including in Panc1 model, which is usually non-responsive to either agent alone. From a mechanistic point of view, our results showed that guadecitabine functioned as a DNMTi with a `memory effect' observed after 5-days of rest, followed by increased caspase 3/7 and LDH activity, thus suggesting that the optimal time for chemotherapy administration could be 5 days post-guadecitabine treatment (53).

The evidence of methylome dysregulation of PDAC has served as foundation for the development of clinical trials employing DNMTs inhibitors alone or in combination with chemotherapy or radiation as treatment for PDAC, and two phase 1 trials of AZA and nab-paclitaxel (NCT01845805) or gemcitabine (NCT01167816) are now ongoing in the metastatic setting. An orally active formulation of AZA, CC-486, has been developed providing a much more desirable route of administration as compared to the subcutaneous delivery of AZA. In the phase I study in patients with myelodysplastic syndromes, chronic myelogenous leukemia or refractory acute myeloid leukemia, CC-486 has proved to be bioavailable, well tolerated with similar response as the subcutaneous version. An investigator initiated, randomized phase II trial, of CC-486 in high risk, resected PDAC patients in now ongoing, with the main goal to decrease recurrence and/or improve response to chemotherapy at the time of recurrence (NCT01845805).

A study by Nagaraju *et al*. investigated the effect of curcumin analogues, EF31 and UBS109, as DNMT1 inhibitors in PDAC cells lines. Treatment resulted in re-expression of CDKN2A and p16 and a significant tumor growth inhibition, and increased the sensitivity to the combination of 5FU and oxaliplatin. However, the first-in-patient study assessing the feasibility of curcumin in combination with gemcitabine in advanced PDAC patients was associated with dose-limiting GI toxicity (54). In an attempt to reduce the toxicity, a nanoparticle-based curcumin (Theracurmin) has also been developed (55). In a phase I/II study evaluated the combination with gemcitabine in gemcitabine-resistant patients, although no increased toxicity from curcumin was reported, no responses were observed (55). Dhillon *et al*. conducted a subsequent monotherapy trial with curcumin in patients with advanced, pretreated PDAC. In this phase II trial, 25 patients received 8 g curcumin daily, which was well tolerated and showed biological activity with stable disease in one patient lasting over 18 months, and a brief but marked, tumor regression in another (56).

Important caveats related to DNMTs inhibitors should be acknowledged. First, DNA methylation is essential for a number of significant physiological pathways and global methylome dysregulation by these drugs may result in important toxicities, underscoring the impellent need to optimize their use. Second, hypomethylating drugs are S phase-dependent and both AZA and DAC have short half-lives, and therefore have low incorporation into DNA in many solid tumors (57-60). Finally, even in myeloid malignancies, primary and secondary resistance to these therapies are common and these drugs are most active when used as frontline therapy, a strategy that has never been investigated in PDAC (58,61).

Post-translational histone modifications

Histone modifications are cardinal component of the regulation of gene expression and constitute a dynamic process that is usually carried out by pairs of enzymes with reverse catalytic functions, essential for maintaining normal cellular function. The concept of "histone code" has indeed emerged to refer to histone-based regulation of gene transcription.

Most histone modifying enzymes act only on one or a select few histone marks to either place (writers), or remove (erasers) the mark on the histone tail or recognize the specific modification (readers) (12,62). Together with DNA methylation, these histone alterations have a pivotal role in regulating transcription, as each histone mark can recruit specific protein complexes that can either express or repress gene transcription. Acetylation

of histone tails on lysine residues, mediated by histone acetyltransferases (HATs), changes the secondary structure of chromatin allowing transcription factors' access to gene promoters. Deacetylation, performed by histone deacetylases (HDACs) enzymes, decreases transcription (11,63). Histone methylation, mediated by histone methyltransferases (HMTs), can occur on lysine, arginine and histidine residues, with lysine (K) methylation being the most characterized process: methylation of K9 and K27 in the histone tail of H3 induces the formation of heterochromatin, with subsequent transcriptional silencing (64,65). Additionally, each residue can be mono-, di-, or tri-methylated, providing another layer of regulation (65).

Current evidence suggests that aberrant histone modification patterns are critically involved in the PDAC tumorigenic process (66-69) and can help define subsets of patients with distinct epigenetic phenotypes and clinical outcomes. A study of three histone marks, H3 lysine 4 dimethylation (H3K4me2), H3 lysine 9 dimethylation (H3K9me2), and H3 lysine 18 acetylation (H3K18ac) suggested their role as independent predictors of poor survival in resectable PDAC patients (70). Enhancer of Zeste Homolog 2 (EZH2), a H3K27 methyltransferase and a component of polycomb protein complex (PRC2), is overexpressed in approximately 68% of PDAC (43). Nuclear localization and high expression of EZH2 is associated with poor-differentiation and shorter survival in metastatic setting.

Histone methylation

The most widely studied histone modifications are lysine alterations, including methylation, which is generally associated with transcriptional activation when occurring in promoter regions (71). G9a, is one of the main writers of this mark and has been found to be overexpressed in PDAC. G9a catalyzes the methylation of histone H3 at lysines 9 and 27 (H3K9 and K27) and works in complex with G9a-like protein (GLP) as well as with a variety of other epigenetic *writers* such as PRC2 (72,73).

Pharmacologic inhibition of G9a results in decreased H3K9 methyl marks, decreased proliferation via G2/M cell cycle arrest, and increased cellular senescence in pancreatic and other cancer cell models. G9a inhibition is even more effective as part of a combination therapy to revert chemoresistance, becoming a promising target for therapeutics (72). Pen and colleagues showed that inhibition of G9a using UNC0638 increased sensitivity to gemcitabine (74). These authors also showed that G9a expression correlated with the expression of the stemness genes including CD133, nestin and Lrg5 and its inhibition attenuates cancer stemness in these models. These findings were validated *in vivo*, where the combination treatment with G9a inhibitor and gemcitabine decreased tumor growth, lymph node invasion and distant metastasis.

Another histone tail modification frequently dysregulated in PDAC is hallmark H3K27 mono- and tri-methylation, mediated by EZH2, the catalytic subunit of PRC2. Drugs targeting EZH2 (UNC1999, GSK126) have shown promising results in PDAC models, (monolayer cells culture, spheroids, organoids and *in vivo* patient-derived xenograft mouse models), being associated with reduced aberrant K27 methylation, re-expression of cell cycle regulator p27Kip1, as well decreased PDAC cells proliferation rates, angiogenesis and increased apoptosis (75-77).

Ougolkov *et al*. noted an increase in the nuclear accumulation of EZH2 in chemo-resistant pancreatic tumor cells and showed that reversal of H3K27 methylation restored sensitivity to chemotherapeutics such as doxorubicin and gemcitabine (78). Co-exposure of DZNep (EZH2 inhibitor) and gemcitabine induced selective cytotoxic additivity in well- and poorly-differentiated PDAC cell lines, without affecting normal human pancreatic ductal epithelial cells (79). Interestingly, authors were able to show that a short priming with DZNep followed by gemcitabine treatment produced the maximal chemosensitization response (80).

Mathison *et al*. tested a combination of Aurora kinase A (AURKA) oncogene inhibitor and H3K9 methyltransferases inhibitor *in vitro* and *in vivo* models of PDAC, and showed that the combined inhibition of a genetic-to-epigenetic pathway was efficacious. Mechanistically, they reported that inhibition of H3K9 methyltransferases after targeting AURKA, arrested cells in G2-M phase, triggered an aberrant mitotic checkpoint response, and ultimately mitotic catastrophe (76). These data support the pathobiological hypothesis that PDAC develops and progresses in response to an interaction between known oncogenes and downstream epigenomic regulators. Increased interest has indeed emerged in the use of H3K27 methyltransferase inhibitors as part of combination therapies to re-sensitize resistant cells lines.

Histone deacetylation

Increased activity of HDAC is common in PDAC and

lead to decreased histone acetylation and consequent gene repression. Jiao and colleagues, showed higher levels of HDAC3 protein expression in PDAC tissues and cell lines as compared to paired normal ones, associated with cell proliferation, migration and invasion, and increase drug resistance (81).

HDAC2 and HDAC7 expression also increased in PDACs, especially in poorly-differentiated cases. Increased expression of HDAC7 can distinguish PDAC from other benign pancreatic neoplasms and is associated with apoptotic resistance of cancer cells via silencing of the apoptotic-inducing NOXA gene and attenuation of TRAIL-induced apoptosis (82-84). HDAC1 is found at increased levels in the vast majority of PDACs and its precursor lesions. HDAC1 and HDAC2/SIN3a are recruited to the TGFBR2 promoter leading to repressed expression of this tumor suppressor gene, in the absence of any genetic alterations. HDAC1/2-mediated transcriptional control regulates the epithelial-mesenchymal transition in PDAC cells, thereby contributing to invasion and metastasis (85).

As a result of the frequently observed dysregulation of HDAC family members in PDAC, and their role in controlling key oncogenic features, inhibition of HDAC has been investigated as potential therapy for PDAC patients. Several natural and synthetic compounds that inhibit HDAC activity are now available, which either target all HDAC family members (pan-HDAC inhibitor) or selectively interfere with subgroups of HDAC isoforms, with interesting activity shown in preclinical models (13,86).

Knockdown of HDAC3 gene through lentivirus-mediated methods inhibits PDAC cells proliferation and enhances the sensitivity to gemcitabine treatment, consistent with the effect of HDAC inhibitor (HDACi) trichostatin A and 4-phenylbutyrate (TSA) (87-90). In PDAC cell lines, treatment with HDACi vorinostat induces growth inhibition and G1 cell cycle arrest via upregulation of p21, with an additive effect on growth inhibition when combined with gemcitabine. Fritsche *et al*. noted that HDAC2 is upregulated in PDAC cells that acquire resistance to etoposide and treatment with HDACi, valproic acid, in combination with etoposide increased apoptosis and restored the etoposide sensitive phenotype in resistant cells (83).

In the clinical setting, monotherapy treatment with HDACi has showed activity in hematological malignancies, with vorinostat, romidepsin and panobinostat reaching approval for the treatment of cutaneous T-cell lymphoma (vorinostat and romidepsin) (91), peripheral T-cell lymphoma (romidepsin) (92) and multiple myeloma (vorinostat and panobinostat) (93). However, although effective in cell lines, HDAC inhibitors in monotherapy have not shown efficacy in early phase clinical trials for PDAC.

Consequently, clinical investigations have been focused on combined approaches with small-molecule inhibitors, chemotherapeutic or immunotherapy agents, with the intent to use HDAC inhibition to manipulate the microenvironment of the tumor to increase the sensitivity to standard therapeutics. A phase I trial tested oral panobinostat combined with gemcitabine in advanced solid tumor patients, including three PDAC patients, of which one had stable disease under treatment (94). A similar trial investigating the combination of mocetinostat with gemcitabine in patients with advanced PDAC was early terminated because of lack of efficacy combined with significant toxicity profile (95).

Romidepsin has been evaluated in combination with gemcitabine in solid tumors, including PDAC. No responses were observed in the ~25% of individuals with PDAC, associated with additive hematological toxicity of the combination (96).

The pan-HDAC inhibitor belinostat has been shown to decrease growth and increase apoptosis in PDAC preclinical models, mainly via blocking the AKT/mTOR pathway. In a phase I clinical trial evaluating belinostat in combination with carboplatin and/or paclitaxel in patients with solid tumors, one partial response was observed among the three PDAC patients enrolled, but as platinums and taxanes have activity in PDAC, it is uncertain what the contribution of the HDACi was (97). In two phase I studies testing the safety and activity of entinostat given alone and in combination with 13-cis-retinoic acid (13-cR), one PDAC patient either was enrolled, achieving stable disease in the first setting, while progressive disease was noted after combined treatment (98). Similarly, a phase I/II study has evaluated the toxicity and efficacy of valproic acid in combination with S-1 in pancreatobiliary patients, reported clinically significant agent-related adverse events in 67% of the patients enrolled, including G3–4 anemia and thrombocytopenia (99).

No objective responses were reported when vorinostat was added to chemoradiation with capecitabine in a phase I dose-finding study of 21 patients with non-metastatic PDAC, although 90% had stable disease (100).

These studies revealed minimal effects in a limited number of PDAC patients for the combination of HDACi as compared to gemcitabine monotherapy (101). Moreover, in all these studies high grade, treatment-related

myelosuppression and gastrointestinal events were common, raising concern about the safety profile of this approach.

As an alternative approach, several trials have been evaluated the combination of HDACi and targeted therapies in PDAC. Studies *in vitro* in PDAC showed a synergistic effect of the combination of the proteasome inhibitor marizomib and vorinostat; however, no responses were found in a phase I clinical trial using this strategy (102). Similarly, no efficacy was shown for the combination of panobinostat with the proteasome inhibitor, bortezomib, in a phase II clinical trial in gemcitabine-resistant PDAC patients, with high rate of grade 3 and 4 toxicities reported (thrombocytopenia and diarrhea) (103).

An ongoing neoadjuvant clinical trial is now investigating the efficacy of vorinostat and sorafenib plus standard therapy (gemcitabine plus nab-paclitaxel or gemcitabine plus radiation) and sorafenib in patients with stage I–III PDAC (NCT02349867).

Unfortunately, combinatory treatment regimens with strong and promising mechanistic synergism in preclinical PDAC models failed in first-in-patient studies, thus reflecting the difficulties of translating these findings into the clinical setting. One of the main difficulties of HDAC inhibition therapy is their global repressive effects with lack of target specificity: the outcome of these drugs is not predictable and can be associated with increased toxicity. Further careful investigations are highly needed to understand the impact of HDAC inhibition on both antitumor activity and toxicity

Nguyen *et al*. advanced the hypothesis that stromal fibroblasts can contribute to the poor efficacy of HDAC inhibition in PDAC. A key mechanism by which CAFs modify the behavior of neighboring tumor cells is via release of proinflammatory factors into the tumor microenvironment (TME) (104). These authors showed that HDACi-treatment of CAFs caused a dose dependent increase in the expression of inflammatory genes, causing a counter-productive and paradoxical tumor supportive phenotype. Combination therapies targeting PDAC stroma may mitigate these unintended effects and enhance their efficacy as anti-tumor drugs.

Finally, epigenetic mechanisms are intertwined as part of a broader spectrum of cellular mechanisms including DNA repair and DNA-damage signaling. Dynamic regulation of acetylation events on H3K56 and H4K16 and recruitment of HDAC1/2 to sites of DNA double-strand breaks (DSBs) have been reported (105).

Chen and colleagues showed that treatment of PDAC cells with the HDACi AR-42 induced ROS and caused DNA damage, particularly double-strand breaks (DSBs), leading to activation of both caspase-dependent and -independent apoptosis pathways. This was associated with decreased cell invasiveness *in vitro* and suppressed tumor growth *in vivo* (106). Agarwal *et al*. showed that the G9a inhibitor, UNC0638, sensitizes PDAC cells to DNA-damaging agents, by impairing DSBs repair (72). Both epigenetic and DNA repair pathways are aberrantly regulated in PDAC, especially in the subtypes carrying germline or sporadic mutations in BRCA genes (107). Targeting epigenetic and DNA repair pathways simultaneously might strongly impede cancer cell proliferation and provide new opportunities for future PDAC combination therapies.

Together, these results suggest that an optimized exposure to epigenetic modulatory drugs (EMD) can sensitize PDAC to other therapeutic agents, and emphasize the promising clinical utilities of epigenetic reversal agents in future PDAC combination therapies (*Table 1*).

Histone acetylation

Acetylation of histones and non-histone proteins of specific lysine residues by HATs neutralizes the positive charge on the amino group, weakening the DNA-chromatin complex and creating an open chromatin configuration, which facilitates gene expression. One study evaluated H4K12 and H3K18 acetylation in PDAC patients' samples by immunohistochemistry and found that these marks were indicators of lower overall survival (111). Decreased expression of p300, a HAT, has been reported in highly metastatic PDCA cell lines, supporting its role as a classical tumor suppressor protein (112). However, the role of histone acetylation in PDAC tumorigenesis and progression is still unclear, and highly dependent on the cellular context.

Careful preclinical investigations are still required and no current studies are investigating drugs targeting these proteins in this context.

Bromodomain epigenome readers and their inhibitors: a novel therapeutic target

While acetylation levels are regulated by HATs ("writers") and HDACs ("erasers"), acetylation marks are recognized by bromodomain-containing proteins ("readers"), such as the bromodomain and extra-terminal motif (BET) family of chromatin adaptors (BRD2, BRD3, BRD4 and BRDT) (113).

Table 1 Clinical trials evaluating EMD alone or in combination with chemotherapy, target therapy and/or radiation in PDAC

Drug group	Drug name	Combination agent(s)	Clinical trial phase, disease stage	Results and best response in PDAC	Clinical trial Ref.
HDAC inhibitors					
HDAC Pan-inhibitors	Panobinostat	Bortezomib	2, metastatic, gemcitabine-resistant PDAC	3 patients with PDAC, all had PD and severe treatment-related toxicity, study was early closed	Wang *et al*. 2012 (103)
HDAC class I and II	Vorinostat	Marizomib	1, metastatic PDAC	3 patients with PDAC, all had PD	Millawrd *et al*., 2012 (102)
		5-FU + radiation	1, non-metastatic PDAC	19 patients had SD, and 2 had PD	Chan *et al*., 2016 (100)
		Capecitabine + radiation	1/2, locally advanced PDAC	Not available, study early terminated	NCT00948688
		Gemcitabine/ Sorafenib +/– radiation	1, non-metastatic PDAC	Ongoing and recruiting	NCT02349867
	Valproic Acid	Epirubicin	1, advanced solid tumors	PR in one PDAC patient	Munster *et al*., 2007 (108)
		Gemcitabine + radiation	2, locally advanced PDAC	Not available, study terminated	NCT01333631
	Romidepsin	Gemcitabine	1, advanced solid tumors	SD in 6/10 patients with PDAC, treatment associated with significant TEAEs	Jones *et al*., 2012 (96)
HDAC class I	Entinostat	13-cis retinoic acid	1, advanced solid tumor	One patient with PDAC enrolled had SD	Pili *et al*., 2012 (98)
		FOLFOX	1b, metastatic PDAC	Not yet recruiting	NCT03760614
	CI-994	Gemcitabine	2, randomized to gemcitabine advanced PDAC	Inferior in OR and survival compared to gemcitabine monotherapy with decreased quality of life	Richards *et al*., 2006 (101)
HDAC class I + IV	Mocetinostat	Gemcitabine	1/2, advanced solid tumors	Phase 1: 12 patients with PDAC, 2 had PR; phase 2: 22 patients with PDAC, 9 patients had SD, but no response observed	Chan *et al*., 2018 (95)
HMT inhibitors	Curcumin	–	2, metastatic PDAC	21 evaluable PDAC, one patient had SD and one had PR	Dhillon *et al*., 2008 (56)
		Gemcitabine	1/2, advanced PDAC	11 evaluable PDAC, 1 PR and 1 SD, but overall treatment discontinued very early due to toxicity	Epelbaum *et al*., 2010 (54)
		Gemcitabine	1/2, metastatic, gemcitabine resistant PDAC	21 enrolled PDAC, 5 had SD	Kanai *et al*., 2011 (55)
DNMT inhibitors	CC-486 (oral azacitidine)	–	2, high risk resected PDAC	Ongoing and recruiting	NCT01845805
		Gemcitabine	1, advanced	Not available, study early terminated	NCT01167816
	Rx-3117	nab-paclitaxel	1,2 first line in PDAC	Ongoing and recruiting	NCT03189914

Table 1 (*continued*)

Table 1 (*continued*)

Drug group	Drug name	Combination agent(s)	Clinical trial phase, disease stage	Results and best response in PDAC	Clinical trial Ref.
BET inhibitors	MK-8628	–	1, advanced solid tumors including PDAC	Completed, no results yet	NCT02259114
	Bay1238097	–	1, advanced solid tumors including PDAC	Study was prematurely terminated because of the occurrence of DLTs	Postel-Vinay *et al*., 2019 (109)
	BI-2536	–	2, advanced PDAC	86 evaluable patients, 2 PR, but both patients discontinued treatment to clinical or non-target lesion progression, OS was 4.9 months	Mross *et al*., 2012 (110)
	INCB057643	Monotherapy (part 1 and part 2) and in combination with standard-of-care (SOC) agents (part 3 and 4)	1/2, advanced solid tumors including PDAC	Active, not recruiting	NCT02711137

BET, bromodomain and extra-terminal; DNMT, DNA methyltransferase; HDAC, histone deacetylase; HMT, Histone methyltransferases; PD, progressive disease; PDAC, pancreatic adenocarcinoma; PR, partial response; SD, stable disease; TEAEs, treatment emergent adverse events; EMD, epigenetic modulatory drug.

These proteins interact with the acetylated lysine residues of the histone tails to facilitate the recruitment of macromolecular transcription complexes necessary for the transcription of specific subset of genes, further enhancing the transcriptional activation resulting from the acetylation marks. These proteins also function as mediators of transcriptional elongation by promoting the recruitment and activation of the positive transcription elongation factor-b complex (P-TEFb) (114,115).

Human BRD4 was initially identified in NUT midline carcinoma (NMC), a rare subtype of squamous cell carcinoma characterized by a translocation most often involving the NUT gene and BRD4 (116). NMC, typically arising from the midline structures of the head, neck, and thorax, is extremely aggressive tumor, with median overall survival of 6.7 months (117). Treatment of NMC cells with BET inhibitors results in proliferation arrest in *in vitro* and *in vivo* models, and treatment with the oral BET inhibitor OTX015/MK-8628 led to significant and rapid tumor regression in 2 NMC patients (118). Now multiple BET inhibitors have shown clinical activity in NUT midline patients (119,120).

While initial reports suggested the transcriptional inhibition of oncogenic c-Myc as the crucial mechanism of BET inhibition antitumor activity (121,122), recent studies have shown the role of the BET proteins in various differentiation pathways and in controlling other cancer-relevant genes such as BCL2, FOSL154, as well as the activity of the EMT-related transcription factor Twist1. These findings underscore the potential of small-molecule inhibitors that specifically target these readers of for solid tumors' treatment and tumor reprogramming (114,123,124). One of the founding BET inhibitor small molecules is JQ1, which was initially described to be a potent suppressor of NMC and B-cell lineage malignancies (115). Expression of BRD2, BRD3, and BRD4 has been detected in both preneoplastic lesions and PDAC and administration of JQ1 blocked acinar-to-ductal metaplasia—a key event in PDAC initiation—the development of PanINs lesions and PDAC cells proliferation. These effects were associated with decrease activation of the pro-survival kinase AKT and with downregulation of inflammatory regulators such as STAT3 and IL6 (125). Wang and colleagues showed that BRD4 was significantly upregulated in PDAC cell lines upon treatment with gemcitabine and combination treatment with BET inhibitors had a synergistic effect (126).

These data suggested that BET proteins play an important role in PDAC growth, progression and chemoresistance, making it a promising target for anticancer treatments in this disease (127). Sahai *et al*. demonstrated

that JQ1 and BRD4 knockdown suppress proliferation of chemotherapy resistant- PDAC cells in an *in vitro* three-dimensional collagen model (128). Later, Garcia *et al*. using five PDAC patient-derived xenograft models, showed that JQ1 treatment was associated with significant tumor growth suppression. No consistent association with decreased c-Myc expression was observed, while significant inhibition of CDC25B expression, a regulator of cell cycle progression, was reported (129). A more recent study confirmed the antitumoral activity of BET inhibitors in PDAC human-derived xenograft tumors (127).

In terms of combination approaches, JQ1 in combination with gemcitabine led to a significant reduction in tumor volume and proliferation in a Kras;p53 mutant PDAC mouse model (125). Functional studies confirmed that BET inhibition alters PDAC' TME by decreasing the protective stroma formed by CAF: inflammatory signals and expression of the tumor-associated stroma markers were all reduced upon JQ1 treatment, consistent with other reports (128,130,131), providing a unique example of simultaneous targeting of both the stromal and neoplastic cells.

Clinical trials using different BETi have been initiated in PDAC (NCT01987362, NCT02259114, and NCT02369029) (*Table 1*). However, first clinical studies testing BET inhibitors in monotherapy in PDAC have been discouraging: a randomized phase II trial in patients with unresectable PDAC using BI-2536, an inhibitor of the Polo-like kinase that has been shown to block BRD4 activity *in vitro* (NCT00710710), yielded poor response rates.

These data are not completely surprising: considering that JQ1 treatment had only a modest effect on survival on its own in preclinical models, paving the pathway for the use of these drugs not as a standalone treatment, but combination with other therapies. Mazur *et al*. showed that JQ1 synergizes with the HDAC inhibitor, vorinostat, in a KrasG12D;p53ko PDAC model. Similar antitumourigenic activity of BETi/HDACi treatment was shown in a preclinical model of lymphoma and acute myelogenous leukaemia and in KRAS mutant lung cancer models (125,132,133). Several mechanisms have been proposed to explain the antitumor activity of combined BETi/HDACi, including de-repression of p57, P-TEFb recruitment and subsequent transcriptional induction and elongation of a defined set of target genes.

In summary, BET proteins clearly play a role in PDAC pathology, but additional studies are required to optimize their value as therapeutic target and biological marker in PDAC.

Epigenetic regulation by noncoding RNAs

Non-coding RNAs (ncRNA) are RNAs that are not translated into proteins and include different classes of small RNAs [<200 bases, like microRNA (miRNA)] and long non-coding RNAs (lncRNAs, >200 bases), whose expression is tissue and stage specific. NcRNA are mainly implicated in translational repression and RNA degradation, but recent findings underscored their interaction with chromatin modifier complexes in gene regulation.

MiRNAs bind complementary regions of mRNAs, usually in the 3' region, and inhibit the process of translation or decrease the stability of the associated mRNA specie. Each miRNA may have many mRNA targets. Numerous miRNAs are abnormally expressed in PDAC and its precursor lesions (134) and miRNA profiling has been shown to be effective in differentiating normal tissue from PDAC and could facilitate early diagnosis. Examples include miR-155 and miR-21. MiR-155 has been used in diagnosing IPMNs in pancreatic juice samples and its levels increase progressively in PanIN2 and PanIN3 lesions (135,136). Similarly, miR-21 expression increases with PanIN grade, with peak expression occurring in hyperplastic PanIN-1/2 lesions and when tested in pancreatic cyst fluid, was found to be an encouraging biomarker to differentiate cancer patients from those with chronic pancreatitis and healthy subjects (137-139).

Extension of these works demonstrated that a number of miRNAs are deregulated in patients with PDCA as compared to healthy controls (140,141). Meta-analyses of these studies have identified a few miRNAs that are reported in multiple studies as consistently altered in PDAC (i.e., miR-21 and miR-23a were identified as upregulated, and miR-148a and miR-375 as downregulated in multiple profiling studies), pointing out the complexity of the miRNA transcriptome (142,143).

MiRNAs levels are also associated with PDAC clinical outcomes. Increased expression of miR-21, miR-155 (140), miR-196a-2 (144), miR-203(145), and miR-183 (146) are associated with poor prognosis, while miR30a-3p, miR-105, miR-127, miR-187, miR-452, and miR-518a-2 predict better survival in PDAC patients with lymph node positive disease (140).

Other studies suggested that miRNAs modulate chemo-resistance to gemcitabine in PDAC (147-149). Treatment of PDAC gemcitabine-resistant cell lines with lentiviral vectors

containing miR-181b mimics resulted in increased sensitivity to the drug. Similar findings were obtained in PDAC xenografts models (150). Wang *et al*., reported that miR-23b is downregulated in radioresistant PDAC cells and its restoration increases the sensitivity to radiation therapy (151).

LncRNA have also emerged as a major mechanism for PDAC tumorigenesis by regulating important cellular behaviors such as cell proliferation, invasion, metastasis and chemoresistance. LncRNAs are also potential diagnostic and prognostic biomarkers in PDAC (35,152). A recent genome-wide study showed that germline variation of lncRNA, LINC00673, might confer susceptibility in development of PDAC (153). LncRNA HOTAIR, PVT, H19 are overexpressed in PDAC and H19 has been associated with tumor grade and metastasis (154-156). Uc.345 is also upregulated in PDAC tissues and correlates with higher stage and decreased overall survival (157). Plasma levels of Linc-pint, a p53-induced lncRNA, are associated with higher risk tumor recurrence, and correlate with poor prognosis (158).

Therefore, ncRNAs are potential diagnostic, prognostic biomarkers of PDAC and there is significant enthusiasm about their role as therapeutic target (159). Restitution of miRNAs through nanoparticle delivery has been investigated preclinically in PDAC. The miR-34a nanocomplexes alone or combined with miR-143/145 nanovectors significantly suppressed the growth of gemcitabine resistant MiaPaCa-2 subcutaneous xenografts and orthotopic PDAC models (160,161). It has also been shown that the combination of gemcitabine with miR-205 is able to overcome drug resistance and inhibit invasion of gemcitabine resistant PDAC cells and animal models (162).

Another promising approach to target small ncRNAs involves antisense oligonucleotides, which function binding miRNAs with high complementarity to inhibit their function. Administration of the combination of anti-miR-21 and anti-miR-221 oligonucleotides significantly reduced tumor growth and metastasis in PDAC models (163). Similar findings were reported with inhibition of miR-132 and miR-212 by antisense miRNA (164).

Several natural agents including isoflavone, curcumin, 3,3'-diindolylmethane (DIM), have been investigated for their effects on the regulation of miRNAs in PDAC. Studies showed that in PDAC cells isoflavone could normalize the levels of several miRNA (including miR-27a, miR-146a, miR-200, miR-34a), resulting in suppressed cancer cell proliferation and invasion through the inactivation of Akt and NF-κB pathway. However, in a phase 2-study in patients with advanced PDAC, the addition of soy isoflavones to gemcitabine and erlotinib did not improve patients' outcome, although the triplet appeared to be well tolerated (165). DIM and curcumin have also shown potential anti-cancer activities in PDAC through miRNA regulations. Treatment of MiaPaCa-2 and Panc-1 cells with either DIM or curcumin resulted in cell growth and migration inhibition via the down-regulation of miR-221 and subsequent induction of PTEN, p27, p57, and PUMA (166). Another phase 1/2a clinical trial investigated the role of intratumoral administration of BC-819 in locally advanced PDAC. BC-819 is a DNA plasmid carrying the gene for the diphtheria toxin- under the regulation of the lncRNA H19 gene promoter, which is overexpressed in PDAC. The maximum tolerated dose of BC-819 was not reached in this study and encouraging results were observed in term of tumor response (167).

Although, ncRNAs are promising therapeutic agents, therapeutic application is still in its infancy and to date there are no miRNA-based therapy approved for PDAC. Several challenges exist for their application, including *in vivo* instability and lack of gene targeting specificity. Additional pre-clinical and proof-of-concept clinical studies are required to better understand the meaning of these ncRNAs in PDAC and their possible value as therapeutic targets.

The impact of epigenetic therapeutics in pancreatic cancer immunity and immunotherapy

The understanding of the role that immune checkpoint molecules, such as cytotoxic T-cell lymphocyte-4 antigen (CTLA-4) receptor and programmed death-1 (PD-1) T cells co-receptor and its ligands PD-L1/PD-L2, play in the maintenance of immunosuppression within the TME has led to the clinical development of monoclonal antibodies targeting these molecules [immune checkpoint inhibitors (ICIs)] (168-170). ICIs have become a major focus of cancer therapy.

Antitumor immune response and therapy effectiveness depends on the ability of cytotoxic T cells (Teff) to infiltrate the tumor and recognize tumor cells and on the amount of tumor antigens and intact antigen presentation machinery (171). Cancer types with higher mutation burden, and consequently higher probability of neo-antigens, frequently show higher response rates to ICIs: in responsive patients, these immune checkpoint blockade therapies have resulted in long-term control of chemotherapy-resistant tumors that

can last years (172,173).

The benefits are much more limited in non-immunogenic tumors lacking T cell infiltrate, such as PDAC, characterized by a hostile and immunosuppressive microenvironment that impedes T cell infiltration and function (174,175).

Recently, it has become apparent that EMD are capable of enhancing tumor immunogenicity and boosting the antitumor immune response, and several studies have already demonstrated synergy between immunotherapies and EMDs for cancer treatment (176). Multiple groups have demonstrated that the administration of low doses of DNMTIs, though not cytotoxic to cells, causes wide promoter DNA demethylation and reprogramming of regulatory pathways in tumor cells, increase expression of genes in the type I IFN, antigen processing and presentation, PD-1/PD-L1 pathways and induce the expression of cancer testis antigens in various cancer types (177,178). Other group showed that DNA hypomethylating agents and HDAC inhibitors can also reactivate endogenous retroviral elements (ERV), thus enhancing an intrinsic immune system response. Specifically, the long terminal repeats (LTRs) of ERVs, normally silenced by DNA methylation, can become re-expressed with these agents, leading to transcriptional expression of thousands of previously non-annotated transcription start sites and subsequent activation of an antiviral innate immune response and creating a state of 'viral mimicry' (179).

Recent work with HDACi has shown the ability of these agents to alter the immunogenicity of the TME by inducing the expression of tumor associated antigens, increasing tumor cell expression of MHC class II, inducing the expression of natural killer cell receptors and ligands and decreasing Tregs and myeloid-derived suppressor cells (MDSCs) in multiple different tumor models (180,181).

BETi also have intrinsic immunomodulatory properties that favor antitumor immunity. Zhu *et al.* were among the first to describe that CD274, the gene encoding PD-L1, was a direct target of BRD4; BETi directly suppressed PD-L1 transcription in cancer and immune cells (182). The potential for BET inhibitors to induce immunogenic cell death has also been suggested (183).

Peng *et al.*, using a mouse model of ovarian cancer, reported increased expression of the Th1-type chemokines CXCL9 and CXCL10 upon EZH2 inhibition and DNMT1i exposure, resulting in increased T-cell trafficking in the TME, which enhanced therapeutic efficacy of PD-L1 checkpoint blockade as well as adoptive T-cell therapy (184). In preclinical models of renal and castration resistant prostate cancer, low dose of HDACi entinostat in combination with IL-2 therapy or a survivin based vaccine inhibited tumor growth, reduced infiltrating regulatory T cells (Tregs), and increased the Teff response (185). A synergistic effect of ICIs in combination with entinostat and AZA was reported also in preclinical models of CRC and breast cancer (186).

In NSCLC patients who previously underwent epigenetic therapy and subsequently began immune checkpoint therapy, all five patients passed the 24-week point without progression with three of these individuals maintaining partial RECIST responses for over 2.5 years (187). Other clinical trials combining the HDACi entinostat and the anti-PD-1 pembrolizumab in NSCLC and melanoma have demonstrated promising activity in anti-PD-L1-resistant patient groups. Of note, responses were observed even in the absence of PD-L1 expression by IHC (188). Multiple other phase I/II trials are underway testing DNMTi and/or HDACi in combination with anti-PD1 therapy in multiple histologies with results expected soon.

Acknowledgments about the precise relationships between epigenetic aberrations, immune system and the consequences for cancer cell phenotypes could have tremendous translational implications in PDAC. The TME in PDAC is remarkable for its profound desmoplasia and absence of Teffs and its T helper 2 cell immunophenotype (189). This allows PDAC to avoid immune surveillance and explains the ineffectiveness of ICIs in numerous studies of metastatic PDAC patients (190-192). Therefore, epigenetic modulation might represent a novel strategy to prime the tumor and TME and reverse immunosuppression in PDAC.

Shakya *et al.* tested DAC in an aggressive stroma-rich mouse model of PDAC and showed that DAC was able to slow disease progression and induce transient tumor growth inhibition. Furthermore, an additive antiproliferative effect on PDAC cells was reported for the combination of DAC plus IFN-γ (193), providing a rationale for future studies combining hypomethylating agents with cytokines and immunotherapy.

Lu and colleagues showed that CD274 promoter is enriched for H3K4 trimethylation (H3K4me3), catalyzed by MLL1 and result in PD-L1 transcription in PDAC cells. In an orthotopic mice model, they showed that inhibition of MLL1 decreases the H3K4me3 levels in the CD274 promoter and PD-L1 expression, and resulted in significant tumor growth suppression when combined with anti-PD-L1 or anti-PD-1 antibody (194).

Table 2 Clinical trials evaluation the combination of EMDs with immune-oncology (IO) therapies in PDAC

EMD class	EMD name	Immunotherapy target	IO agent name	Clinical trial phase, disease stage	Status	Clinical trial Ref.
HDAC inhibitor	Entinostat	PD-1	Nivolumab	2, advanced PDAC and cholangiocarcinoma	Ongoing, recruiting	NCT03250273
DNMT inhibitor	Guadecitabine	PD-L1	Durvalumab	1b, advanced HCC, PDAC, cholangiocarcinoma	Ongoing, recruiting	NCT03257761
DNMT inhibitor	Azacitidine	PD-1	Pembrolizumab	2, advanced PDAC	Ongoing, recruiting	NCT03264404

DNMT, DNA methyltransferase; EMD, epigenetic modulatory drug; HCC, hepatocellular carcinoma; HDAC, histone deacetylase; IO, immune-oncology; PDAC, pancreatic adenocarcinoma; PD-1, programmed cell death; PD-L1, programmed death-ligand.

Our laboratory has explored the role of HDAC inhibition in immunocompetent murine PDAC models, and we demonstrated that the HDACi, entinostat, shifted MDSCs from a myeloid-MDSC-dominant population to the less immunosuppressive G-MDSCs subtype. The functional capability of these cells was also impaired, with the remaining MDSCs expressing less Arginase-1 and less PD-L1 (195). Combination therapy of entinostat with anti-PD1 agent or anti-CTLA4 antagonist antibody significantly improved survival as compared to either agent alone. Based on these preclinical data, a phase 2 study evaluating the clinical efficacy of entinostat plus anti-PD-1 in unresectable or metastatic PDAC has been initiated (NCT03250273).

Several challenges need to be considered when developing epigenetic-immune therapy combinations, including the optimal drug sequence, whether the treatments should be sequential or concurrent, continuous or intermittent, and the optimal dose (196). However, multiple lines of evidence presented here suggest that exploiting cancer epigenome may result in increased immunogenicity and overcome resistance to immune therapies, supporting the hypothesis that EMD may prime an ICI insensitive cancer into a sensitive one, and clinical trial have been initiated (*Table 2*).

Conclusions

The role of epigenetics in PDAC carcinogenesis is now better defined and has been linked to increased cancer cell stemness, altered cellular metabolism, differentiation, and chemoresistance (197). Unlike genetic alterations, epigenetic plasticity allows rapid, dynamic and potentially reversible changes that favor tumor growth and progression, as well as immune escape and resistance to therapies (7,13).

However, how exactly alterations in epigenome affect PDAC development and progression, is still not fully understood (41).

The overall satisfactory tolerance of epigenetic drugs with minimal overlapping toxicities with other classes of drugs, together with their intrinsic immunomodulatory effects, make them promising therapeutic approach to combine with conventional therapies and immune therapies.

However, there is no doubt that much still need to be done to optimize the use of epigenetic drugs before translating these agents into the clinical practice. The discrepancy noted between the promising preclinical data and the modest clinical efficacy reported in early phase clinical trials in PDAC patients raises important concerns (198). In the era of personalized medicine, a better understanding of which subset of patients could benefit most from certain EMD treatment is highly needed. Appropriate preclinical models should be used to explore the molecular rational of combinatorial regimens and set the stage for future clinical trials. The use of dedicated pharmacodynamics companion biomarkers in these studies may guide the determination of the optimal dosage, schedule and population. Accordingly, comprehensive translational studies should be carried out to determine whether the observed effect of EMDs in PDAC is linked to the effect of the drug on the tumor, on the stroma and/or on specific subsets of immune cells.

In conclusion, our increasing knowledge of the genetic and epigenetic aberration that drive PDAC pave the pathway for novel, promising and exciting therapies in this setting. Laboratory-based studies, clinical and translational studies are warranted to better understand the complex interactions of PDAC genetics, epigenetics and immunology to allow the translation of these findings into clinical practice.

Acknowledgments

None.

Footnote

Conflicts of Interest: N Ahuja has licensed pancreas cancer biomarkers to Cepheid and has received grant funding from Astex Inc. She has served as a consultant for Ethicon. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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doi: 10.21037/apc.2019.10.01
Cite this article as: Baretti M, Ahuja N, Azad NS. Targeting the epigenome of pancreatic cancer for therapy: challenges and opportunities. Ann Pancreat Cancer 2019;2:18.

