Review

Molecular Cancer Therapeutics

ATM Dysfunction in Pancreatic Adenocarcinoma and Associated Therapeutic Implications

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) remains one of the most lethal solid malignancies with very few therapeutic options to treat advanced or metastatic disease. The utilization of genomic sequencing has identified therapeutically relevant alterations in approximately 25% of PDAC patients, most notably in the DNA damage response and repair (DDR) genes, rendering cancer cells more sensitive to DNA-damaging agents and to DNA damage response inhibitors, such as PARP inhibitors. ATM is one of the most commonly mutated DDR genes, with somatic mutations identified in 2% to 18% of PDACs and germline mutations identified in 1% to 34% of PDACs. ATM plays a complex role as a cell-cycle checkpoint kinase, regulator of a wide array of downstream proteins, and responder to DNA damage for genome stability. The disruption of ATM signaling leads to downstream reliance on ATR and CHK1, among other DNA-repair mechanisms, which may enable exploiting the inhibition of downstream proteins as therapeutic targets in *ATM*-mutated PDACs. In this review, we detail the function of ATM, review the current data on ATM deficiency in PDAC, examine the therapeutic implications of ATM alterations, and explore the current clinical trials surrounding the ATM pathway.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) remains one of the most lethal solid malignancies with fewer than 10% of patients surviving 5 years. Disease incidence is increasing, and PDAC is projected to be the second most common cause of cancer-related death by 2030 (1, 2). The high mortality is due to the majority of patients presenting with locally advanced or metastatic disease at the time of diagnosis. Unfortunately, despite recent improvements in outcomes with newer chemotherapy regimens, the median survival remains less than 1 year (3, 4). Comprehensive genetic analysis is being pursued to identify mutational pathways for potential treatment options, and a recent study utilizing genomic sequencing identified therapeutically relevant (highly actionable) alterations in 27% of PDAC samples (5). These findings were consistent with several other publications, all of which have demonstrated findings of actionable targets in 17% to 48% of PDAC samples (6–11). One commonality to these large-scale next-generation sequencing efforts is the identification of mutational defects in the genes that regulate the DNA damage response and repair (DDR) system, found in 17% to 25% of PDACs.

During the cell cycle, there is a replication of over 6 billion base pairs of DNA. Such genomic replication is subject to numerous insults and replication stressors, which rely on essential response and repair mechanisms to ensure DNA's integrity. Furthermore, the chemotherapies used to treat cancers, particularly pancreatic cancer, result in specific types of DNA damage. For example, alkylating agents, such as platinums, and topoisomerase inhibitors, such as irinotecan, cause double-strand DNA breaks (DSB), whereas antimetabolites such as 5-fluorouracil and gemcitabine cause singe base-pair damage that can lead to single-strand DNA breaks (12). Deficiencies in DDR mechanisms have revealed targets for therapy, and research has led to FDA-approved treatments targeting cancers that harbor such deficiencies. For example, BRCA1 and BRCA2 play an integral role in the maintenance of genomic integrity, and germline mutations in either gene lead to increased risks for breast, ovarian, pancreatic, and prostate cancers (13). However, the presence of a *BRCA1/2* mutation also predicts for an improved response and improved overall survival with platinum-based chemotherapy in both triple-negative breast cancers and PDAC (14, 15). Exploiting this DNA-repair defect not only improves sensitivity to chemotherapy, but also allows targetable therapy through the inhibition of the poly [adenosine diphosphate (ADP)–ribose] polymerase (PARP), leading to the accumulation of single-strand breaks which compromise DNA double-strand integrity at the replication fork. PARP inhibitors increase progression-free survival in advanced *BRCA1/2*-mutated ovarian and breast cancer (16–18), and are now FDA approved for these diseases. Additionally, responses to PARP inhibitors are also frequently seen in *BRCA*-mutated castrate-resistant prostate cancer with, for example, a response rate of 88% in a 50-patient trial; and in *BRCA*-mutated pancreatic cancer, with responses of 16% to 22% in small trials of 19 and 23 patients, respectively (16, 19–21).

ATM also plays a critical role in DDR. The ataxia telangiectasia mutated (ATM) gene, located on chromosome 11q 22–23, was first identified in 1995 during the evaluation of the ataxia telangiectasia syndrome. Germline mutations of *ATM* result in a

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well-characterized syndrome, as well as an increased predisposition for breast, pancreatic, and prostate cancers (22–28). Relevant here, mutations in the *ATM* gene, whether germline or somatic, are found in up to ∼6% of PDACs (further details below), and thus may represent a more prevalent DDR mutation than *BRCA1/2* (7). In this review, we detail the function of ATM, review the current data on ATM deficiency in PDACs, and examine the therapeutic implications of ATM alterations.

Function of ATM

The ATM gene consists of 66 exons that encode a PI3K-related serine/threonine protein kinase that plays a central role in the response to, and ultimately the repair of DNA DSB. Structurally, this large protein (350 kDa) contains serine or threonine residues susceptible for phosphorylation, followed by a glutamine amino acid located near its hydrophobic target region. Similar sites for posttranslational modifications (PTM) are found in the ataxia telangiectasia and RAD 3-related (ATR) kinase and in the DNA protein kinase (DNA-PK) proteins (29). ATM has important functions in the cell, including the maintenance of (i) telomere length (30, 31) and (ii) the mitotic spindle structure during mitosis (32). However, this review solely focuses on the central role of ATM in the process of DDR, including as it relates to targeted therapies in cancer. As depicted in Fig. 1, in order to repair damaged DNA, the MRE11-RAD50-NBS1 (MRN) complex acts as the primary sensor for DSBs and creates a physical bridge between the two broken ends (33). ATM can then interact directly with NBS1 (part of the MRN complex) through the direct binding of the C-terminus of NBS1 to several of the HEAT repeats that reside in ATM (34). It is believed that several PTMs are required for subsequent ATM activation. For instance, ATM has been shown to be activated through acetylation of K3016 by TIP60, a histone acetyltransferase that binds to ATM through recognition of the C-terminal FATC domain (35). ATM also requires autophosphorylation at S1981, which allows the kinase domain to dissociate from the FAT domain, enabling, in turn, the kinase to become active (36). These modifications allow ATM to transition from an inactive homodimer into an active monomer in response to DNA damage (36). This mechanism has been supported in the literature, but also questioned by others, demonstrating the need for further work in the field to clearly identify the role of S1981 and other ATM autophosphorylation events (29, 36, 37). Once activated, ATM phosphorylates multiple substrates, protein kinases, and sensor proteins in order to carry out DSB repair and also regulate normal cell-cycle processes, such as apoptosis and checkpoint activation (36, 38, 39).

ATM plays a role in the signaling required to initiate DNA repair, and thus, ATM defects can lead to genomic instability and malignancy. Hereditary and sporadic *ATM* mutations span the functional domains of the entire *ATM* gene (Fig. 2). These mutations occur mostly in the C-terminal end, which interacts with the PI3 kinase domain. This domain is involved with acetylation and activation of ATM (40). DDR is impaired when the ATM protein is dysfunctional, and loss of this DDR mechanism designed for DSBs can possibly lead, over time, to the accumulation of mutations, which can, in theory initiate the process of tumorigenesis. For instance, germline point mutations in *ATM* result in increased risks of breast cancers, specifically those associated with the S49C and S707P mutations. Melanoma, prostate, and oropharyngeal cancers are specifically associated with the S49C mutation. Although thyroid or endocrine cancers are generally associated with the S707P mutation (41, 42). Next-generation sequencing has also revealed somatic *ATM* mutations in many tumor types, including PDAC (Fig. 2; ref. 40).

Inactivating ATM Variants in Pancreatic Cancer

Multiple studies have reaffirmed the importance of *TP53*, *KRAS*, *CDKN2A*, and *SMAD4* mutations in PDAC (7, 11, 43, 44). Inherited risks of PDAC are also well established to be in large part due to germline mutations in *BRCA1/BRCA2* and *CDKN2A*, identified in 7.4% of familial pancreatic cancers ($N = 727$; ref. 45), as well as individuals affected by Lynch syndrome (mutations in the genes: *MLH1*, *MSH2*, *PMS2*, *and MSH6*; ref. 46). Additionally, a study of familial PDAC patients showed that deleterious *ATM* mutations were significantly higher than the control group, suggesting ATM's role in malignancy (25). This knowledge has led to germline and somatic mutations in *ATM* as being identified and added to the catalog of predisposing gene mutations.

A genomic characterization of PDAC revealed 37% of the 71 samples carried alterations in DNA-repair genes (6). This significant discovery has also been confirmed in a recent large PDAC profiling study that identified targetable alterations in 50% of 640 PDAC patients, of which 8.4% expressed BRCA1/2 or ATM mutations (5). A review of the International Cancer Genome Consortium, a large database of sporadic PDACs, in 2015 identified *ATM* mutations in 9% to 18% of PDACs, with an average of 12% of the 591 samples (47). An additional large population study that identified the prevalence of homologous recombination–related gene mutations in 15.4% of PDACs (48). This study also revealed that *ATM*, *ATRX*, and *CHEK2* mutations are present in 1.3% of 50,000 tumor samples. These mutations were most prevalently identified in PDAC (48). Further genomic studies support the high prevalence of *ATM*, *CHEK2*, and *ATR* mutations in PDAC (8). Our literature search, capturing 5,234 pancreatic cancer patients overall, shows that the total prevalence of ATM mutations (germline or somatic) in PDAC is 6.4% (range, 1%–34%; refs. 5, 6, 8, 9, 11, 25, 47–57). Importantly, in one study that showed that nearly 10% of PDAC patients carried a germline *ATM* mutation, in 44% of these patients a somatic second hit was identified (58). Although this loss-of-heterozygosity (LOH) seems to occur frequently in tumors arising from patients with germline *ATM* mutations (e.g., as seen in breast and pancreatic cancers; refs. 59, 60), the necessity of LOH to confer therapeutic sensitivity (i.e., to platinums and DDR inhibitors, as discussed below) is uncertain.

ATM Deficiency: A Therapeutic Opportunity?

It is now well established that tumor cells with underlying defects in DDR are exquisitely sensitive to DNA-damaging agents, notably platinums, and more recently to PARP inhibitors in a phenomenon known as synthetic lethality (61). Similarly, inactivating mutations in *ATM* can also set up synthetic lethality in the presence of DNA-damaging agents (62). Historically, ataxia telangiectasia patients were found to be profoundly radiosensitive at the chromosomal level, suggesting the link between ATM and DNA repair (63). Preclinical data have demonstrated that

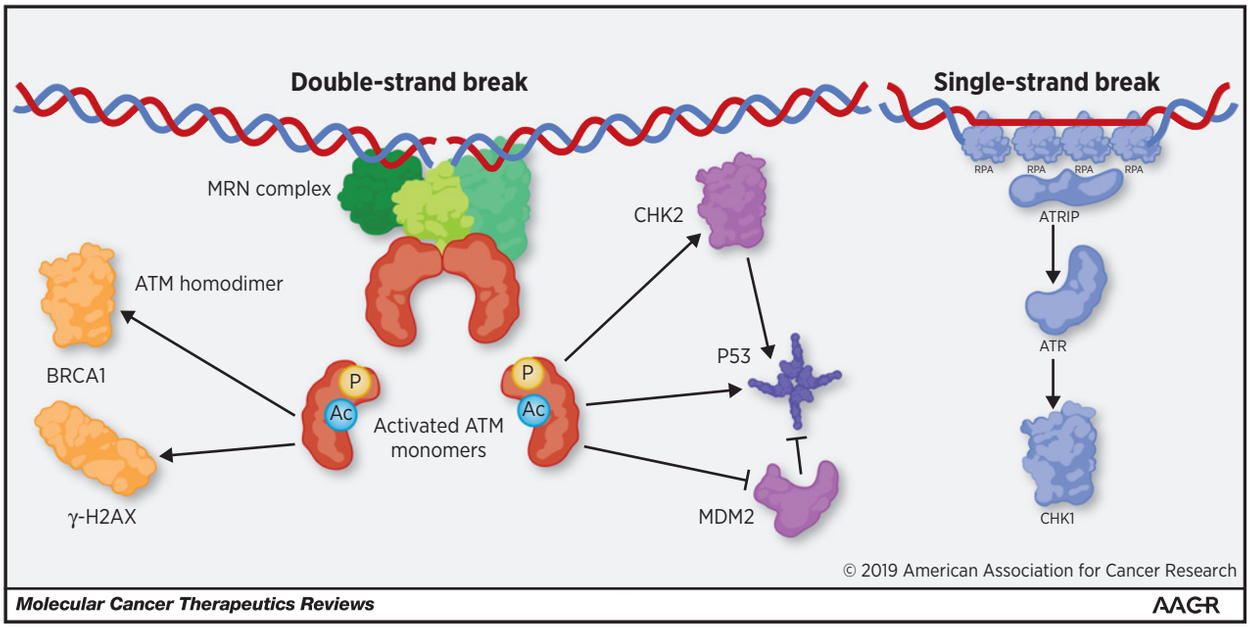

Figure 1.

ATM functions and other related pathways for DNA repair. ATM is recruited to DSBs by the MRN complex through direct interaction of NSB1 with ATM's HEAT repeats. ATM is then activated through autophosphorylation, and acetylation by TIP60; this activation allows ATM to dissociate to the active monomeric state. ATM monomers can then signal for DNA repair through BRCA1 and γ-H2AX. ATM can also signal for cell-cycle arrest and/or apoptosis through the activation of p53 through direct phosphorylation and indirect activation through CHK2 and MDM2. In parallel, ATR is recruited to long stretches of single-strand DNA caused by single-strand breaks, the resection of DSBs, or replication stress. PARP1 is another factor that is critical for the repair of single-strand breaks.

knockdown of ATM results in radiosensitization (64, 65). Additional studies in the laboratory have demonstrated that ATM alterations in malignant cells can sensitize cells to platinum drugs (44, 66), and outside of platinum therapy, preclinical studies have also demonstrated ATM suppression in p53-deficient mouse fibroblasts sensitizes them to doxorubicin (67). Previous work has also demonstrated that ATM deficiency in *p53*-deficient cell lines causes a modest increase in 5-FU sensitivity (68). Lastly, one study took advantage of *ATM*-mutated PDAC cells in a mouse model and showed that treatment with the PARP inhibitor olaparib or the ATR inhibitor VE-822 led to dramatic accumulation of DSBs and reduced tumor cell viability *in vitro* and *in vivo* (62). The authors noted the compensation of alternate signaling routes to bypass ATM deficiency, including ATR in the replicative stress response. Thus, ATR inhibition was efficient in promoting intolerable mitotic damage, an effect that was enhanced when combined with gemcitabine (62).

The clinical experience in PDAC patients with confirmed *ATM*, *ATR*, or *CHEK2* mutations is very limited, and focused on the efficacy of oxaliplatin-based chemotherapy. One case series ($N = 71$) utilizing real-time whole-exome sequencing demonstrated that a majority of patients with such mutations experienced a partial response or stable disease with oxaliplatin-based chemotherapy (6). In this case series, 80% of those with *ATM*, *ATR*, or *CHEK2* mutations were treated with an oxaliplatin-based chemotherapy and 62.5% demonstrated partial response or stable disease on first follow-up scans (6). Another small study ($N = 13$) demonstrated a 37.5% response rate to oxaliplatin-based chemotherapy regimens in patients with DDR-mutated tumors (69). There was also a significantly longer progression-free survival compared with those patients whose tumors were DDR-nonmutated (20.8 months vs. 1.7 months, respectively $P = 0.049$; ref. 69). Specifically, 4 of 30 patients had known pathogenic *ATM* mutations, with at least one patient experiencing a prolonged partial response of nearly 40 months on 5-FU, irinotecan, and oxaliplatin (FOLFIRINOX; ref. 69). There are currently multiple ongoing trials targeting ATM-deficient tumors, including and especially with PARP inhibitors (Table 1).

Because ATM, ATR, and CHK1 are all important for resolving DNA damage (Fig. 1), utilizing an underlying DDR defect and inducing synthetic lethality by inhibiting an additional kinase is an innovative way to induce cancer cell death. The use of small-molecule inhibitors of ATM, ATR, and CHK1 is a promising avenue of cancer treatment due to the malignant cells' rapid and unregulated cell division. There are currently phase I and II clinical trials utilizing ATM or ATR inhibitors as monotherapy as well as in combination with chemotherapy (70, 71).

ATM inhibitors

The first compound described to inhibit ATM was wortmannin (72); however, there are now a host of newer, more potent compounds that inhibit ATM. One of the newer generation of ATM inhibitors published in 2004 was KU55933. This compound was shown to inhibit downstream ATM phosphorylation after radiation, and it also enhanced responses to the topoisomerase inhibitors etoposide, camptothecins, and doxorubicin (73). A similar sensitization to topoisomerase inhibitors was later demonstrated with the ATM inhibitor AZ31, which was shown to increase the efficacy of irinotecan in resistant tumors in PDX models (74). KU60019 is another compound that was introduced in 2009 as an improved analogue of KU55933 (75), and early work demonstrated that KU60019 is a potent radiosensitizer (75).

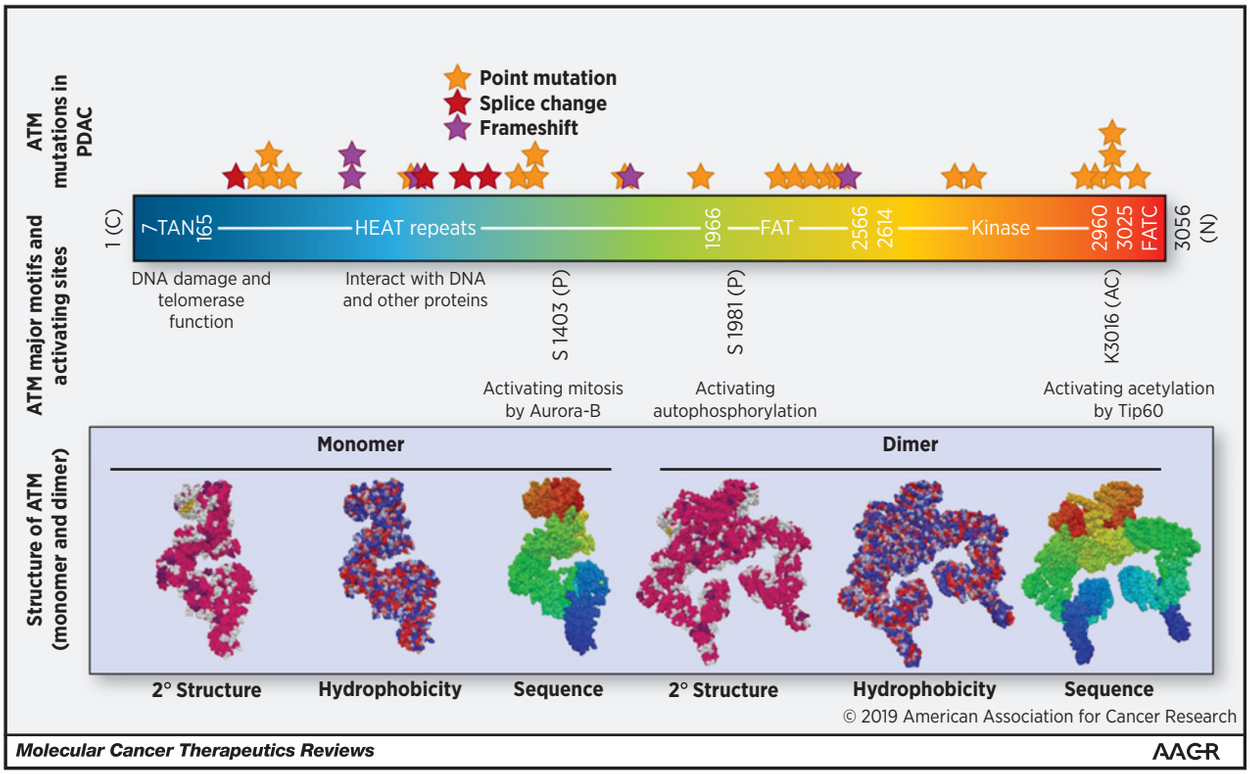

Figure 2.

ATM structure–function domains and frequent mutations in PDAC. ATM has several important domains that are critical for ATM function as either a monomer, dimer, or both. The TAN domain is critical for telomerase function and recruitment to DSBs. This recruitment is also dependent on interactions between the ATM HEAT repeats and NBS1 (part of the MRN complex; refs. 31, 34). The FAT domain normally inhibits the kinase activity as a dimer, but after DNA damage induced autophosphorylation at S1981 and subsequent dissociation of the dimer the kinase domain becomes active (36). The FATC domain is critical for interaction with TIP60, and TIP60 acetylation of ATM at K3016 is necessary for ATM activation. Mutational analysis of PDAC patients with *ATM* mutations from Cbio-portal (date of accession January 21, 2019) did not show significant clustering or hotspot mutations in *ATM*, but the number of patients was low ($N = 34$).

This molecule is currently being studied in in clear-cell renal cell carcinoma in combination with another known sensitizing agent, CX4945, which is an inhibitor of the protein kinase CK2 (76). The rationale for this combination comes from a compound screen where CK2 and ATM inhibitors were found to be highly synergistic in renal cancer. Interestingly, when CK2 inhibitors were tested in isogenic *ATM*-proficient and -deficient mouse cell lines, there was little difference in downstream effectors of DNA repair, such as AKT1 and BID, although overall viability in the ATM proficient and deficient cell lines treated with CK2 inhibitors was not assessed (77). This brings to light an important point when comparing the efficacy of interventions performed in combination with ATM inhibitors, as compared with these same interventions performed in patients with *ATM*-deficient tumors. The results may potentially be divergent as tumors with constitutive deficiency in ATM may have adapted to chronic loss of ATM function as opposed to acute loss as induced by ATM inhibitors. Conversely, other interventions may be both synergistic with ATM inhibitors and more potent in *ATM*-deficient patients.

Beyond the preclinical explorations of ATM inhibitors, currently two ATM inhibitors in clinical trials are being investigated in combination with other therapies (Table 1). AZD0156, an oral ATM inhibitor, is currently in clinical trials in combination with olaparib or FOLFIRI (78). These combinations are rational because, as previously mentioned, ATM inhibitors have been shown to sensitize cells to PARP inhibitors, and also to both 5-FU and irinotecan (61, 62, 68). AZD1390, another oral ATM inhibitor that penetrates the blood–brain barrier, is currently being tested in combination with radiation, given that radiation has been demonstrated to be more effective in ATM-deficient cancers (65, 79, 80). Importantly, in considering the potential adverse events for this trial, knockout of *ATM* in healthy tissue as compared with cancerous tissue was shown to induce less radiation sensitivity (80). This work demonstrated that increased sensitivity to radiation through ATM inhibition was primarily seen in cells that were rapidly replicating. As ATM inhibitors are further explored in the clinic, it will, of course, be important to monitor the side effects of ATM inhibitors particularly in combination with other therapies.

Increased sensitivity of ATM-deficient tumors to PARP inhibitors has previously been shown (64, 75, 81), and in a clinical trial of 124 patients, it has been demonstrated that dosing with olaparib and paclitaxel was more effective at increasing overall survival in patients with less ATM activity (HR, 0.35; 80% CI, 0.22–0.56; $P = 0.002$; median OS, not reached vs. 8.2 months; ref. 82). Unfortunately, the subsequent phase III trial with 525 patients did not enrich for patients with ATM-deficient tumors and was a negative study (83). Nevertheless, there are multiple

Table 1. ATM-relevant trials

DDR target	Drug name	NCT identifier	Phase	Study size	ATM status considered	Study title (pancreatic cancer eligible trials are in bold)
ATM	AZD0156	NCT02588105	I	83	No	Study to Assess the Safety and Preliminary Efficacy of AZD0156 at Increasing Doses Alone or in Combination with Other Anticancer Treatment in Patients with Advanced Cancer (AToM)
	AZD1390	NCT03423628	I	132	No	A Study to Assess the Safety and Tolerability of AZD1390 Given with Radiation Therapy in Patients with Brain Cancer
ATR	M6620 (VX-970)	NCT02723864	I	60	No	**Veliparib (ABT-888), an Oral PARP Inhibitor, and VX-970, an ATR Inhibitor, in Combination with Cisplatin in People with Refractory Solid Tumors**
	M6620 (VX-970)	NCT02595931	I	51	No	**VX-970 and Irinotecan Hydrochloride in Treating Patients with Solid Tumors That Are Metastatic or Cannot Be Removed by Surgery**
	M6620 (VX-970)	NCT03641313	II	28	Exploratory objective	ATR Kinase Inhibitor M6620 and Irinotecan in Treating Patients with Progressive, Metastatic, or Unresectable TP53-Mutant Gastric or Gastroesophageal Junction Cancer
	M6620 (VX-970)	NCT03517969	II	130	No	ATR Kinase Inhibitor VX-970 and Carboplatin with or without Docetaxel in Treating Participants with Metastatic Castration-Resistant Prostate Cancer
	M6620 (VX-970)	NCT02567409	II	90	No	Cisplatin and Gemcitabine Hydrochloride with or without ATR Kinase Inhibitor M6620 in Treating Patients with Metastatic Urothelial Cancer
	M6620 (VX-970)	NCT02595892	II	70	No	Gemcitabine Hydrochloride Alone or with M6620 in Treating Patients with Recurrent Ovarian, Primary Peritoneal, or Fallopian Tube Cancer
	M6620 (VX-970)	NCT02627443	I	111	No	Carboplatin and Gemcitabine Hydrochloride with or without ATR Kinase Inhibitor VX-970 in Treating Patients with Recurrent and Metastatic Ovarian, Primary Peritoneal, or Fallopian Tube Cancer
	M6620 (VX-970)	NCT02487095	I/II	70	No	Trial of Topotecan with VX-970, an ATR Kinase Inhibitor, in Small Cell Cancers and Extrapulmonary Small Cell Cancers
	M6620 (VX-970)	NCT03641547	I	65	No	M6620 plus Standard Treatment in Oesophageal and Other Cancer (CHARIOT)
	M6620 (VX-970)	NCT02567422	I	45	No	M6620, Cisplatin, and Radiation Therapy in Treating Patients with Locally Advanced HPV-Negative Head and Neck Squamous Cell Carcinoma
	M6620 (VX-970)	NCT02589522	I	46	No	VX-970 and Whole Brain Radiation Therapy in Treating Patients with Brain Metastases from Non–small Cell Lung Cancer, Small Cell Lung Cancer, or Neuroendocrine Tumors
	M6620 (VX-970)	NCT03718091	II	223	Yes	M6620 (VX-970) in Selected Solid Tumors
	AZD6738	NCT03682289	II	68	No	**Phase II Trial of AZD6738 Alone and in Combination with Olaparib**
	AZD6738	NCT03462342	II	86	No	Combination ATR and PARP Inhibitor (CAPRI) Trial with AZD6738 and Olaparib in Recurrent Ovarian Cancer (CAPRI)
	AZD6738	NCT03787680	II	47	No	Targeting Resistant Prostate Cancer with ATR and PARP Inhibition (TRAP Trial)
	AZD6738	NCT03328273	I/II	62	No	A Study of AZD6738 and Acalabrutinib in Subjects with Relapsed or Refractory Chronic Lymphocytic Leukemia (CLL)
	AZD6738	NCT03669601	I	50	No	AZD6738 and Gemcitabine as Combination Therapy (ATRiUM)
	AZD6738	NCT03770429	Ib	52	No	AZD6738 for Patients with Progressive MDS or CMML
	AZD6738	NCT02264678	I	250	Yes	Ascending Doses of AZD6738 in Combination with Chemotherapy and/or Novel Anticancer Agents
	BAY1895344	NCT03188965	I	219	Yes	**First-in-human Study of ATR Inhibitor BAY1895344 in Patients with Advanced Solid Tumors and Lymphomas**
CHK1/2	Prexasertib	NCT02873975	II	50	Yes	**A Study of LY2606368 (Prexasertib) in Patients with Solid Tumors with Replicative Stress or Homologous Repair Deficiency**
	Prexasertib	NCT03057145	I	24	No	**Combination Study of Prexasertib and Olaparib in Patients with Advanced Solid Tumors**
	Prexasertib	NCT03495323	I	28	No	A Study of Prexasertib (LY2606368), CHK1 Inhibitor, and LY3300054, PD-L1 Inhibitor, in Patients with Advanced Solid Tumors
	Prexasertib	NCT02203513	II	153	No	A Phase II Single-Arm Pilot Study of the Chk1/2 Inhibitor (LY2606368) in BRCA1/2 Mutation-Associated Breast or Ovarian Cancer, Triple-Negative Breast Cancer, High-Grade Serous Ovarian Cancer, and Metastatic Castrate-Resistant Prostate Cancer
	Prexasertib	NCT02808650	I	65	No	Prexasertib in Treating Pediatric Patients with Recurrent or Refractory Solid Tumors
	Prexasertib	NCT03735446	I	28	No	Prexasertib in Combination with MEC in Relapsed/Refractory AML and High-Risk MDS – A Phase I Trial
	SRA737	NCT02797964	I/II	170	Chk1 or ATR or other related gene	**A Phase 1/2 Trial of SRA737 in Subjects with Advanced Cancer**
	SRA737	NCT02797977	I/II	140	Yes	A Phase 1/2 Trial of SRA737 in Combination with Gemcitabine plus Cisplatin or Gemcitabine Alone in Subjects with Advanced Cancer

(Continued on the following page)

Table 1. ATM-relevant trials (Cont'd)

DDR target	Drug name	NCT identifier	Phase	Study size	ATM status considered	Study title (pancreatic cancer eligible trials are in bold)
Additional Trials Targeting ATM-Deficient Tumors						
PARP	Olaparib	NCT02576444	II	64	No	**OLAParib COmbinations (OLAPCO)**
	Olaparib	NCT03842228	Ib	102	Yes	**Copanlisib, Olaparib, and Durvalumab in Treating Patients with Metastatic or Unresectable Solid Tumors**
	Olaprib	NCT03009682	II	28	Yes	Olaparib Monotherapy in Relapsed Small Cell Lung Cancer Patients with HR Pathway Gene Mutations Not Limited to BRCA 1/2 Mutations, ATM Deficiency or MRE11A Mutations (SUKSES-B)
	Olaparib	NCT03012321	II	70	Yes	Abiraterone/Prednisone, Olaparib, or Abiraterone/Prednisone + Olaparib in Patients with Metastatic Castration-Resistant Prostate Cancer with DNA-Repair Defects
	Olaparib	NCT03786796	II	20	Yes	Study of Olaparib in Metastatic Renal Cell Carcinoma Patients with DNA-Repair Gene Mutations (ORCHID)
	Olaparib	NCT03570476	Pilot	15	Yes	Olaparib Before Surgery in Treating Participants with Localized Prostate Cancer
	Olaparib	NCT03375307	II	60	Yes	Olaparib in Treating Patients with Metastatic or Advanced Urothelial Cancer with DNA-Repair Defects
	Olaparib	NCT02734004	I/II	427	No	A Phase I/II Study of MEDI4736 in Combination with Olaparib in Patients with Advanced Solid Tumors (MEDIOLA)
	Talozaparib	NCT03565991	II	200	Yes	**Javelin BRCA/ATM: Avelumab plus Talazoparib in Patients with BRCA or ATM-Mutant Solid Tumors**
	Talozaparib	NCT02286687	II	150	Yes	**Study of the PARP Inhibitor BMN 673 in Advanced Cancer Patients with Somatic Alterations in BRCA1/2, Mutations/Deletions in PTEN or PTEN Loss, a Homologous Recombination Defect, Mutations/ Deletions in Other BRCA Pathway Genes and Germline Mutation in BRCA1/2 (Not Breast or Ovarian Cancer)**
	Talozaparib	NCT03330405	Ib/2	242	No	**Javelin Parp Medley: Avelumab plus Talazoparib In Locally Advanced or Metastatic Solid Tumors**
	Talozaparib	NCT03377556	II	64	Yes	Lung-MAP: Talazoparib in Treating Patients with HRRD Positive Recurrent Stage IV Squamous Cell Lung Cancer
	Talozparib	NCT02401347	II	58	Yes	Phase II Talazoparib in BRCA1 + BRCA2 Wild-Type and Triple-Neg/ HER2-Negative Breast Cancer/Solid Tumors
	Niraparib	NCT03209401	I	146	Yes	**Niraparib plus Carboplatin in Patients with Homologous Recombination Deficient Advanced Solid Tumor Malignancies**
	Niraparib	NCT03207347	II	47	Yes	**A Trial of Niraparib in BAP1 and Other DNA Damage Response (DDR) Deficient Neoplasms (UF-STO-ETI-001)**
	Rucaparib	NCT02952534	II	360	Yes	A Study of Rucaparib in Patients with Metastatic Castration-resistant Prostate Cancer and Homologous Recombination Gene Deficiency (TRITON2)

NOTE: The table summarizes currently open clinical trials that directly or indirectly target ATM-deficient tumors. The trials were captured from a search on clinicaltrials.gov on March 2, 2019. Clinical trials that potentially accept pancreatic cancer patients are shown in bold.

ongoing trials of PARP inhibitor–based therapy targeting patients, at least in part, with ATM-deficient tumors (Table 1).

ATR inhibitors

ATR is a phosphoinositide 3-kinase-related protein kinase that primarily responds to and repairs single-strand DNA breaks. It also shares functional sequences with ATM and DNA-PK, which respond to DSBs (29, 76). Upstream protein phosphorylation by ATM and autophosphorylation at the T1989 site stimulates ATR activity as well as TopBP1, which contains an ATR-activation domain to stimulate the kinase's activity (29, 84). The ATR kinase responds to a wide array of cell stressors, maintains DNA's integrity during replication, and is essential for proliferating cell survival. In the rapidly dividing cancer cell, there exists a high degree of replicative stress, creating an environment in which, as preclinical research has shown, suppression of ATR activity further increases replication stress leading to cell death (84). Furthermore, although normal dividing cells utilize ATM-dependent pathways for assistance in DNA repair, cancer cells, which are often deficient in ATM/p53 signaling, may rely solely on the ATR pathway for survival (67, 85, 86). This was demonstrated in genetically engineered mouse models of cancer, in which 90% genetic reduction of ATR expression suppressed the development of fibrosarcomas and acute myeloid leukemias with minimal side effects in normal tissues. This work affirmed the tumor selectivity of ATR inhibition (84). Moreover, inhibition of ATR selectively sensitizes tumor cells, but not normal cells, to radiation and chemotherapy (87).

Thus, small-molecule inhibitors of ATR may be particularly potent in PDACs with somatic mutations in *ATM* given that the lack of ATM's function may lead to increased dependence on ATR, and ATR inhibition could thus significantly promote cancer cell death. The ATR inhibitor VE-821 sensitizes cancer cells but not normal cells to chemotherapy (87), and these effects were synergistic in ATM-deficient cells (87). Another ATR inhibitor, AZD6738, causes accumulation of DNA damage, S phase arrest, and apoptosis in ATM dysfunctional gastric cells while not affecting those with functional ATM (88). Similar preclinical studies also suggest synthetic lethality between ATR inhibition with VE-822 and ATM deficiency in PDAC as well as lung adenocarcinoma cell lines, reaffirming the actionable molecular dependencies on ATR (62, 81). VE-822 has also been shown to potentially synergize with cisplatin in ATM-deficient esophageal squamous cells (89). This effect of potentiating the cytotoxicity of cisplatin

and gemcitabine is also seen with AZD6738 in ATM-deficient non–small cell lung cancer cells (90). Several ongoing trials of ATR inhibitor-based therapies are listed in Table 1.

CHK1 and CHK2 inhibitors

Downstream to and activated by ATR is the checkpoint kinase 1 (CHK1) pathway. CHK1 promotes proteasomal degradation of CDC25A in response to genome stress (29). The combined activity of ATR, CHK1, and CDC25A results in cell-cycle arrest and stabilization of replication stress at DNA forks. The inhibition of this complex leads to a decreased rate of fork progression, massive fork collapse in S phase cells, and ultimately cell death (82). Preclinical studies utilizing the CHK1 inhibitors MK8776 and LY2603618, with gemcitabine-based chemoradiation, showed synergistic effects to induce apoptosis of PDAC cells (91, 92). The combination of gemcitabine, a CHK1 inhibitor, PF-477736, and Lutetium-177–labeled anti-EGFR antibody leads to extensive DNA damage, apoptosis, and tumor degeneration in patient-derived xenografts (93). Additional preclinical studies with a tumor stem cell marker Doublecortin-like kinase 1 (Dclk1) inhibitor, LRRK2-IN-1 (LRRK), showed decreased expression of phosphorylated Chk1 (94). This same study demonstrated the combination of gemcitabine with LRRK significantly reduced cell survival compared with treatment with gemcitabine alone (94). Thus, CHK1 and Dclk1 are both potential targets in ATM-deficient malignancies as they also play a large role in single-strand break DNA repair.

CHK1 activation is primarily dependent on ATR at stalled replication forks and single-strand DNA, whereas CHK2 is activated mainly by ATM induced by DNA DSBs. One preclinical study examined the antitumor effects of a CHK2 inhibitor, NSC109555, in combination with gemcitabine. This combination increased apoptosis in pancreatic cells (95). Clinical trials of CHK1/2 inhibitors are also listed in Table 1.

Conclusions

There are currently no FDA-approved targeted therapies for patients with pancreatic cancer. However, genomic profiling of pancreatic adenocarcinomas is revealing therapeutically relevant alterations, and 17% to 25% of pancreatic cancers harbor mutations in the DDR pathway. Therapy targeted toward inhibiting the DNA damage response, including with PARP inhibitors, is proving to be highly effective particularly in DDR-deficient cancers, as has been established in *BRCA1/2* mutated cancers. However, DDR is a highly complicated process, involving several overlapping pathways. It is reasonable to hypothesize that, depending upon the specific DDR mutation, there may be different optimal therapies to be utilized. PARP inhibitors are showing early promise in PDACs that harbor *BRCA1/2* or *PALB2* mutations, but consistently the most common DDR gene mutated in PDAC is actually *ATM*. It will be critical in the coming years to explore what DDR-targeted therapies might work best in ATM-deficient tumors. As with any therapeutic breakthrough, the future exploration of the complexity of the DDR pathway also justifies the need for a better understanding of compensatory and resistance mechanisms that may arise in the setting of ATM/ATR/CHK1–targeted therapies.

ATM deficiency may provide sensitivity for other elements of conventional therapies for PDAC, including radiation (96) and oxaliplatin. However, emerging targeted strategies, including immunotherapeutic combination approaches (97), will likely provide even better matches for ATM-deficient tumors. For example, mechanistically, it seems reasonable to consider that PARP inhibitors may be effective in treating *ATM*-deficient tumors. But potentially more promising might be the combination of a PARP inhibitor with an ATR inhibitor in ATM-deficient PDAC—essentially exploiting a new node of synthetic lethality in the DDR pathway. Similarly, there is a mechanistic reason to explore the role of CHK1 inhibition in ATM-deficient tumors. In both cases, understanding the need for inducing DNA damage with DNA-damaging chemotherapy will be critical as well. There are several ongoing clinical trials as discussed above, but clinical trials in PDAC in which there is such a high unmet need, and where ATM deficiency is common, would be ideal.

Additionally, recent genetic studies have revealed that specific ATM genotypes correlate to susceptibility to different diseases including cancer, which may provide valuable clinical information with regard to early detection, the subtyping of, and the treatment of PDACs (98). These genetic studies may complement and/or be evaluated in published genetically engineered mouse models (47, 62, 99) that have identified ATM's various roles (i.e., EMT, genetic instability, and metastases) in the progression model of PDAC. Moving forward, the research community should evaluate novel agents and combination therapies discussed above in these isogenic, *in vivo* models with the ultimate aim of classifying each *ATM* pathogenic genotype observed in patients with an optimally tailored, matched targeted therapeutic strategy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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