

ATM Mutations in Cancer: Therapeutic Implications

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Abstract

Activation of checkpoint arrest and homologous DNA repair are necessary for maintenance of genomic integrity during DNA replication. Germ-line mutations of the ataxia telangiectasia mutated (*ATM*) gene result in the well-characterized ataxia telangiectasia syndrome, which manifests with an increased cancer predisposition, including a 20% to 30% lifetime risk of lymphoid, gastric, breast, central

nervous system, skin, and other cancers. Somatic *ATM* mutations or deletions are commonly found in lymphoid malignancies, as well as a variety of solid tumors. Such mutations may result in chemotherapy resistance and adverse prognosis, but may also be exploited by existing or emerging targeted therapies that produce synthetic lethal states. *Mol Cancer Ther*; 15(8); 1781–91. ©2016 AACR.

Introduction

Defective DNA repair is a hallmark of cancer and results in genomic instability and accumulation of other genetic abnormalities (1, 2). Hereditary mutations of genes involved in DNA repair, such as ataxia telangiectasia mutated (*ATM*), breast cancer (*BRCA*) 1 or 2, and *TP53*, result in markedly increased susceptibility to a variety of cancers. Likewise, somatic mutations in these genes are among the most commonly found aberrations in cancer and are associated with inferior outcomes and chemotherapy resistance. Cancers with mutations in genes encoding proteins involved in DNA repair may be more sensitive to treatments that induce synthetic lethality by inducing DNA damage or inhibiting complementary DNA repair mechanisms. For example, *BRCA1*-mutated tumors may be sensitive to platinum therapy; indeed, 80% response rates, including 45% complete responses in *BRCA1*-mutated breast cancer, have been reported with cisplatin (3). Interrupting DNA damage repair with mitomycin C also may also be effective; a patient with *PALB2*- (a partner and localizer of *BRCA2*) mutated pancreatic cancer achieved a 36+-month response on mitomycin C therapy (4). Patients with breast or ovarian cancer with mutations of *BRCA1* have been treated with inhibitors of PARP with early clinical success (5). These and similar strategies may also be of particular utility in treating patients with cancer that have *ATM* mutations, which are commonly identified in next-generation sequencing (6). Herein, we review the normal structure and function of the *ATM* gene; the spectrum, frequency, and significance of somatic mutations; and preclinical and early clinical data with agents that may best target *ATM* aberrant cancers.

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doi: 10.1158/1535-7163.MCT-15-0945

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Function and Structure of ATM

The *ATM* gene was first cloned in 1995 through studies of ataxia telangiectasia (A-T) syndrome (7). Located on chromosome 11q 22–23, it includes 66 exons with a 9168 base pair coding sequence, and encodes a PI3K-related serine/threonine protein kinase (PIKK) that helps maintain genomic integrity. *ATM* plays a central role in the repair of DNA double-strand breaks (DSB), which can be induced by ionizing radiation, chemotherapy drugs, or oxidative stress, or occur during normal physiologic events like meiotic recombination or rearrangement of antibody genes during B-cell maturation (8, 9).

Repair of DSBs involves an extensive network of signals, including *sensor/mediator* proteins, downstream *transducer* protein kinases, and *effector* proteins (Fig. 1; refs. 10, 11). The MRE11–RAD50–NBS1 (MRN) complex is considered the primary sensor of DSBs. It forms a physical bridge spanning the DSB ends, takes part in DSB end resection, and recruits and retains *ATM* at DSB sites. *ATM* can be considered the main *transducer* in the DSB repair process. It recruits and cooperates with other sensor proteins, including 53BP1 (p53-binding protein) and *BRCA1* (breast cancer type 1). Along with this spatial localization at the site of DSBs, *ATM* becomes catalytically activated. The precise mechanism of this activation is not fully established, but has been shown to involve dissociation of *ATM* homodimers into active monomers, autophosphorylation of *ATM* at Ser1981 and other sites, and acetylation (12–14).

Once activated, *ATM* phosphorylates many downstream *effectors*, which in turn phosphorylate their own targets. The PIKK domain of *ATM* recognizes serine-glutamine (SQ) and threonine-glutamine (TQ) motifs of many proteins, including ones involved in cell-cycle checkpoint arrest (e.g., Chk1 and Chk2), DNA repair (*BRCA1* and *RAD51*), and apoptosis (p53; ref. 15). It also has been determined that there are in fact over 700 targets phosphorylated following DSBs, and that *ATM* modulates networks not immediately involved in DNA repair like the insulin-like growth factor or other metabolic and stress-response pathways (16). The plethora of *ATM* targets is likely a means of coordinating multiple pathways at times of DNA repair or genomic stress. There are also redundancies and collaboration between *ATM* and other

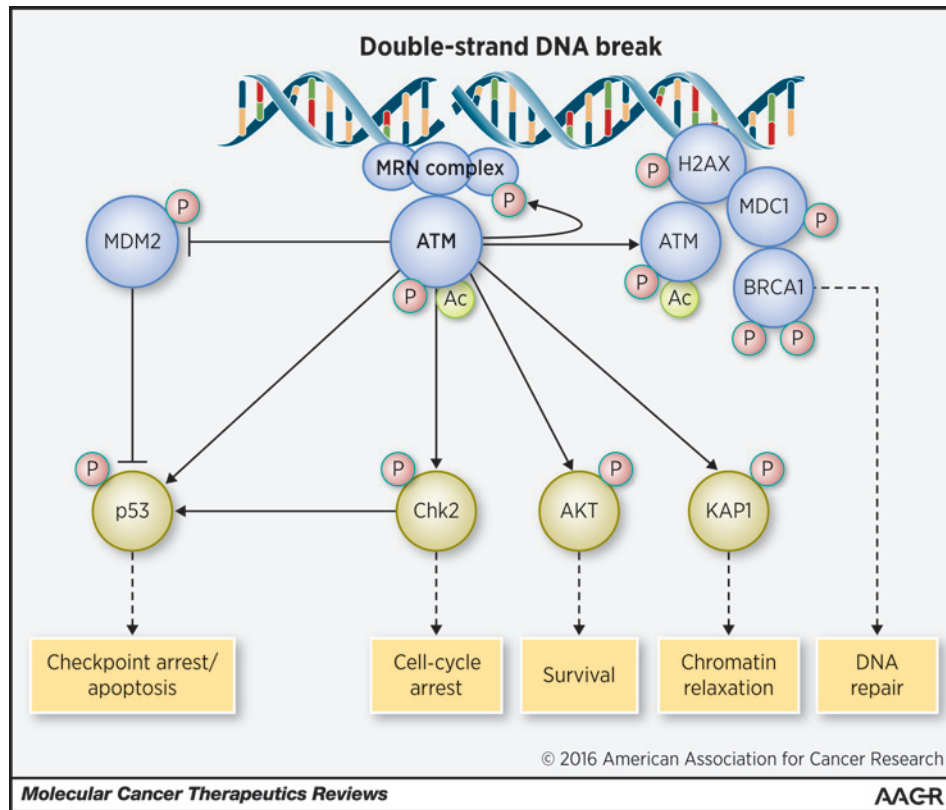


Figure 1.

ATM function in repair of double-stranded DNA breaks. Following DNA DSBs, the MRN complex senses and initiates DNA repair and recruits ATM. As ATM is brought to the site of DNA damage, it dissociates from homodimers into active monomers and is catalytically activated by autophosphorylation at Ser1981 and other sites, as well as acetylation at Lys3016. ATM also interacts with and phosphorylates other sensor proteins, MDC1 (mediator of DNA damage checkpoint protein 1), and histone H2A.X (γ H2A.X). There are also positive feedback loops at these steps, as ATM phosphorylates these sensor proteins, resulting in a rapid accumulation of these and other proteins at the DSB sites. Once activated, ATM serves as a transducer and phosphorylates and activates other protein kinases. Examples include phosphorylation of checkpoint kinase 2 (CHK2) at Thr68, which in turn modulates its own substrates, resulting in cell-cycle arrest. ATM also activates p53, by direct ATM-mediated phosphorylation at Ser15, Ser46, and others, through CHK2 (which also phosphorylates p53, though at Ser20), and indirectly by phosphorylating (Ser394) and thus disrupting mouse double minute 2 homolog (MDM2) and other E3 ubiquitin ligase–negative regulators of p53. ATM also has effects on chromatin relaxation, which aids in repair, through phosphorylation of KRAB-associated protein 1 (KAP1) and others. Finally, ATM can result in prosurvival signaling that is balance with the apoptotic signals, including phosphorylation of AKT, and activation of NF- κ B (not shown). It should be noted that ATM phosphorylates and activates a plethora of other protein kinases—over several hundred—in addition to the main elements of DSB repair shown here.

members of the PIKK family, including catalytic subunit of the DNA-dependent protein kinase (DNA-PKc) and ATM-related (ATR), which are activated in responses to other sources of genotoxic stress. These redundancies may represent therapeutic targets for treating cancers that have lost ATM function.

A-T syndrome

Germ-line mutations of *ATM* cause the autosomal recessive A-T syndrome. These mutations occur in each functional domain of the gene, without a predominantly recurring site of mutation (Fig. 2; refs. 17, 18). Germ-line mutations manifest with a variety of phenotypic characteristics, including neurodegeneration, cerebellar ataxia, immunodeficiency, hypogammaglobulinemia, gonadal dysgenesis, and radiosensitivity (19). A-T syndrome also increases cancer susceptibility, most likely due to impaired DNA damage repair and genomic instability. In a retrospective analysis of 279 patients with A-T syndrome enrolled in the French National Reference Center for Primary Immune Deficiencies, there were 69 patients with cancers (24.5%; ref. 20). The majority had

hematologic cancers; 8 patients had acute leukemia, 12 had Hodgkin lymphoma, 38 had non-Hodgkin lymphoma, and 3 patients had T-cell prolymphocytic leukemia. There were eight carcinomas, which developed at higher median ages than the hematologic cancers. The overall cumulative incidence of cancer by age 40 was 38.2% in this cohort. The authors noted that immunodeficiency was also associated with cancer risk, and that Epstein-Barr virus (EBV) reactivation was highly prevalent in the lymphoma cases. An earlier retrospective analysis by Reiman and colleagues had similar observations, with cancer occurring in about 25% of the 296 individuals with A-T syndrome in their cohort, primarily lymphoma or leukemia, though breast, gastric, and other solid tumors also occurred at high rates (21). The approximate incidence of various cancers in A-T syndrome is in Table 1.

Germ-line ATM heterozygosity and cancer susceptibility

Germ-line *ATM* heterozygosity occurs in about 1% of the population and appears to increase cancer susceptibility

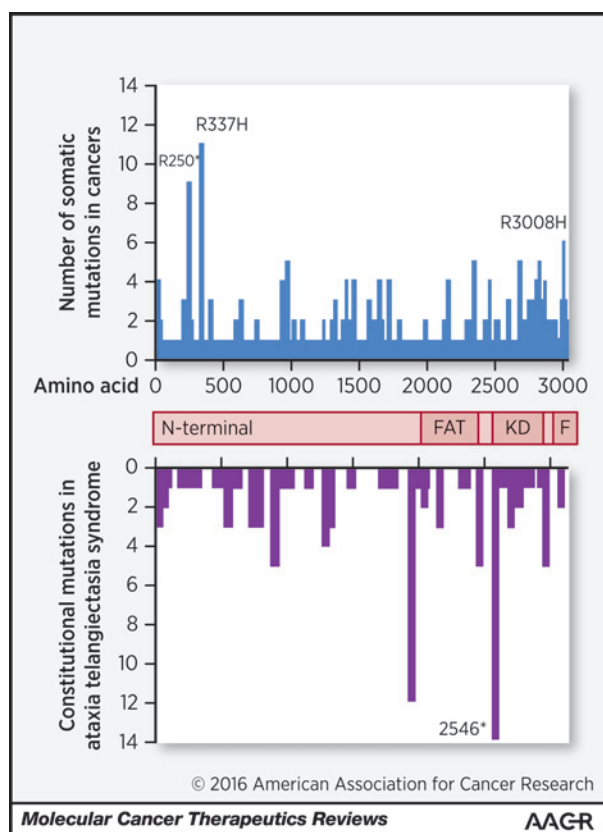


Figure 2.

Spectrum of hereditary ATM mutation and somatic ATM mutations in cancers. *ATM* is one of the most commonly aberrant genes in sporadic cancers. Similar to studies of ataxia telangiectasia syndrome (lower), somatic mutations found in sporadic cancers (top) span the entire *ATM* gene and occur in each of the functional domains, which are shown in schematic form, and are mostly in the C-terminal end. The N-terminus contains HEAT sequences that mediate protein-protein interactions. The FRAP-ATM-TRRAP (FAT) domain (amino acids 2097–2488) stabilizes the C-terminal region of ATM and interacts with the PI3 kinase domain (KD, aa 2623–2974). The FAT carboxy-terminal (FATC, abbreviated as F in the figure) domain (aa 3026–3055) is thought to be involved in acetylation and activation of ATM (18, 48, 49).

(22). Studies of family members known to be heterozygous for *ATM* gene mutations showed an approximate 2- to 3-fold risk of cancer, and a 5- to 9-fold risk of breast cancer in

women (22, 23). In particular, the relative risk of breast cancer in those younger than age 50 was increased (24). Likewise, Broeks and colleagues evaluated 82 patients who developed breast cancer at a young age (less than 45 years old), many of whom had bilateral breast cancer. In this cohort, 8.5% had *ATM* germ-line mutations (25). However, this association has been subject to some controversy. A study of young patients with breast cancer (and no known family history of A-T syndrome) detected heterozygous truncating *ATM* mutations in only 0.5% of cases, less than the rate predicted by the preceding studies, and even less than the general population, suggesting that such heterozygous *ATM* mutations did not predispose those individuals to breast cancer (26). The slightly different patient characteristics may account for the discrepant results between these studies.

Several large population studies have evaluated the rate of specific *ATM* point mutations. Stredrick and colleagues analyzed approximately 2,800 breast cancer cases and controls. The *ATM* Ser49Cys mutation was found in approximately 2% of subjects and was more common in the breast cancer than control populations, with an OR of 1.69 ($P = 0.004$; ref. 27). Dombernbowsky and colleagues also evaluated S49C, as well as *ATM* Ser707Pro heterozygosity, in over 10,000 individuals, approximately 20% of whom developed cancer during prospective observation (28). S49C was associated with an increased incidence of melanoma (HR 4.8), prostate cancer (HR 2.3), and oropharyngeal cancer (HR 3.4); S707P was associated with an increased risk of thyroid or endocrine cancers (HR 10). However, these missense mutations were not associated with an increased breast cancer risk compared with the general population (HR 0.8 and 0.6 for S49C and S707P, respectively) or overall cancer risk (HR 1.2 and 0.8). Fletcher and colleagues expanded the analysis of *ATM* variants to over 26,000 breast cancer cases and controls (29). In this large population, heterozygosity for one of 5 *ATM* variants evaluated (S49C and S707P as above, and also F858L, P1054R, and L1420F) was associated with a small increased risk of breast cancer (OR 1.05). The risk was greater for rare homozygotes (OR 1.51). Collectively, these studies support a role of *ATM* point mutations in breast cancer predisposition, though this effect may be limited to a small subset of cases, such as young patients with familial or bilateral disease.

Other studies have identified functional variants associated with increased lung cancer or thyroid cancer risk (30, 31). Finally, inherited *ATM* mutations are also felt to play a role in familial pancreatic ductal adenocarcinoma (32–34).

Table 1. A-T syndrome and cancer risk

Tumor type	Approximate incidence in A-T syndrome	Notes/references
Non-Hodgkin's lymphoma	13%	(20, 97)
Leukemia	2%–8%	Mostly T-cell origin (20, 21)
Hodgkin's lymphoma	4%	(20, 21)
Breast cancer	2%–3%	(20, 21)
Adenocarcinoma of stomach	<1%	Single case reported (21)
Dysgerminoma	<1%	Single case reported (21)
Gonadoblastoma	<1%	Single case reported (21)
Medulloblastoma	<1%	Single case reported (21)
Pancreatic carcinoma	<1%	(21)
Thyroid carcinoma	<1%	2 cases reported (21)
Total (All types)	25%	

Radiosensitivity and chemosensitivity and ATM

Radiosensitivity is also a hallmark of the A-T syndrome (35, 36). Increased toxicity to radiotherapy has been reported in patients with A-T syndrome and heterozygous carriers of *ATM* mutations, probably due to defective DNA repair and genomic instability in normal tissues (37–40). However, heterozygous germ-line mutations apparently do not contribute to secondary cancers following radiotherapy (41–43). Similarly, toxicity from systemic chemotherapy may be increased in patients with heterozygous germ-line *ATM* mutations, albeit mildly. Patients with heterozygous germ-line *ATM* variants treated with carboplatin and paclitaxel had more myelosuppression compared with patients with wild-type *ATM*, but this effect was modest (e.g., platelet nadir 112 vs. 180 thousand/microliter; ref. 44). This may be compared with the data on patients with germ-line *BRCA* mutations, who do not experience a significant increase in chemotherapy-related toxicity (45). Finally, *ATM* SNPs have been correlated with regimen-related gastrointestinal toxicity in patients after hematopoietic stem cell transplantation (46).

Somatic ATM Mutations in Cancer

Next-generation sequencing efforts have revealed that *ATM* is among the most commonly aberrant genes in sporadic

cancers. The COSMIC database lists 167 distinct somatic mutations in *ATM* (excluding variants of unknown origin) that have been observed in a broad range tumors and hematologic malignancies (47). Similar to studies of A-T syndrome, these somatic mutations span the entire *ATM* gene and occur in each functional domain (Fig. 2; refs. 48, 49).

Somatic mutations in *ATM* occur in many tumor types, though are more often found in hematologic malignancies (Fig. 3). Forty-six percent of cases of T-cell prolymphocytic leukemia have inactivating mutations in both *ATM* alleles (50, 51). *ATM* mutations observed in approximately 45% of mantle cell lymphoma cases; such mutations generally are truncating mutations or missense mutations within the region of the gene encoding the PI3K domain (52). Aberrations of the *ATM* gene are also common in B-cell chronic lymphocytic leukemia (CLL). Loss of heterozygosity through deletion of chromosome 11q23 occurs in approximately 20% of cases. Such mutations are associated with relatively short progression-free survival following chemotherapy and adverse prognoses (53–56). Next-generation sequencing studies identified point mutations of *ATM* in 9% of cases of CLL (57). *ATM* expression also can be down regulated in Reed Sternberg cells derived from classical Hodgkin lymphoma. In one study, the *ATM* protein was not detectable in Reed Sternberg cells

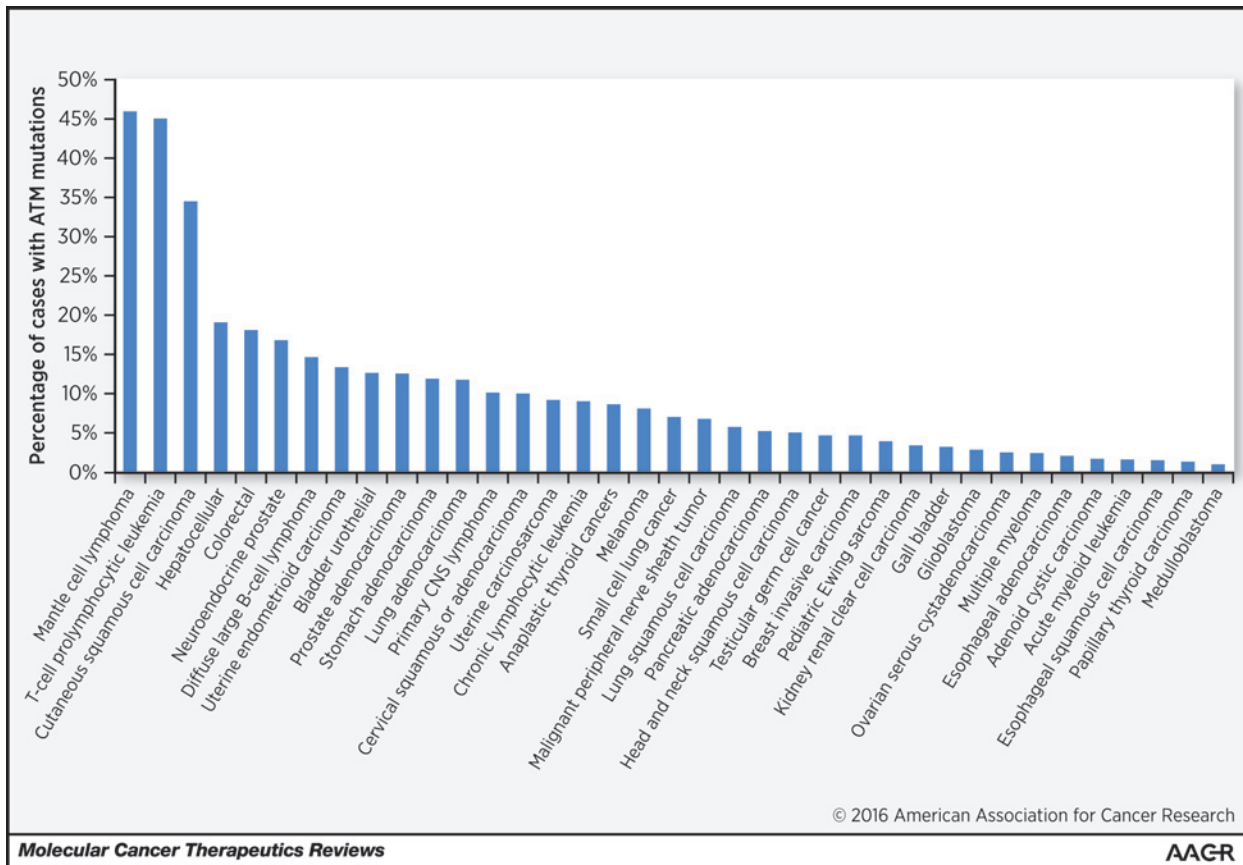


Figure 3.

Cross-cancer alteration summary for *ATM*: compiled from cBioPortal, accessed March 14, 2016, as well as next-generation sequencing studies that were published separately (49, 50, 52, 110, 111).

compared with normal germinal center B cells in 17 of 18 cases, despite no loss of heterozygosity, pathogenic mutations, or hypermethylation of the *ATM* promoter. It is speculated that the reduced *ATM* expression is due to alterations of upstream regulators (58).

Although somatic missense or nonsense mutations of *ATM* have not been commonly detected in breast cancer, COSMIC reports a high rate of copy-number variation, mainly LOH (391 of 852 analyzed specimens; ref. 47). In addition, a high rate of deletion or methylation of either *ATM* or *MCPH1*, a proximal regulator that recruits *ATM* to the sites of DNA DSBs, was reported in breast cancer samples (96%, 121 of 126). In prostate cancer, targeted next-generation sequencing has revealed an 8% incidence of *ATM* mutations (59). *ATM* is also one of the most commonly mutated genes in pancreatic cancer (60) or lung adenocarcinoma (61). In colorectal cancer, loss of *ATM* protein expression is associated with worse prognosis, based on immunohistochemical analysis of stage II/III cancers (62), and a high percentage of *ATM*-mutated cases has been reported by COSMIC (47). COSMIC also reports point mutations in 1% to 5% of endometrial, kidney, liver, esophageal, ovarian, salivary gland, gastric, thyroid, and urinary tract cancers, though most are classified as variants of unknown origin.

Not every identified somatic mutation correlates with one known to cause A-T syndrome, and the functional impact of each somatic mutation is not established. Therefore, it is difficult to predict whether a certain mutation is deleterious or a variant without functional significance. Furthermore, it is not clear at what allele frequency deleterious mutations affect the tumor behavior or response to therapy. As referenced above, heterozygous germ-line *ATM* mutations appear to impart some vulnerability to chemotherapy and radiotherapy toxicity. To this end, many groups have designed assays to measure *ATM* protein function (36, 54, 63, 64).

Therapeutic Opportunities in ATM-Deficient Cancers: Inducing Synthetic Lethality

Although *ATM* deficiency increases genomic instability through loss of DSB DNA repair, it also increases the dependence of cancer cells on other repair mechanisms, specifically repair of replication stress that is incurred by dividing cells. Stalled replication forks or unrepaired single-strand breaks (SSB) that convert into DSBs are not repaired in cells deficient in *ATM* or other double-strand DNA repair mechanisms. Based on this rationale, inhibition of other kinases involved in SSB DNA repair has been considered a potential mechanism for synthetic lethality in cancers lacking *ATM* (Table 2). In addition to this rationale, there is precedent for pharmacologic inhibition of such kinases that can be extrapolated from animal studies where a defective kinase is more detrimental to the animal than a nonexpressed kinase. For instance, *ATM* knock-out mice are viable and recapitulate A-T syndrome, whereas mutated "kinase dead" *ATM* results in embryonic lethality (65). Kinase inhibitors may have effects similar to the kinase-dead mutants; they not only inactivate the kinase target, but also leave the catalytically inactive kinase to block access to site of DNA damage from other proteins that would initiate alterna-

tive repair pathways. Many of the agents have been primarily evaluated for the potential capacity to sensitize cancer cells to radiotherapy or chemotherapy-induced DNA damage. However, in cancers lacking functional *ATM*, single-agent activity may be anticipated.

Platinum drugs

Cancers with deficits in double-strand DNA repair pathways are sensitized to platinum drugs that induce such double-strand DNA breaks. This has been demonstrated in the context of *BRCA1*- or *BRCA2*-mutated cancer, particularly breast and ovarian cancer. Cisplatin chemotherapy had a high response rate in patients with *BRCA1* mutations and breast cancer, both in the metastatic and neoadjuvant settings (3, 66). This principle also has been extended to other mutations that affect double-strand DNA repair. For example, a mutation of *PALB2* has been described to sensitive pancreatic cancer to mitomycin C treatment by disrupting *BRCA1* and *BRCA2* interactions (4). Similarly, *ATM* mutations are predicted to result in increased sensitivity to platinum chemotherapy (6, 67). However, a recent preclinical study in gynecologic cancer cell lines found that *ATM* inhibition enhanced the response to ionizing radiation, but did not enhance the *in vitro* killing of the cells by platinum drugs (68). This might suggest that *BRCA* plays a more important role in homologous recombination than does *ATM* (69). Larger retrospective analyses of *ATM*-aberrant cancers and platinum sensitivity may help clarify this issue.

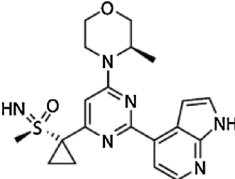
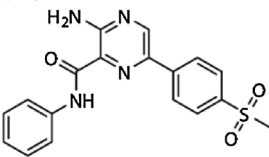
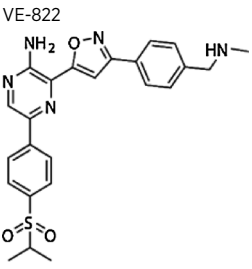
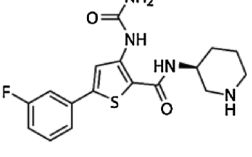
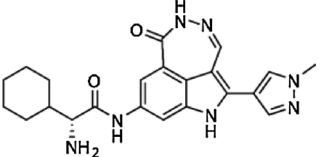
PARP inhibitors

Similar to the platinum agents, the evaluation of inhibitors of PARP1 in *BRCA1*- or *BRCA2*-mutated cancers can be considered as proof of the principle that inhibition of endogenously arising DNA damage alone can result in clinical benefit in cancers that lack double-strand DNA repair. Inhibitors of PARP have been evaluated in clinical trials in a variety of settings, both alone and in combination with other agents, though they have not been evaluated specifically in *ATM*-deficient tumors in clinical trials. Preclinical evaluation of olaparib in *ATM*-deficient leukemic cells from patients with CLL suggested single-agent activity (70). Similar findings were described in mantle cell lymphoma and gastric cancer (71, 72). Furthermore, a phase II double-blind study of paxitaxel with or without olaparib for patients with gastric cancer stratified patients between low or undetectable *ATM* levels versus normal *ATM* levels. A greater improvement in overall survival was seen in the *ATM*-low subgroup, consistent with the preclinical observations (73).

Targeting ATR

The ATR-checkpoint kinase 1 (Chk1) pathway is another potential target for therapy in *ATM*-deficient cancers, as it is a primary mediator of SSB DNA repair. A synthetic lethal siRNA screen confirmed that mantle cell lymphoma cells with loss of *ATM* function have increased sensitivity to ATR inhibition (74). Preclinical data support the notion that inhibition of this pathway may have therapeutic activity against this and a variety of other cancers too. The compounds VE821, VE822, and AZD6738 each can inhibit ATR and are in various stages of preclinical development (75, 76). In particular, VE821 and VE822 have been noted to sensitize pancreatic tumor cells to DNA-damaging modalities, including chemotherapy and

Table 2. Drugs that may have selective activity in ATM-deficient cancers

Molecule	Mechanism	Stage of testing	Tumor types	Notes on selective activity in ATM mutation/results	References
ATR inhibition					
AZD6738 	ATR inhibitor	Phase I	Lymphoid malignancies; head and neck cancer	Single-agent activity in ATM-deficient but not ATM-wild-type xenografts. Phase I trial in head neck cancer planned.	(77–79, 98)
VE-821 	ATR inhibitor	Preclinical	To be determined	Selective killing of ATM-deficient lung cancer cell lines. (IC ₅₀ approximately 10 μmol/L vs. not reached for 24-hour culture). Radiosensitization of cancer cell lines; phase I trials planned.	(99, 100)
VE-822 	ATR inhibitor	Preclinical	To be determined	Selective pancreatic cancer radiosensitization/chemosensitization.	(75)
PARP inhibition					
Olaparib	PARP inhibitor	Approved	Ovarian cancer, breast cancer, gastric cancer	Synthetic lethality in BRCA-deficient breast and ovarian cancer, or ATM-deficient gastric cancer and chronic lymphocytic leukemia.	(70, 71, 73)
Checkpoint inhibition					
AZD7762 	Chk1/2 inhibitor	Phase I	Advanced solid cancers (with chemotherapy)	Results pending.	(101–103)
CBP501	Chk1/2 inhibitor	Phases I–II	Advanced solid tumors, mesothelioma	25% response with cisplatin, 0% overall response rate as single agent; studies in unselected patients.	(104, 105)
PF-00477736 	Chk1/2 inhibitor	Phase I	Advanced solid malignancies (with gemcitabine)	Study terminated (NCT00437203).	(90, 106)

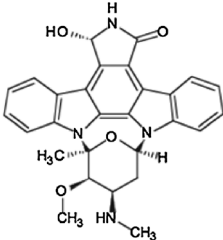
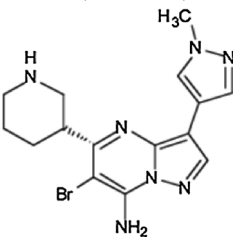
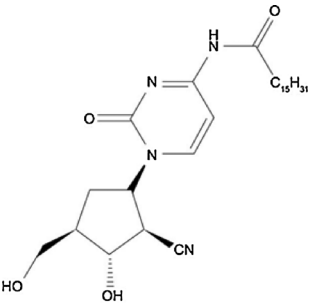
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radiotherapy. Of note, these studies were not limited to ATM-deficient cell lines.

AZD6738 is a potent, selective inhibitor of ATR, and the first specific ATR inhibitor dosed in man. Preclinical studies with AZD6738 demonstrated single-agent activity across cancer cell lines, but enhanced activity in cell lines with ATM deficiency.

In vivo, more antitumor activity was seen in ATM-deficient than ATM-wild-type xenograft models, and importantly, measurement of the DNA damage marker gamma-H2AX showed persistent staining in tumor tissues only, but not normal bone marrow or gut tissue (77). Notably, ATM-deficient lung cancer xenografts were particularly sensitive to a combination of cisplatin and

Table 2. Drugs that may have selective activity in ATM-deficient cancers (Cont'd)

Molecule	Mechanism	Stage of testing	Tumor types	Notes on selective activity in ATM mutation/results	References
UCN-01 (7-hydroxystaurosporine)	Nonselective Chk1/2 inhibitor	Phases I-II	Solid (pancreatic, ovarian, breast, melanoma; leukemia/myelodysplastic syndrome)	10% partial response rate in combination with chemotherapy; minimal single-agent activity in unselected patients.	(82, 84, 85, 87, 107)
					
MK-8776 (SCH900776)	Selective Chk1 inhibitor	Phase I	Solid tumors or lymphoma (with gemcitabine), leukemia (with cytarabine)	33% complete response rate in relapsed/refractory acute leukemia; long-term responses reported in combination with gemcitabine; unselected patients.	(87, 88)
					
Other					
Platinum drugs	DNA cross-linking	Approved		Activity specifically in ATM-mutated cancers is not yet clear.	(6, 66, 67)
Sapacitabine	Nucleoside analogue induces DNA SSBs	Phases I-II	Elderly acute myeloid leukemia and myelodysplastic syndrome, chronic lymphocytic leukemia	50- to 100-fold sensitization in cell lines with defective homologous repair. 28% response rate in phase I; 33% 1-year survival in phase II; unselected patients.	(94, 95, 108, 109)
					

AZD6738 (78). In studies with primary leukemic cells from patients with chronic lymphocytic leukemia, AZD6738 had superior cytotoxicity in CLL cells induced to divide (79), and in *ATM*- or *TP53*-mutated CLL cells (80). A first-in-man study was initiated in *ATM*-deficient non-Hodgkin lymphoma, and a subsequent study will be initiated in combination with radiotherapy for the treatment of patients with head and neck cancer, based on evidence that ATR inhibition can sensitize *ATM*-deficient cancer cells to ionizing radiation (68, 81).

CHK1 inhibitors

Chk1 is a serine/threonine kinase that is downstream of ATR in the DNA damage-induced cell-cycle arrest. Therefore, inhibitors of Chk1 may have particular activity either as chemosensitizers or as monotherapy in cancers deficient in *ATM* based on the same rationale as ATR inhibitors, and inhibitors of Chk1 are in various stages of clinical development. UCN-01 (7-hydroxystaurosporine) is a nonspecific Chk1 and Chk2 inhibitor that also inhibits Akt phosphorylation and sensitizes p53-defective cancer cells to DNA-damaging agents (82, 83). Clin-

ical trials have shown limited activity. In a study with advanced of melanoma, there were no responders in 17 treated patients (84). In a separate study of UCN-01 and irinotecan for the treatment of triple-negative breast cancer, the overall response rate was only 4%, and the clinical benefit rate (complete response + partial response + stable disease greater than or equal to 6 months) was only 12%. The incidence of *ATM* deficiency is not reported in either of these studies, though many patients in the breast cancer study had *TP53* mutations that should have imparted sensitivity to Chk1 inhibition. Of particular note, Chk1 inhibition by UCN-01 was not observed in all tumors, and so the low response rate was thought to be due in part to the pharmacokinetics of UCN-01 (85). Initial trials also showed a high binding affinity of UCN-01 to plasma proteins (86).

MK-8776 (also known as SCH-900776) is a more specific Chk1 inhibitor with potent Chk1 inhibition than Chk2 inhibition (500-fold). Like UCN-01, it enhances the rate of cell death induced by chemotherapy agents. Preclinical studies showed a 20- to 70-fold reduction of the growth-inhibitory

concentration of hydroxyurea, and similar sensitization with gemcitabine and cytarabine. Some cell lines, including cell lines with mutation of TP53, were highly sensitive to SCH900776 alone (87). SCH900776 was tested in phase I clinical trials, including acute leukemia, where it resulted in a 33% complete response rate in combination with cytarabine (88), and recently evaluated in combination with gemcitabine in patients with advanced solid tumors. Treatment was well tolerated. Of the 30 treated patients, 2 patients had partial responses, and 13 (43%) had stable disease. Some patients had disease stability for greater than 10 months, including 1 patient each with pancreatic adenocarcinoma, spindle cell sarcoma, and cholangiocarcinoma (89). It was not reported if any of these patients had somatic mutations in the *ATM* gene.

Other inhibitors of Chk1, including PF-00477736 (90) and AZD7762, sensitized cells to chemotherapy-induced cell death in preclinical studies with cancer cells or xenografts. Of note, pancreatic cancer stem cells became more sensitive to gemcitabine following culture with AZD7762, prompting the authors to suggest that Chk1-mediated DNA damage response was greater in stem cells than in nonstem cells in pancreatic cancer (91). AZD7762 also inhibited radiation damage repair and conferred radiosensitivity on p53-mutant breast cancer cell lines, similar to other agents of the class (92). Notably, a patient with refractory small-cell cancer who had a complete response that proved durable for 3 years was found to have a hemizygous mutation of *RAD50* that attenuated *ATM* signaling (93). This outlier response supports the hypothesis that patients with acquired mutations in the *ATM* pathway are ideal candidates for agents that induce synthetic lethality.

Nucleoside analogues

The oral nucleoside analogue sapacitabine has also been proposed as a means to achieve synthetic lethality in *ATM*-deficient tumors. Sapacitabine is an orally bioavailable pro-drug of the nucleoside analogue 2'-C-cyano-2'-deoxy-1-beta-D-arabino-pentofuranosylcytosine (CNDAC). CNDAC incorporates into DNA and induces SSBs, which are converted into DSBs when cells go through a second S phase. These lethal DSBs are mainly repaired through homologous recombination, which is lost in the setting of *ATM* deficiency (94). Sapacitabine has been evaluated in the treatment of elderly patients with acute myeloid leukemia (AML) including a randomized, phase II study, in which patients received oral sapacitabine as a single agent. This was found to be active and tolerable with 1-year survival rates up to 35% in the

cohorts with the optimal dosing schedule. Cytopenias, febrile neutropenia, and infections were the most common adverse events (95). Sapacitabine is also under investigation in the treatment of patients with CLL, specifically with del (11q), in combination with cyclophosphamide and rituximab (NCT01253460).

Conclusions

Aberrancies of the *ATM* gene are among the most commonly occurring somatic mutations in cancer and generally have been associated with inferior prognosis. Novel therapies are in development that may improve the response to therapy of patients with *ATM*-deficient cancers. These agents inhibit checkpoint proteins or ATR, or are nucleoside analogues such as sapacitabine that induce synthetic lethality. *ATM* aberrations may also sensitize cancers to platinum drugs, similar to the effect of *BRCA1* mutations. Although initial response rates to some of these agents such as the ATR or checkpoint inhibitors have been modest, subsequent (and ongoing) studies that prospectively identify patients with *ATM* mutations may allow for more optimized drug development. Another area of investigation might relate to immunotherapy and possible usefulness of agents that target the PD1/PDL1 axis. With mismatch repair gene alterations in colon cancer, there is a high response rate to these molecules, presumably due to the high rate of mutation-associated neoantigens (96). The question arises as to whether or not abnormalities in other DNA repair genes (such as *ATM*) that interfere with the integrity of the genome increase the aberrations in the mutanome and similarly result in sensitivity to immunotherapeutic agents.

Disclosure of Potential Conflicts of Interest

R. Kurzrock has ownership interest in Novena, Inc. and Curematch; reports receiving commercial research grants from Foundation Medicine, Genentech, Guardant, Merck Serono, Pfizer, and Sequenom; and is a consultant/advisory board member for Sequenom. No potential conflicts of interest were disclosed by the other authors.

Grant Support

This study was funded in part by the Joan and Irwin Jacobs Fund philanthropic fund (to R. Kurzrock) and the Tower Cancer Research Foundation (to M. Choi)

Received December 3, 2015; revised April 13, 2016; accepted April 25, 2016; published OnlineFirst July 13, 2016.

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