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Developing effective combination therapy for pancreatic cancer: An overview

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Abstract

Pancreatic cancer is a fatal disease. The five-year survival for patients with all stages of this tumor type is less than 10%, with a majority of patients dying from drug resistant, metastatic disease. Gemcitabine has been a standard of care for the treatment of pancreatic cancer for over 20 years, but as a single agent gemcitabine is not curative. Since the only therapeutic option for the over 80 percent of pancreatic cancer patients ineligible for surgical resection is chemotherapy with or without radiation, the last few decades have seen a significant effort to develop effective therapy for this disease. This review addresses preclinical and clinical efforts to identify agents that target molecular characteristics common to pancreatic tumors and to develop mechanism-based combination approaches to therapy. Some of the most promising combinations include agents that inhibit transcription dependent on BET proteins (BET bromodomain inhibitors) or that inhibit DNA repair mediated by PARP (PARP inhibitors).

Graphical abstract

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Declaration of Competing Interest

The authors declare that there is no competing interest related to this review manuscript.

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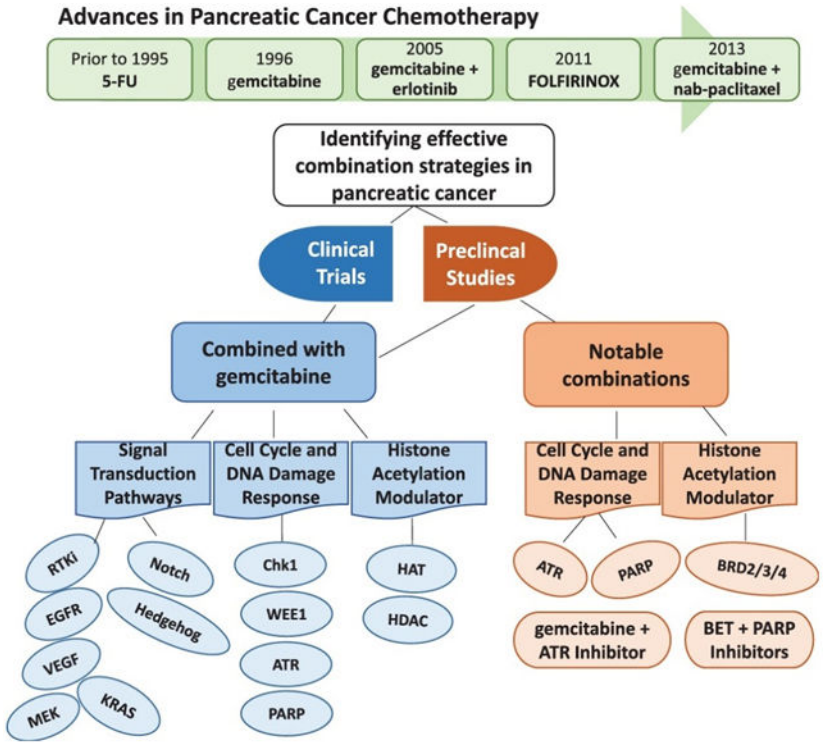

Keywords

Pancreatic cancer; BET bromodomain inhibitors; PARP inhibitors; gemcitabine

1. Introduction

Pancreatic cancer is a highly aggressive lethal neoplasm, with a 5-year survival of <10% (1). While cancer associated deaths declined ~ 27% between 1991 and 2016, the incidence of and deaths associated with pancreatic cancer have continued to increase (2). Pancreatic cancer is expected to become second only to lung cancer as the leading cause of cancer related deaths in the United States by the year 2030 (3). The greatest non-modifiable risk factor associated with pancreatic cancer is age, with a median age at diagnosis in the United States of 70 years (4). Other well recognized risk factors include smoking, obesity, and type II diabetes (4-7). Pancreatic ductal adenocarcinoma (PDAC) is the most common histological type of pancreatic cancer. Although our overall understanding of the biology of this disease continues to expand, pancreatic cancer is frequently diagnosed at late stage due to non-specific symptoms and lack of early detection methods.

In addition to environmental and lifestyle risk factors, several genetic conditions also increase risk for PDAC. These include hereditary pancreatitis and Peutz-Jeghers syndrome (8-10). PDAC tumors also commonly harbor germline or sporadic mutations in *KRAS* (95%), *CDKN2A* (90%), *TP53* (75%), and *SMAD4* (50%) (11-13). Additional pathways or processes frequently altered in these tumors include proteins associated with Notch, WNT, and Hedgehog signaling, as well as proteins that contribute to DNA damage checkpoints and

DNA repair (14). Histologically, these tumors are characterized by a unique desmoplastic stroma tumor microenvironment which is thought to minimize access of systemically administered anti-tumor agents to the tumor, support tumor cell proliferation, and contribute to chemoresistance (15).

This review includes a brief history of approaches to the treatment of pancreatic cancer, a summary of the current standards of care, and a discussion of new drugs and drug combinations being evaluated preclinically and clinically. We propose that the more effective agents and combinations are those that target specific characteristics common to pancreatic tumors.

2. Standard of Care for Patients with Pancreatic Cancer

Surgical resection remains the only potentially curative treatment for patients with PDAC, but only 20% of these patients have surgically resectable disease at diagnosis (16). The remaining 80% of patients are diagnosed with locally advanced or metastatic disease and are considered ineligible for surgery. It wasn't until the 1980s that conventional cytotoxic chemotherapeutics were introduced as standards of care for PDAC patients, when it was reported that the addition of a fluoropyrimidine such as 5-fluorouracil (5-FU) to radiation improved 1-year survival (17). Following this advance, the standard of care for these patients did not change for nearly two decades, until the nucleoside analog gemcitabine was shown to improve 1-year survival from 2% with 5-FU to 18% with gemcitabine (18). Subsequent combination therapies relied on combining other well-characterized conventional cytotoxic agents with the new standard of care, gemcitabine. Clinical trials were initiated to evaluate the efficacy and safety of gemcitabine in combination with cytotoxic agents such as cisplatin or the 5-FU prodrug capecitabine (19, 20). Although the combination of gemcitabine + cisplatin improved 6 month survival, this combination also increased the incidence of grade 3 and 4 toxicities (20). Similarly, a phase III trial in which gemcitabine + capecitabine was compared with gemcitabine alone as adjuvant therapy, the addition of capecitabine increased median overall survival from 25.5 to 28 months (P=0.032), but again with increased grade 3 and 4 toxicities (19). Recent phase I-II clinical trials demonstrate the utility of an albumin-bound form of paclitaxel (nab-paclitaxel, Abraxane) + gemcitabine in patients with advanced disease, and phase I-III trials with this combination are ongoing for patients with metastatic disease or as adjuvant therapy for patients with resectable disease (21-23). Another combination regimen that was introduced in 2010 and determined to be more effective than gemcitabine alone for the treatment of metastatic disease was FOLFIRINOX (leucovorin, fluorouracil, Irinotecan, oxaliplatin; PubChem CID: 136171075). Overall survival for patients treated with FOLFIRINOX was 11.1 months compared to 6.8 months with gemcitabine as a single agent (24). However, while the efficacy of FOLFIRINOX is greater than gemcitabine alone, the toxicity of this regimen limits its use to patients with an Eastern Cooperative Oncology Group (ECOG) performance status score of 0, 1 or 2, due to increased toxicity (24, 25).

3. Targeted Agents that Augment Gemcitabine Efficacy

In addition to identifying combinations of conventional agents that can be combined with gemcitabine to improve survival for patients with pancreatic cancer, recent studies attempt to identify targeted agents that can be effectively combined with this standard of care. This strategy has been facilitated by rapid advances in genetic, molecular, proteomic, and data analysis techniques that can be used to identify new therapeutic targets for pancreatic cancer. The following sections focus on agents that target dysfunction of pathways associated with overexpression or mutations in EGFR, VEGF, Notch, Hedgehog, MEK and KRAS proteins that occur commonly in pancreatic tumors. Table 1 summarizes combinations discussed in section 3.

3.1. Epidermal growth factor receptor (EGFR) inhibitors

EGFR belongs to the tyrosine kinase family of growth factor receptors, and is overexpressed in up to 95% of pancreatic tumors (26). This overexpression correlates with advanced and metastatic disease (27); and, therefore, has been evaluated as a potential therapeutic target for this tumor type. The small molecule EGFR inhibitor erlotinib (OSI-774, Tarceva) binds to the ATP binding domain of EGFR and inhibits EGFR-associated tyrosine kinase activity (28). Erlotinib (PubChem CID: 176870) was demonstrated in phase III clinical trials to increase 1-year survival of patients with pancreatic cancer from 17% with gemcitabine alone to 23% with gemcitabine + erlotinib (29). The FDA approved the use of this combination for locally advanced, unresectable or metastatic pancreatic cancer in 2005 (30).

Cetuximab (IMC-225, ABX-EGFR, PubChem SID: 46507042) is a monoclonal antibody that binds to the extracellular domain of EGFR and inhibits EGFR downstream signaling (31). Phase I, II and III clinical trials with this antibody in combination with gemcitabine did not increase overall survival (OS) (32, 33). For example, in phase III trials, a combination of cetuximab (400 mg/m^2 followed by 250 mg/m^2 weekly) + gemcitabine (1,000 mg/m^2 weekly, for 7 weeks of an 8-week cycle and 3 weeks of each 4-week cycle) was associated with a 6.3 month median survival compared to a 5.9 month median survival with gemcitabine as a single agent (33). A recent review of similar published studies also concluded that cetuximab adds no benefit to standards of care for patients with pancreatic cancer (34).

3.2. Vascular endothelial growth factor (VEGF) inhibitor

Among VEGF members, VEGF-A is the most well characterized. VEGF-A is a secreted protein that binds to VEGF receptors (VEGFRs), primarily VEGFR-2, on endothelial cells, to facilitate downstream signaling and increase vascular permeability (35, 36). Early immunohistochemical studies show that VEGF-A is expressed in up to 65% pancreatic tumors, and that this expression correlates with local disease progression (36, 37). A second generation VEGF-A inhibitor, the recombinant humanized monoclonal antibody bevacizumab (Avastatin, PubChem SID: 46504473), binds to VEGF-A to limit the interaction of VEGF-A with VEGFR (38). Unfortunately, randomized and controlled phase III trials that included 602 patients with pancreatic cancer demonstrated that bevacizumab did not meet the designated primary end point of the study and the trial was discontinued

(39). Although clinical trials have not demonstrated the utility of this monoclonal antibody for the treatment of pancreatic cancer, preclinical studies are ongoing to evaluate its potential utility in treating other tumor types (40, 41).

3.3. Notch inhibitors

In normal cells, Notch signaling plays a key role in embryonic development, cell proliferation and differentiation (42). In tumor cells, the role of Notch signaling in tumor development and progression is somewhat controversial. The literature indicates that this pathway may contribute to both oncogenesis and tumor suppression in tumor types that include pancreatic cancer (42). Notch signaling is thought to be required for Ras-dependent oncogenesis (43), and proteins involved in this pathway are expressed at higher levels in pancreatic tumors compared to normal pancreas (42). Gamma secretases (γ-secretases), a family of enzymes responsible for proteolytic processing of Notch receptors, have been investigated as potential therapeutic targets (44). Preclinical studies of one such inhibitor, PF-03084014 (nirogacestat, PubChem CID: 46224413), showed that PF-03084014 + gemcitabine suppressed the growth of subcutaneous pancreatic tumors and also suppressed metastatic tumor growth in orthotopic models of pancreatic cancer (45). PF-0384014 is in phase III clinical trials for desmoid tumors and aggressive fibromatosis; but that trial does not include patients with pancreatic cancer (NCT03785964). Data from a phase I trial in 2018 with a second γ-secretase inhibitor, MK-0752 (PubChem CID: 9803433), demonstrated that MK-0752 + gemcitabine in PDAC concluded these two agents are well tolerated by these patients at doses of MK-0752 1,800 mg/m^2 weekly + gemcitabine 1,000 mg/m^2 on days 1, 8,15 of a 28-day cycle (46).

3.4. Hedgehog inhibitors

The hedgehog (Hh) pathway contributes to embryonic development as well as to the development of tumors such as basal cell carcinoma (47). Three ligands activate Hedgehog signaling: Sonic hedgehog (Shh), Indian hedgehog (Ihh) and Desert hedgehog (Dhh), each of which binds to a plasma membrane receptor called patched (Ptch) to activate signaling (48). In several tumor types, Hh pathway activation contributes to tumor development (49). In pancreatic cancer, Shh a downstream target of oncogenic *KRAS*^{G12D}, is overexpressed and is activated by mutant *KRAS* (50, 51). Shh regulates the binding of the G protein-coupled receptor protein Smoothened (Smo) to Ptch1. Inhibitors of Smo would be anticipated to inactivate downstream events that facilitate cell proliferation (49). Inhibitors of Smo include vismodegib (GDC-0449, Erivedge; PubChem CID: 24776445) and sonidegib (LDE225, Odomzo; PubChem CID: 24775005). Vismodegib was approved for the treatment of advanced basal cell carcinoma in 2012 (52). However, vismodegib + gemcitabine was not superior to gemcitabine as a single agent in a phase Ib/II trial in patients with metastatic pancreatic cancer, with an overall survival of 6.3 months compared to 5.4 months for gemcitabine alone (53). Sonidegib was approved for the treatment of locally advanced basal cell carcinoma in 2015 (54). A phase Ib trial of sonidegib (400 mg daily) + gemcitabine (1,000 mg/m^2 on days 1, 8, 15) on a 28-day cycle showed that although this combination was well-tolerated, median progression free survival (PFS) for this combination did not differ from that with gemcitabine alone (NCT01487785) (55). Similarly, a phase II clinical trial combining gemcitabine + nab-paclitaxel ± visdemogib in

newly diagnosed patients with metastatic pancreatic cancer did not support further investigation of Hh inhibitors in this disease setting (NCT01088815) (56). Although the literature does not provide strong support for combining Hh inhibitors with gemcitabine for the treatment of pancreatic cancer, there are ongoing trials to evaluate the toxicity and efficacy of the Smo inhibitor sonidegib + the anti PD-1 monoclonal antibody pembrolizumab for patients with advanced solid tumors including metastatic and refractory PDAC (NCT04007744). The results of these trials have not yet been reported.

3.5. Mitogen-activated protein kinase (MAPK)/ extracellular signal-regulated kinase (ERK) kinase (MEK) inhibitors

Over ninety percent of pancreatic tumors harbor *KRAS* mutations that constitutively activate this oncogene and contribute to pancreatic tumorigenesis and progression (57). Based on the hypothesis that targeting proteins downstream of KRAS such as RAF, MEK or ERK would inhibit tumor development and progression, inhibitors of these effectors have been developed. Preclinical studies with dual MEK1/2 kinase inhibitors demonstrate that, for example, when PDAC cells were exposed sequentially to the MEK1/2 inhibitor pimasertib (AS-703026, MSC1936369B, PubChem CID: 44187362) followed by gemcitabine, this combination was synergistic *in vitro* and inhibited ribonucleotide reductase subunit 1 (RRM1) (58). Further, *in vivo* data demonstrated that pimasertib + gemcitabine delayed the growth of orthotopic pancreatic tumors, compared to either drug as a single agent ($P<0.05$). Another preclinical study with the MEK1/2 inhibitor trametinib (PubChem CID: 11707110) showed that a 2-week regimen of trametinib + gemcitabine was more effective than trametinib alone in patient-derived orthotopic pancreatic cancer (59, 60). However, while preclinical data appeared encouraging, clinical trials of MEK1/2 inhibitors + gemcitabine have not supported the use of this combination. For example, a randomized phase II trial of pimasertib (AZD6244, PubChem CID: 10127622) + gemcitabine as front line treatment for metastatic pancreatic cancer patients did not meet its primary end point for progression free survival (PFS) (NCT01016483) (61). Another trial comparing trametinib + gemcitabine with gemcitabine alone showed a similar result: trametinib + gemcitabine did not improve overall survival (OS), PFS or duration of response (DOR), compared to gemcitabine alone (NCT01231581) (62). The literature indicates that MEK inhibitors + gemcitabine does not warrant further clinical evaluation for treatment of patients with pancreatic cancer.

3.6. Prenylation inhibitors

Prenylation is a posttranslational modification of proteins such as GTPases by farnesyl or geranylgeranyl transferases (63). Farnesyltransferase belongs to the family of prenylation enzymes (64). Farnesylation of Ras proteins, including KRAS, is required for protein function, and oncogenic KRAS and its effector signaling contribute to pancreatic cancer initiation, development and progression (65, 66). Therefore, farnesylation was one of the first proteins proposed as a potential therapeutic target for pancreatic cancer, and inhibitors of farnesylation have been in development since the early 1990s (67). Over the last two decades, farnesylation inhibitors have been evaluated for their ability to inhibit the oncogenic function of KRAS and to induce apoptosis in pancreatic cancer cells (68). One of these inhibitors, tipifarnib (Zarnestra, R115777; PubChem CID: 159324), was evaluated in a phase II trial for pancreatic cancer patients with advanced ‘systemic therapy-naïve’ disease

(69). This randomized, double-blind, placebo-controlled phase II study compared tipifarnib + gemcitabine with placebo + gemcitabine. Tipifarnib (200mg BID) was given on an oral daily dosing schedule and gemcitabine (1,000 mg/m^2 weekly dosing) was administered for 7 consecutive weeks on an 8 week cycle, followed by 3 consecutive weeks on a 4 week cycle (69). This combination did not prolong overall survival, compared to placebo + gemcitabine. Evaluation of potential uses for this agent is ongoing in a phase II trial for patients with HRAS-mutant head and neck cancer (NCT02383927) (70).

Interestingly, when farnesyltransferase is inhibited KRAS is geranylgeranylated (66). Therefore, it was suggested that simultaneous inhibition of both prenylation processes might have anti-proliferative effects. To address this hypothesis, Lobell et al. (2001), for example, compared the cytotoxicity of farnesnyltransferase inhibitors FTY-I or -II and the geranylgeranyl transferase inhibitors GGTI-I or II as single agents or in combination in multiple *in vitro* models that included the PSN-1 pancreatic cancer cell line (65). These investigators observed potent inhibition of pancreatic cancer cell proliferation by the combination. However, subsequent preclinical *in vivo* studies by this group demonstrated that the combination was toxic, and anti-tumor efficacy could not be evaluated (65). More recently, Sebti et al. (2018) reported that the 'dual farnesyl and geranylgeranyl transferase inhibitor' FGTI-2734 (PubChem CID: 49783195) was effective in inhibiting tumor growth in *KRAS* mutant patient-derived xenograft models of pancreatic cancer (71). In depth clinical evaluation of this dual inhibitor in *KRAS* mutated pancreatic cancer is anticipated.

4. Agents targeting DNA Damage Response Pathways

Gemcitabine remains the standard of care for patients with pancreatic cancer. While gemcitabine may have multiple mechanisms of action, it is well documented to be a DNA damaging agent (72). Intuitively, effective combination therapies might include agents that augment or complement the efficacy of this front-line agent by inhibiting repair of the DNA damage induced by gemcitabine. The predominant approaches toward this end have focused on either inhibiting DNA repair itself or on inhibiting the function of cell cycle checkpoint regulators to allow progression of cells through the cell cycle even though DNA damage is present. Either approach would be envisioned to induce apoptosis, when combined with a DNA damaging agent such as gemcitabine. This section provides a rationale and summary for preclinical and clinical efforts to develop inhibitors of cell cycle regulatory proteins Chk1, WEE1, or ATR or indirect inhibition of DNA repair using PARP inhibitors that can be effectively combined with gemcitabine. Table 2 summarizes combinations discussed in section 4.

4.1. Checkpoint kinase 1 (Chk1) inhibitors

Phosphorylation of Chk1 at Ser317 and/or Ser345 by protein kinases activates Chk1. Activation of Chk1, in turn, deactivates CDC25A and arrests cells in the G2 phase of the cell cycle (73). DNA damaging agents, such as gemcitabine, induce phosphorylation of Chk1 and arrest cell cycle progression, to allow repair of DNA damage before progression through the cell cycle. Inhibition of Chk1 would be predicted to minimize cell cycle arrest and allow cells to proceed through the cell cycle in the presence of DNA damage, resulting in

accumulation of damage and induction of apoptosis. Parsels et al. (2009) evaluated this strategy using pancreatic cancer cell line models and the small molecule Chk1 inhibitor PD-321852 in combination with gemcitabine (73). This study demonstrated that PD-321852 1) increased gemcitabine-induced apoptosis up to 17-fold at IC_{50} concentrations, 2) increased gemcitabine-induced levels of the DNA damage marker γH2AX ($P<0.05$), and 3) inhibited the formation of gemcitabine-induced RAD51 foci >10-fold (74). Further, Engelke, et al. (2013) reported that a second Chk1 inhibitor, MK-8776, sensitized homologous recombination repair proficient pancreatic cancer cell lines to gemcitabine + radiation *in vitro* and *in vivo* (75). A phase I dose escalation trial of the Chk1 inhibitor MK-8776 with or without gemcitabine for patients with advanced solid tumors recommended doses of MK-8776 200 mg/m^2 + gemcitabine 1,000 mg/m^2 for phase II trials (NCT00779584) (76). A subsequent phase II trial was conducted to assess the impact on overall survival of the Chk1 inhibitor LY2603618 with or without gemcitabine in 99 patients with stages II-IV pancreatic cancer, but LY2603618 + gemcitabine failed to demonstrate better outcome than LY2603618 (NCT00839332) (77). The study concluded that the data did not support further investigation of this combination for the treatment of pancreatic cancer patients.

4.2. Wee1-like protein kinase (WEE1) inhibitors

WEE1 belongs to the Ser/Thr protein kinase family that inhibits cyclin dependent kinase 1 (CDK1) activity, to prevent cells from proceeding through the G2 phase of the cell cycle (78). WEE1 is upregulated or activated by genotoxic stress as would be mediated by cytotoxic agents, and this upregulation or activation results in G2/M arrest (79). The WEE1 inhibitor MK-1775 (AZD1775, Adavosertib) sensitized DNA damage and repair (DDR)-proficient pancreatic cancer cells to gemcitabine + radiation (80).

Interestingly, Rajeshkumar et al. (2011) showed that MK-1775 + gemcitabine had greater antitumor efficacy in p53 mutated patient-derived xenograft models of pancreatic cancer than in p53 wild type models (81). The combination of MK-1775 + gemcitabine + radiation in patients with locally advanced pancreatic cancer was well tolerated in a completed phase I trial (NCT02037230) (82).

4.3. Ataxia-telangiectasia and RAD3-related (ATR) kinase inhibitors

ATR kinase contributes to the DNA damage response (DDR) pathway by detecting single- and double-strand DNA breaks and other types of genotoxic stress, and transducing signals to effector proteins to initiate DNA repair and halt cell cycle progression (83, 84). Chk1 is thought to be a direct downstream effector of ATR; therefore, an ATR inhibitor would be predicted to allow cell cycle progression in the presence of DNA damage. Prevo et al. reported that the ATR inhibitor VE-821 sensitized pancreatic cancer cells to gemcitabine ($P<0.05$) and to radiation ($P<0.01$) *in vitro* (85). Further, Fokas et al. showed that the ATR inhibitor VE-822 + gemcitabine + radiation delayed the progression of pancreatic tumor xenografts, compared to the gemcitabine + radiation ($P<0.001$) (85, 86). Wallez et al. (2018) used murine and human pancreatic cancer cell lines to demonstrate that a second ATR inhibitor, AZD6738 (Ceralasertib, PubChem CID: 54761306), inhibited Chk1 activation and augmented the effect of gemcitabine (87). Further, combined administration of gemcitabine

+ AZD6738 induced regression of allografts derived from *KRAS*- and *TP53*-mutant KPC mouse pancreatic cancer cell lines (87). The authors suggested further clinical investigation of this combination is warranted. AZD6738 is in a phase II trial as a single agent and also in combination with the PARP inhibitor olaparib in patients with solid tumors including all stages of pancreatic cancer (NCT03682289). Plummer et al. (2017) reported a phase I trial of ATR inhibitor VX-970 (M6620) in combination with gemcitabine in patients with advanced solid tumors including 2 pancreatic cancer patients (NCT02157792) (88). This trial is currently active.

4.4. Poly (adenosine diphosphate [ADP]-ribose) polymerase (PARP) inhibitors

PARP proteins are involved in many essential cell functions, including cell proliferation and death (89, 90). Of particular relevance to this review is the role of PARP enzymes in DNA repair. In 2005, the Ashworth group described the utility of PARP inhibitors in BRCA mutant cancer cells, based on the concept of 'synthetic lethality' (91-94). Synthetic lethality refers to conditions in which cells remain viable when each of two (or more) genes is dysfunctional, but cell death occurs when these genes are simultaneously dysfunctional. BRCA1/2 and PARP proteins both have DNA repair functions: BRCA1 and BRCA2 participate in homologous recombination repair of DNA double-strand breaks and PARP proteins function as DNA repair enzymes when mutations render BRCA1/2 nonfunctional (92, 95). Therefore, inhibiting PARP would be predicted to sensitize cancer cells with BRCA mutations, and selectively target cells with this genotype.

Preclinical data from multiple laboratories provide compelling data to support this concept (92, 96). Accordingly, the PARP inhibitor olaparib (PubChem CID: 23725625) was approved for the treatment of BRCA mutated ovarian cancer in 2018 (NCT02000622) and the PARP inhibitor talazoparib was approved for metastatic breast cancer in 2019 (NCT01945775) (97). The first phase I trial with olaparib + gemcitabine in patients with advanced pancreatic cancer was reported in 2015 (98). This randomized, dose escalation study showed that olaparib (100 mg BID, days 1-14) plus gemcitabine (600 mg/m^2, days 1, 8, and 15) every 4 weeks was tolerated and without unexpected toxicities. These data support further clinical evaluation of olaparib + gemcitabine for patients with pancreatic tumors. A phase III trial to evaluate olaparib monotherapy in BRCA mutant metastatic pancreatic cancer demonstrated a median progression free survival of 7.4 months, compared to 3.8 months for placebo (P=0.004) (NCT02184195) (99). Additional studies using PARP inhibitors in combination with other targeted agents are discussed below.

5. Histone Acetylation Modulators

In addition to genetic lesions such as mutations in *KRAS* and *TP53*, other factors that impact therapeutic efficacy include the family of enzymes that regulate acetylation and deacetylation of lysine residues on chromatin associated histones (100, 101). Transcription of genes is regulated in part by acetylation and deacetylation of histones, a primary determinant of chromatin structure (102). In general, acetylated histones are thought to confer a more 'open' structure that supports transcription (103). Recent evidence suggests that the rate of acetylation/deacetylation also contributes to efficiency of transcription (104,

105). While it seems counterintuitive that both acetylation inhibitors and deacetylation inhibitors would have antiproliferative effects, preclinical data clearly support this conclusion. This section will discuss studies that focus on agents that inhibit the function of enzymes that acetylate or deacetylate chromatin-associated histones, and will also discuss the potential utility of each type of inhibitor.

Table 3 summarizes combinations discussed in sections 5 and 6.

5.1. Histone Acetyltransferase (HAT) inhibitors

HATs are enzymes that add acetyl groups to lysine residues on histones or other proteins (106, 107). One of the synthetic small molecule HAT inhibitors that has been evaluated *in vitro* using pancreatic cancer cell lines is the p300 inhibitor C646 (108). Ono et al. (2016) reported that siRNA-mediated down regulation of p300 inhibited pancreatic cancer cell proliferation and increased levels of the apoptosis markers cleaved caspase 3,8,9 and cleaved PARP; therefore, inhibition of p300 activity would be anticipated to increase apoptosis in pancreatic cancer cells. Notably, C646 enhanced gemcitabine cytotoxicity in *in vitro* models (108); but preclinical studies or clinical trials with C646 have not yet been reported. The natural product curcumin is also thought to inhibit p300 HAT activity (109). The results of a phase II study with pancreatic cancer patients who received curcumin + gemcitabine have also not yet been reported (NCT00192842).

5.2. Histone Deacetylase (HDAC) inhibitors

The FDA has approved four HDAC inhibitors: vorinostat, romidepsin, belinostat and panobinostat. More than fifteen additional inhibitors are in phase I-III clinical trials (110).

5.2.1. Vorinostat (suberoylanilide hydroxamic acid [SAHA], Zolinza, PubChem CID: 5311)—Vorinostat is a pan HDAC inhibitor initially approved for the treatment of cutaneous T cell lymphoma (111). Two of the first studies to evaluate vorinostat in pancreatic cancer cell lines were those of Garcia-Morales et al. in 2005 and of Arnold et al. in 2007 (112, 113). These studies demonstrated that vorinostat inhibited the proliferation of pancreatic cancer cells and induced apoptosis. In 2008, Zhang et al. demonstrated that the tyrosine kinase inhibitor sorafenib + vorinostat was synergistic in several cell line models including pancreatic cell lines (114). Information regarding vorinostat in the clinical setting is relatively limited. A phase I trial with the combination of sorafenib + vorinostat with gemcitabine and radiation in pancreatic cancer patients is ongoing (NCT02349867). Data from a second phase I trial with vorinostat (100-400 mg daily) + capecitabine (100 mg QID on the days of radiation) + radiation (total of 30 Gy) determined an maximum tolerated dose (MTD) for vorinostat of 400 mg daily, and a median overall survival of 1.1 years (95% confidence interval 0.78-1.35) for patients with advanced pancreatic cancer (115)

5.2.2. Romidepsin (Istodax, PubChem CID: 5352062)—The HDAC inhibitor romidepsin was approved for the treatment of cutaneous T-cell lymphoma in 2006 (116). A phase I dose escalation trial of romidepsin + gemcitabine with advanced solid tumors including pancreatic cancer recommended doses of romidepsin of 12 mg/m^2 and of

gemcitabine of 800 mg/m^2 (NCT00379639) (117). The authors suggest that this combination will require additional trials to evaluate safety and efficacy.

5.2.3. CI-994 (Tacedinaline, PubChem CID: 2746)—A phase II randomized clinical trial performed for the combination of gemcitabine + CI-994 for patients with advanced pancreatic cancer showed that the combination had no advantage over gemcitabine as a single agent in this cohort of patients with advanced disease (NCT00004861) (118).

5.2.4. MGCD0103 (Mocetinostat, PubChem CID: 9865515)—Data are not yet available for the phase I/II clinical trial of MGCD0103 + gemcitabine (NCT00372437) in patients with solid tumors.

The paucity of published preclinical and clinical data precludes conclusions regarding the utility of HAT inhibitors and HDAC inhibitors as single agents or in combination with gemcitabine, for treating patients with pancreatic cancer.

6. Bromodomain and extra-terminal (BET) bromodomain inhibitors

BET bromodomain proteins bind to acetylated lysine residues on specific chromatin-associated histones (103, 119). Proteins of this family (BRD2, BRD3, BRD4, BRDT) regulate transcription by controlling the binding of BET-dependent transcriptional complexes to promoter and enhancer regions of specific genes (103, 119). BET inhibitors are considered to function as acetylated lysine (K-Ac) mimetics that competitively inhibit the association of BET proteins with histones. This inhibition minimizes the recruitment of BET-dependent transcriptional complexes and inhibits transcription of a large subset of genes (103, 119, 120). The subset of genes affect by each family member may be cell type selective. BET bromodomain inhibitors such as JQ1 and I-BET762 were initially reported about ten years ago, and numerous published studies evaluate the potential use of this agent particularly in tumor types that overexpress the oncogene c-Myc (120-122). One of the first studies to evaluate the utility of BET inhibitors in pancreatic cancer cell lines *in vitro* showed that JQ1 or I-BET151 inhibited tumor cell proliferation in three-dimensional collagen (123). This study also determined that these BET inhibitors decreased expression of FOSL1 and high mobility group AT-Hook 2 (HMGA2) proteins in pancreatic cancer cells. Notably, HMGA2 is reported to confer resistance to gemcitabine in pancreatic cancer cells (124, 125).

Subsequently, our laboratory reported that the BET inhibitor JQ1 suppressed the growth of five patient-derived xenograft (PDX) models of PDAC (126). Using tumor models derived from independent primary human tumor specimens, we demonstrated that JQ1 suppressed tumor growth and decreased expression of the G2/M cycle regulator CDC25B. However, JQ1 as a single agent did not induce tumor regressions, and work is ongoing to evaluate the efficacy and toxicity of JQ1 in combination with other agents. Recently published results with JQ1 + olaparib are described below.

6. 1. JQ1 (BET inhibitor, PubChem CID: 46907787) + olaparib (PARP inhibitor, PubChem CID: 23725625): Preclinical study

In the preclinical studies mentioned above in which we observed that JQ1 as a single agent inhibited the growth of early passage tumors of human origin, we also observed that a well-tolerated regimen of JQ1 increased levels of the DNA damage marker γH2AX *in vivo* (127). Further, and consistent with the observed increase in DNA damage, this regimen of JQ1 inhibited DNA repair proteins Ku80 and RAD51. Based on these observations, we hypothesized that simultaneous inhibition of DNA repair by decreasing expression of Ku80 and RAD51 with JQ1 and inhibition of PARP activity with targeted small molecule PARP inhibitors would comprise effective 'therapy' (Figure 1). Sequential administration of the PARP inhibitor olaparib followed by the BET inhibitor JQ1 suppressed pancreatic tumor growth in two PDX models, and the efficacy of the combination was greater than either drug alone (127). No toxicity was observed. These preclinical data are encouraging, and suggest that combinations of BET inhibitors + PARP inhibitors warrant further investigation.

6. 2. JQ1 (BET inhibitor) + vorinostat (HDAC inhibitor): Preclinical study

Mazur et al. reported that the BET inhibitor JQ1 25 mg/kg twice daily + the HDAC inhibitor vorinostat (SAHA) 25 mg/kg daily increased the median survival of *Kras:p53* mutant genetically engineered mouse models of pancreatic cancer, compared to vehicle ($P<0.001$) or JQ1 alone ($P<0.05$) (128). This regimen also decreased tumor volume in two patient-derived PDX models of PDAC, compared to vehicle or JQ1 alone. No systemic toxicity or weight loss was observed with JQ1 + vorinostat.

7. Other Combinations Using Targeted Agents

7.1. MEK inhibitor + PI3K/mTOR (Phosphoinositide 3 kinase/Mammalian target of rapamycin) inhibitor

Alagesan et al. (2015) evaluated the combination of a MEK and a PI3K inhibition in a *Kras:p53* mutant genetically engineered mouse model (GEMM) of pancreatic cancer (129). These investigators identified the MEK1/2 inhibitor AZD6244 (Selumetinib, PubChem CID: 10127622) as an effective inhibitor of pancreatic cancer cell viability using high-throughput screening (129). Subsequently, these investigators evaluated the efficacy of AZD6244 as a single agent or in combination with the PI3K inhibitor BKM120 (Buparlisib, PubChem CID: 16654980) or GDC-0941 (Pictilisib, PubChem CID: 17755052) in GEM models of PDAC (129). The combination of AZD6244 + BKM120 delayed development of detectable PDAC tumors from 31.5 days in the control group to 71 days in the group receiving the combination. Further, AZD6244 + BKM120 or AZD6244 + GDC-0941 reduced tumor size in 80% of mice compared to either drug alone. Notably these results were obtained when treatment was delayed until mice were 12 weeks of age, to mimic treatment of late stage PDAC. These investigators concluded that simultaneous inhibition of MEK and PI3K may have utility in pancreatic cancer patient populations.

A few clinical trials have been conducted with combinations of a MEK and a PI3K inhibitor for locally advanced or metastatic solid tumors, including pancreatic cancer. Trial NCT01347866 evaluated the PI3K/mTOR inhibitor PF-05212384 (Gedatolisib, PubChem

CID: 44516953) + the MEK inhibitor PD-0325901 (PubChem CID: 9826528) or PF-05212384 + irinotecan. No results are available from that study, as it was terminated subsequent to internal prioritization review but not due to safety or efficacy concerns. Another phase I dose escalation trial NCT01390818 evaluated the combination of MEK inhibitor pimasertib (AS703026, MSC1936369B) with PI3K/mTOR inhibitor voxtalisib (SAR245409, PubChem CID: 16123056) in patients with locally advanced or metastatic solid tumors. Data from this study indicated that the combination was tolerated and a phase II trial was planned (130).

7.2. MEK inhibitor + EGFR inhibitor

Based on their earlier findings that MEK inhibition activated PI3K and EGFR pathways, Mirzoeva et al. (2013) evaluated the potential utility of simultaneous inhibition of MEK and EGFR pathways (131). These investigators demonstrated that MEK inhibitor (CI1040, PubChem CID: 6918454) + the EGFR inhibitor erlotinib were synergistic *in vitro* in PDAC cell lines. Further, PD0325901 7.5 mg/kg daily + erlotinib 35 mg/kg daily x 34 days inhibited the growth of HPAF-II cell line-derived xenograft tumors, compared to the control group or to groups that received a single agent. The effect of the combination was regarded as additive (131). Escalation of these doses to 12.5 mg/kg of PD-0325901 and 50 mg/kg for erlotinib was not tolerated, and the study was stopped at day 18. Based on these *in vitro* and preclinical findings, a phase II trial was conducted with the MEK1/2 inhibitor selumetinib + the EGFR inhibitor erlotinib for patients with chemorefractory advanced pancreatic ductal adenocarcinoma (132). Results from this trial indicated that 41% of patients had stable disease for over 6 weeks, with a median progression-free survival of 1.9 months and a median overall survival of 7.3 months. While the effect of the combination in this cohort of patients with advanced disease was modest, strong supporting mechanistic, *in vitro* and preclinical data suggest that it may be worthwhile to evaluate similar combinations in patients with lesser stage disease.

7.3. Anti-EGFR monoclonal antibody (Cetuximab) and anti-HER-2 monoclonal antibody (Trastuzumab)

Several reports in the literature suggest a potential anti-tumor effect for cetuximab + trastuzumab. Larbouret et al. (2007) documented that EGFR (HER-1) is expressed in 45-95% and HER-2 is expressed in 43-69% of pancreatic tumors (133). Worthylake et al. (1999) showed that HER-2 overexpression activates EGFR signaling by inhibiting EGFR internalization (134). Based on the possibility that simultaneous inhibition of both EGFR (HER-1) and HER-2 receptors would be synergistic and decrease the viability of pancreatic cancer cells, Larbouret et al. (2007) used cetuximab and trastuzumab to inhibit HER-1 and HER-2 activity, respectively, in a pancreatic cancer cell line-derived xenograft model. The combination had greater anti-tumor activity than either antibody alone (133). Based on this report, a phase I - II trial (THERAPY trial) in patients with metastatic pancreatic cancer was initiated. The phase I trial escalated the dose of trastuzumab with a fixed dose of cetuximab of 400 mg/m^2 on day 1, weekly (135). The primary endpoint of the phase II trial was to achieve an objective response rate (ORR) of 5-20%, but results did not meet this criterion. Investigators who conducted these phase I-II trials suggested that definitions of dose limiting toxicities for ‘non-cytotoxic agents’ such as monoclonal antibodies may require different

evaluation criteria than for cytotoxic agents, as adverse events such as the cutaneous lesions associated with these antibodies are not life-threatening, and traditional dose escalation studies may be inappropriate for therapeutic antibodies.

7.4. Immune checkpoint inhibitors

An emerging field of investigation for the treatment of solid tumors focuses on inhibiting the function of proteins that suppress the immune response, thereby allowing activation of endogenous immune response pathways. This type of inhibitor is termed an immune checkpoint inhibitor. Inhibition of immune checkpoint proteins such as PD-1 allows T cell activation, to facilitate the immune suppressive and anti-proliferative functions of these cells (136, 137). Thus far, most immune checkpoint inhibitors are monoclonal antibodies. Nivolumab, pembrolizumab and pidizumab, for example, bind to and inhibit the function of the immune checkpoint protein PD-1 (programmed cell death protein-1). Durvalumab inhibits PD-L1 (programmed death-ligand 1); ipilimumab and tremelimumab inhibit CTLA-4 (cytotoxic T lymphocyte-associated protein 4) (138, 139). More than ten phase I and II trials with immune checkpoint inhibitors (nivolumab, pembrolizumab or ipilimumab) are ongoing (140). A major advantage to this approach is the specificity of the therapeutic monoclonal antibody for its target protein.

However, unlike the clinical successes seen with these inhibitors for patients with, for example, lung adenocarcinoma and breast cancer, the strategy of inhibiting immune checkpoints to allow immunologic responses has been less successful in patients with pancreatic cancer (141). For example, O'Reilly et al. (2019) reported the first phase II trial of durvalumab ± tremelimumab in patients with metastatic PDAC (142). All 64 patients that participated in that trial tolerated the combination well, but no clinical benefit was observed. The authors of that study suggested that combining an immune checkpoint inhibitor with agents having a different mechanism of action might be superior to using two immune checkpoint inhibitors (142). Multiple ongoing trials evaluate combinations of immune checkpoint inhibitors + gemcitabine to other chemotherapeutic agents (143). It would be of interest to determine if, for example, sequential administration of gemcitabine followed by an immune checkpoint inhibitor(s) would prolong stable disease or tumor regressions produced by gemcitabine. Table 4 summarizes combinations discussed in section 7.

8. Conclusion

This review provides a brief overview of preclinical and clinical efforts in the past two decades to develop effective combination therapies for the treatment of pancreatic cancer. While numerous combinations have been evaluated, gemcitabine has remained the standard of care since 1996, with FOLFIRINOX or gemcitabine + nab-paclitaxel as alternative front line combinations for patients with unresectable PDAC (144). However, most of the trials or approaches to develop novel combination therapies have been marginally successful in the clinic, and overall survival for this tumor type has improved little.

Recent laboratory studies have identified many combinations that are not worth pursuing. However, we propose that two combinations in particular appear to deserve additional evaluation. These combinations are: 1) the BET inhibitor JQ1 + the PARP inhibitor olaparib,

which was evaluated in two PDX models of PDAC (see section 6.1); and 2) the ATR inhibitor AZD6738 + gemcitabine (see section 4.3). With respect to the first of these combinations, our laboratory observed that a nontoxic regimen of JQ1 + olaparib induced stable disease and was more effective than either drug as a single agent ($P<0.0001$ to <0.05) in PDX models of PDAC (127). More than ten phase I-II trials are ongoing to evaluate BET inhibitors for the treatment of different types of solid tumors. Trials to evaluate BET inhibitors + with other classes of agents such as PARP inhibitors are anticipated. With respect to the second of these combinations, Wallez et al. observed that the ATR inhibitor AZD6738 + gemcitabine induced regressions in PDAC allograft models, and multiple phase I-II trials are underway using ATR inhibitors such as VX-970 or AZD6738 in combination with other classes of therapeutic agents in hematologic and solid tumor settings (87). A trial with AZD6738 + gemcitabine in patients with pancreatic cancer is also anticipated.

It is well accepted that combination therapies are usually more effective than monotherapies. We propose that effective combination therapies for pancreatic cancer will result from studies that focus on identifying agents that target genetic and molecular lesions common to pancreatic tumors and that synergize with gemcitabine, the current standard of care for this tumor type.

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Abbreviations:

PDAC	pancreatic ductal adenocarcinoma
5-FU	5-fluorouracil
EGFR	epidermal growth factor receptor
VEGF	vascular endothelial growth factor
MEK	mitogen-activated protein extracellular signal-regulated kinase (MAP2K)
ERK	extracellular receptor kinase (MAPK1)
Chk1	Checkpoint kinase 1
CDK1	cyclin dependent kinase 1
DDR	DNA damage and repair
ATR	ataxia-telangiectasia and RAD3-related
PARP	poly (adenosine diphosphate [ADP]-ribose) polymerase
HAT	histone Acetyltransferase

HDAC	histone Deacetylase
BET	bromodomain and extra terminal
PDX	patient-derived xenograft
PI3K	phosphoinositide 3 kinase
mTOR	mammalian target of rapamycin
GEMM	genetically engineered mouse model
PD-1	programmed cell death protein-1
PD-L1	programmed death-ligand 1

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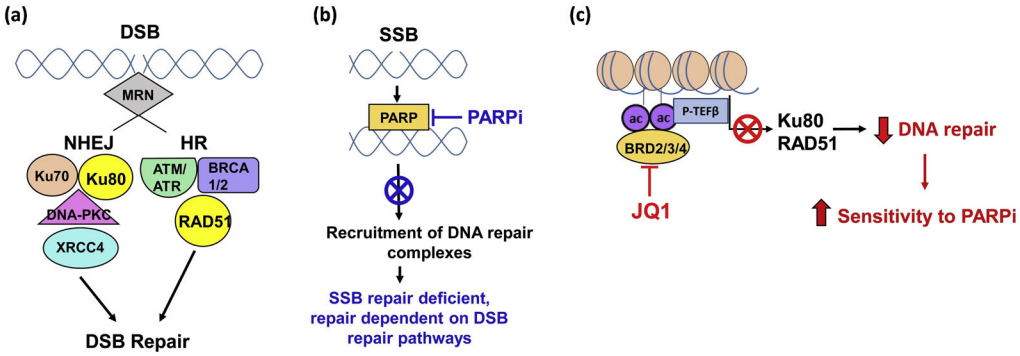

Figure 1. Model depicting complementary cytotoxicity of PARP inhibitors (PARPi) and JQ1 (BET bromodomain inhibitor).

(a) Schematic DNA double strand break (DSB) repair pathways: NHEJ and HR. **(b)** PARP recruits base excision repair (BER) enzymes to SSB loci. PARP inhibitors reduce SSB BER. Cells with reduced SSB repair result in DSB. **(c)** JQ1-mediated inhibition of BET protein (BRD2/3/4) activity inhibits expression of DSB repair proteins Ku80 and RAD51. Decreased DSB DNA repair sensitizes cells to PARP inhibitors. Multiple ongoing preclinical studies focus on the hypothesis that simultaneous inhibition of the expression or activity of enzymes that contribute to NHEJ, HR, and BER have complementary anti-tumor efficacy. **Black font = normal pathway. Red font = effect of JQ1. Blue font = effect of PARPi.** (Abbreviations: NHEJ, nonhomologous end joining; HR, homologous recombination; DSB, double-strand break; SSB, single-strand break; ac, acetylation).

Table 1.

Summary of preclinical and clinical studies of gemcitabine in combination with targeted agents to the most commonly dysregulated cell signal transduction pathways in pancreatic cancer.

Combination	Type of Study	NCT #	Status	Reference
EGFR Inhibitors				
gemcitabine + erlotinib	Clinical (Phase III)	NCT00026338	Completed	29, 30
gemcitabine + cetuximab	Clinical (Phase III)	NCT00075686	Completed	32,33,34
VEGF Inhibitor				
gemcitabine + bevazucimab	Clinical (Phase II)	NCT00028834	Completed	39
Notch Pathway (γ-Secretase Inhibitors)				
gemcitabine + PF-0384014 (Nirogacestat)	Preclinical			45
gemcitabine + MK-0752	Clinical (Phase I)	NCT01098344	Completed	46
Hedgehog Pathway (Smoothened inhibitors)				
gemcitabine + vismodegib	Clinical (Phase 1 b/II)	NCT01064622	Completed	53
gemcitabine + sonidegib	Clinical (Phase 1b)	NCT01487785	Completed	55
gemcitabine + nab-paclitaxel + visdemogib	Clinical (Phase II)	NCT01088815	Completed	56
sonidegib + pembrolizumab	Clinical (Phase I)	NCT04007744	Not yet recruiting (expected to begin in 02/15/2020)	
MEK Inhibitors				
gemcitabine + pimasertib	Preclinical			58
	Clinical (Phase II)	NCT01016483	Completed	61
gemcitabine + trametinib	Preclinical			59, 60
	Clinical (Phase II)	NCT01231581	Completed	62
Prenylation Inhibitors				
gemcitabine + tipifarnib	Clinical (Phase II)	NCT00005832	Completed	69

Table 2.

Summary of preclinical and clinical studies of agents targeting cell cycle checkpoint regulatory proteins and DNA damage and repair proteins.

Combination	Type of Study	NCT #	Status	Reference
Chk1 Inhibitor				
gemcitabine + PD321852	Preclinical			74
gemcitabine + MK-8776 + radiation	Preclinical			75
	Clinical (Phase I)	NCT00779584	Completed	76
gemcitabine + LY2603618	Clinical (Phase I/II)	NCT00839332	Completed	77
WEE1 Inhibitor				
gemcitabine + MK-1775 (Adavosertib) + radiation	Preclinical			80, 81
	Clinical (Phase I)	NCT02037230	Completed	82
ATR Inhibitors				
gemcitabine + VE-822 + radiation	Preclinical			86
gemcitabine + AZD6738 (Ceralasertib)	Preclinical			87
gemcitabine + VX-970	Clinical (Phase I)	NCT02157792	Active, Not recruiting	88
AZD6738 (Ceralasertib) + olaparib	Clinical (Phase II)	NCT03682289	Recruiting	
PARP Inhibitors				
gemcitabine + olaparib	Clinical (Phase I)	NCT00515866	Completed	98

Table 3.

Summary of preclinical and clinical studies evaluating the therapeutic efficacy of histone acetylation modulators in pancreatic cancer.

Combination	Type of Study	NCT #	Status	Reference
HAT inhibitors				
gemcitabine + C646	Preclinical			108
gemcitabine + curcumin	Clinical (Phase II)	NCT00192842	Completed, results not reported	109
HDAC Inhibitors				
vorinostat + sorafenib	Preclinical			114
gemcitabine + vorinostat + sorafenib + radiation	Clinical (Phase I)	NCT02349867	Recruiting	
vorinostat + capecitabine + radiation	Clinical (Phase I)	NCT00983268	Completed	115
gemcitabine + romidepsin	Clinical (Phase I)	NCT00379639	Completed	117
gemcitabine + CI-994 (Tacedinaline)	Clinical (Phase II)	NCT00004861	Completed	118
gemcitabine + MGCD0103 (Mocetinostat)	Clinical (Phase I/II)	NCT00372437	Completed, results not posted	
BET Inhibitors				
JQ1 + olaparib	Preclinical			127
JQ1 + vorinostat	Preclinical			128

Table 4.

Summary of preclinical and clinical studies evaluating combination of targeted agents in pancreatic cancer.

Combination	Type of Study	NCT #	Status	Reference
Combination of targeted agents				
selumetinib + BKM120 (Buparlisib) or GDC-0941 (Pictilisib)	Preclinical			129
PF-05212384 (Gedatolisib) + PD-0325901 or PF-05212384 + irinotecan	Clinical (Phase I)	NCT01347866	Terminated	
pimasertib + voxtalisib	Clinical (Phase I)	NCT01390818	Completed	130
erlotinib + CI1040	Preclinical			131
selumetinib + erlotinib	Clinical (Phase II)	NCT01222689	Completed	132
cetuximab + trastuzumab	Preclinical			133
	Clinical (Phase I/II)	NCT00923299	Completed	135
durvalumab + tremelimumab	Clinical (Phase II)	NCT02558894	Completed	142