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REVIEW ARTICLE

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Emerging strategies for cancer therapy by ATR inhibitors

Kimiyoshi Yano | Bunsyo Shiotani

Laboratory of Genome Stress Signaling, National Cancer Center Research Institute, Tokyo, Japan

Correspondence
Bunsyo Shiotani, Laboratory of Genome Stress Signaling, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan.
Email: bshiotan@ncc.go.jp

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Abstract

DNA replication stress (RS) causes genomic instability and vulnerability in cancer cells. To counteract RS, cells have evolved various mechanisms involving the ATR kinase signaling pathway, which regulates origin firing, cell cycle checkpoints, and fork stabilization to secure the fidelity of replication. However, ATR signaling also alleviates RS to support cell survival by driving RS tolerance, thereby contributing to therapeutic resistance. Cancer cells harboring genetic mutations and other changes that disrupt normal DNA replication increase the risk of DNA damage and the levels of RS, conferring addiction to ATR activity for sustainable replication and susceptibility to therapeutic approaches using ATR inhibitors (ATRis). Therefore, clinical trials are currently being conducted to evaluate the efficacy of ATRis as monotherapies or in combination with other drugs and biomarkers. In this review, we discuss recent advances in the elucidation of the mechanisms by which ATR functions in the RS response and its therapeutic relevance when utilizing ATRis.

KEYWORDS
ATR inhibitor, ATR kinase, biomarker, cancer therapy, DNA replication stress, replication stress tolerance

1 | INTRODUCTION

DNA replication is one of the fundamental cellular processes that duplicate genomic DNA during each cell cycle. Although faithful DNA replication is necessary for the preservation of genome integrity, the replication fork is constantly challenged by a wide variety of factors, resulting in altered progression of replication forks, reduced replication fidelity, and DNA breaks. These phenomena during DNA replication are collectively referred to as DNA replication stress (RS), which is a major cause of genome instability.[1] RS is characterized by many different causes, and the definition of RS is constantly evolving. Exogenous factors include DNA lesions that are caused by ultraviolet light, ionizing radiation, and chemical agents such as alkylating agents and cross-linking agents, while endogenous stress includes reactive oxygen species, metabolic aldehydes, misincorporated ribonucleotide, and abnormal DNA secondary structures. These lesions

Abbreviations: APOBEC3, apolipoprotein B mRNA editing enzyme catalytic subunit 3; ARID1A, AT-rich interactive domain-containing protein 1A; ATM, ataxia telangiectasia mutated; ATRIP, ATR-interacting protein; BRCA, breast cancer susceptibility; CDC25, cell division cycle 25; CDC45, cell division cycle 45; CDK, cyclin-dependent kinase; CENP, centromere protein; cGAS, cyclic GMP-AMP synthase; Chk1, checkpoint kinase 1; CI, confidence interval; CRISPR, clustered regularly interspaced short palindromic repeat; DDK, DBF4-dependent kinase; DSB, double strand break; ERCC1, excision repair cross-complementation group 1; ETAA1, Ewing's tumor-associated antigen 1; EXO1, exonuclease 1; FOXM1, Forkhead box M1; HGSOC, high-grade serous ovarian cancer; LADC, lung adenocarcinoma; MLL, mixed lineage leukemia; MRE11, meiotic recombination 11; PALB2, partner and localizer of BRCA2; PARP, poly(ADP-ribose) polymerase; PCAF, p300/CBP-associated factor; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; PFS, progression-free survival; PLK, polo-like kinase; Polη, DNA polymerase eta; PrimPol, primase and DNA directed polymerase; RNF4, ring finger protein 4; RPA, replication protein A; SCLC, small cell lung cancer; SMARCA4, SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily A, member 4; SMARCAL1, SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily A like 1; SPOP, speckle-type POZ protein; STING, stimulator of interferon genes; SWI/SNF, switch/sucrose non-fermentable; TopBP1, DNA topoisomerase II binding protein 1; UPF2, up-frameshift suppressor 2; XRCC1, X-ray repair cross complementing protein 1; XRCC3, X-ray repair cross complementing protein 3.

are generally, but not exclusively, physical obstacles to replication fork progression.[2] Accumulating evidence has revealed that the activation of oncogenes, such as Ras, Myc, and Cyclin E, induces RS by aberrant replication initiation, RNA:DNA hybrids (R-loops), replication–transcription collisions, and defective nucleotide metabolism,[3] and defects in DNA damage response (DDR) systems lead to DNA synthesis slowdown and/or replication fork stalling because of irreparable DNA lesions. Subsequently, RS caused by these genetic alterations contributes to the further acquisition of genetic mutations and chromosomal aberrations, which promote tumor development.[4] Therefore, RS is considered one of the hallmarks of cancer.

To safeguard the RS, cells have evolved the DDR network. Ataxia telangiectasia and Rad3-related (ATR) kinase acts as a master regulator of the RS response. RS usually results in stretching ssDNA, which serves as a platform to recruit ATR kinase. Activated ATR stabilizes and restarts the stressed replication fork, suppresses origin firing, activates the cell cycle checkpoint, and facilitates DNA repair. Therefore, ATR has long been considered a tumor suppressor to maintain genome stability in normal cells. However, the mutation in the *ATR* gene is not common in cancer cells, probably because of its essential role in DNA replication. Importantly, recent studies have shown that ATR signaling contributes to RS tolerance and protects cells from deleterious and chronic RS induced by oncogenes during tumor development. Thus, cancer cells are more highly dependent on ATR signaling compared with normal cells, highlighting the potential of targeting ATR in cancer therapy. When ATR is inhibited, large amounts of ssDNA arise in the genome, resulting in massive fork collapse and cell death, which is termed replication catastrophe. In the absence of a functional checkpoint by ATR inhibition, cells prematurely enter mitosis with increased DNA damage, triggering a mitotic catastrophe. Here, we discuss recent advances in the elucidation of ATR signaling, preclinical data on ATR inhibitors (ATRis) that have led to their entry into clinical trials, and potential biomarkers for predicting ATRi efficacy.

2 | ATR SIGNALING PATHWAY IN RESPONSE TO RS

2.1 | Mechanisms of ATR activation

A broad spectrum of genomic insults that activate ATR, including replication interference, DSBs, and other types of DNA lesions, commonly expose ssDNA, which is immediately bound and protected by RPA.[5] The RPA-coated ssDNA is directly recognized by ATRIP, a regulatory partner protein of ATR, which recruits the ATR–ATRIP complex to ssDNA.[6] The recruitment of multiple ATR–ATRIP complexes to RPA–ssDNA promotes the autophosphorylation of ATR at T1989, one of the first markers of ATR activation.[7] In addition, two ATR activator proteins, TopBP1 and ETAA1, are also recruited to ssDNA and stimulate ATR kinase activity by directly interacting with the ATR-activating domain. TopBP1 is recruited at ssDNA–dsDNA junctions where the Rad17 complex and Rad9–RAD1–HUS1 (9–1–1) complexes form the scaffold for TopBP1 recruitment.[8,9] Unlike TopBP1, ETAA1 directly accumulates at the RPA–ssDNA via its two RPA-binding motifs.[10,11] Alternatively, a fraction of ATR that is recruited to the RPA–ssDNA distal to ssDNA–dsDNA junctions is activated through Nbs1 in a TopBP1-dependent manner at replication-associated DSBs.[12] The detailed mechanisms of ATR activation have recently been reviewed elsewhere.[13,14] These multiple modes of ATR activation can allow ATR to phosphorylate different substrates, thereby carrying out diverse functions in the DDR, as discussed below (Figure 1).

2.2 | Cell cycle checkpoint

One crucial function of ATR signaling is to regulate the G2/M cell cycle checkpoint following DNA damage. In response to DNA damage, Chk1 is phosphorylated by ATR at multiple sites, stimulating the kinase activity of Chk1. Activated Chk1 phosphorylates and inactivates CDC25 phosphatases (known as CDC25A, CDC25B, and CDC25C), which positively regulate CDK activity by removing its inhibitory phosphorylation.[15] Chk1 also phosphorylates and activates Wee1 kinase, which negatively regulates CDK1 activity by adding inhibitory phosphorylation.[16] Interestingly, a recent study showed that, until the S phase ends, ETAA1-mediated ATR activation restricts CDK1-dependent FOXM1 phosphorylation and prevents mitotic gene expression by enforcing the S/G2 checkpoint.[17] These signaling pathways delay cell cycle progression for recovery from DNA damage and stalled replication forks.

2.3 | Origin firing

Inhibition of CDK activity by ATR–Chk1 signaling not only regulates the cell cycle checkpoint but also limits origin firing. The origin recognition complex initially loads MCM2–7 complexes as inactive double hexamers onto DNA.[18] Helicase activation requires CDC45 binding, which occurs following the CDK-dependent phosphorylation of Treslin and DDK-mediated phosphorylation of the MCM2–7 complex.[19] Following DNA damage, ATR–Chk1 signaling downregulates the kinase activities of CDK and DDK, and thereby prevents CDC45 loading and helicase activation to limit origin firing.[14] In another mechanism, ATR phosphorylates and stabilizes MLL on chromatin, where it methylates histone H3 lysine 4 at the late replication origin and inhibits CDC45 loading.[20] Based on these mechanisms, ATR inhibition triggers unscheduled origin firing, generates excessive ssDNA that exhausts the nuclear pool of RPA, and increases fork breakage, resulting in replication catastrophe.[21]

2.4 | RS tolerance

Acute and severe RS often give up continuous DNA synthesis, resulting in senescence and cell death. However, cells have evolved RS tolerance (also known as DNA damage tolerance) mechanisms

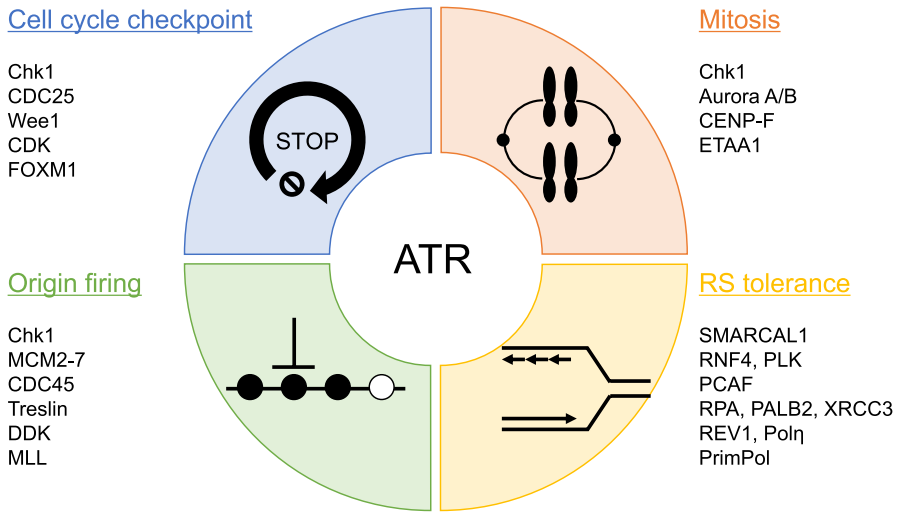

FIGURE 1 Ataxia telangiectasia and Rad3-related (ATR) signaling network in the DNA damage response (DDR). ATR kinase plays a critical role in regulating DDR, particularly during DNA replication. ATR activates the cell cycle checkpoint pathway that halts the cell cycle in response to DNA damage or replication stress (RS). ATR regulates DNA replication by inhibiting the replicative helicase, preventing further origin firing. When a replication fork stalls, ATR phosphorylates some proteins that are involved in stabilizing the fork, preventing its collapse, promoting repair, and restarting forks to promote RS tolerance mechanisms. ATR also functions in mitosis to ensure chromosome segregation. These functions are controlled by the indicated representative factors directly or indirectly regulated by ATR or Chk1. By coordinating these processes, ATR helps to maintain genomic integrity.

aimed at allowing sustainable DNA replication to overcome lesions, which support cell survival. RS tolerance mechanisms mainly include fork reversal/template switching, translesion DNA synthesis (TLS), and repriming. These pathways are regulated by fine-tuned mechanisms based on the genetic background and the extent of DNA damage (for more detail, see these reviews[22,23]). ATR also plays a key role in the regulation of RS tolerance and contributes to fork stability.

Fork reversal can be conceptually divided into the following two steps: first, the formation of four-way junction structures by coordinately annealing the two newly synthesized daughter strands, and second, fork restart through the removal of DNA lesions or template switching. ATR phosphorylates the DNA translocase SMARCAL1, thereby limiting its fork regression activity and preventing aberrant fork processing through SLX4-associated nucleases.[24] In ATR-deficient cells, suppression of RNF4 and/or PLK reduces SLX4-mediated DSB formation.[25] PCAF-mediated histone H4 acetylation at stalled forks promotes fork degradation by MRE11 and EXO1 nucleases in BRCA-deficient cells.[26] ATR phosphorylates PCAF to limit its association and excessive fork degradation. These findings suggest that ATR functions in replication fork protection to prevent aberrant remodeling of stalled forks, thereby avoiding excessive nucleolytic processing of the replicating genome.

Another aspect of RS tolerance regulated by ATR involves the restart of replicated forks. ATR phosphorylates two of the TLS polymerases, REV1 and Pol η, suggesting that ATR may facilitate lesion bypass through continuous DNA synthesis without repairing the lesion.[27–29] ATR also phosphorylates several proteins that promote RAD51-dependent replication restart pathways including template switching, fork reversal and repair, and homologous recombination. At the stressed fork, ATR phosphorylates RPA facilitating recruitment of PALB2 and BRCA2.[30] When DSBs occur, ATR also phosphorylates PALB2 enhancing interaction with BRCA1, promoting RAD51 filament formation.[31] In addition, ATR-dependent XRCC3 phosphorylation is required for chromatin loading of RAD51 and homologous recombination (HR)-mediated recovery of collapsed replication forks.[32] These findings suggest that ATR contributes to fork remodeling and restart by manipulating HR factors. Other processes that promote fork restart involve PrimPol-mediated repriming, which reinitiates DNA synthesis beyond a DNA lesion, leaving unreplicated ssDNA gaps to be filled post-replicatively through either TLS or template switching. A recent report showed that the expression level and repriming activity of PrimPol are controlled in an ATR-dependent manner.[33] In addition, chemically induced RS induces PrimPol phosphorylation dependent on ATR and Chk1, indicating that ATR–Chk1 signaling-dependent phosphorylation of PrimPol is a critical switch to turn on its repriming activity.[34] Importantly, PrimPol phosphorylation reduces undamaged cell fitness but recovers damaged cell fitness, suggesting that PrimPol needs to be tightly regulated during DNA replication to prevent aberrant repriming, fork speeding, and chromosomal breakage, which can increase the risk of genomic instability. Collectively, ATR signaling not only protects the stalled fork from nuclease-mediated degradation but also seems to promote PrimPol-dependent repriming to overcome RS.

2.5 | Mitosis

While ATR inhibition during DNA replication increases genomic instability in the S phase, cells upon uncontrolled replication

prematurely enter mitosis and overlap DNA synthesis with chromosome condensation, resulting in mitosis defects.[35] The mitosis defects in ATR-absent cells are partially rescued by CDK1 inhibition, suggesting that ATR activates the cell cycle checkpoint to minimize the level of unreplicated DNA prior to mitotic onset. Surprisingly, a recent study revealed that ATR localizes to centromeres through Aurora A-regulated association with CENP-F, allowing ATR to engage RPA-coated centromeric R-loops, and activated ATR at centromeres stimulates Aurora B through Chk1, preventing chromosome instability.[36] In addition, TopBP1- and ETAA1-dependent phosphoproteomics revealed TopBP1 to be a primary ATR activator for RS, while ETAA1 regulates mitotic ATR signaling.[37] Although these findings clearly indicate a mitosis-specific ATR role, the functions of ATR in mitosis remain largely unknown. Therefore, further understanding the action of ATR inhibition in mitosis will provide a rationale for ATRi therapy.

3 | CANCER THERAPEUTICS WITH ATRis

In 2011, the first potent and selective ATRi, VE-821, was discovered by Vertex Pharmaceuticals. VE-821 induces cancer cell killing and reversibly inhibits cell cycle progression in normal cells, suggesting that ATR is a promising target for cancer therapy.[38] Subsequently, bioavailable berzosertib (M6620, VE-822, VX-970, recently licensed to Merck KGaA), an improved analog of VE-821, entered clinical trials. In 2013, AstraZeneca also developed a preclinical ATRi, AZ20, and its improved analog, ceralasertib (AZD6738), which can be orally administered. Other orally administered ATRis, gartisertib (M4344, VX-803) and tuvusertib (M1774) by Merck, elimusertib (BAY1895344) by Bayer, camonsertib (RP-3500, recently licensed to Roche) by Repare, ART0380 by Artios, IMP9064 by Impact, ATG-018 by Antengene, and ATRN-119 by Aprea were discovered. These ATRis are currently being tested preclinically and have progressed to phase I/II clinical trials as monotherapies and in combination with DNA-damaging agents or molecular targeted drugs (Table 1).

3.1 | Monotherapy and chemotherapy combinations

Berzosertib is the first-in-class ATR inhibitor and has been tested as a monotherapy. Berzosertib monotherapy was well tolerated, with no dose-limiting toxicities observed and a durable complete response was observed in a patient with advanced-stage colorectal cancer harboring ATM loss and *ARID1A* mutation.[39] Elimusertib monotherapy was well tolerated, with antitumor activity including partial responses against cancers with ATM loss.[40]

Early clinical trials with ATRis were often conducted using them in combination with chemotherapy-inducing RS. Antimetabolites (e.g., gemcitabine) inhibit the elongation of newly synthesized DNA strands by blocking nucleotide synthesis and by acting as nucleotide analogs that lead to chain termination. DNA crosslinkers (e.g., cisplatin and carboplatin) and topoisomerase inhibitors (e.g., topotecan and irinotecan) generate obstacles to fork progression. Consequently, these chemotherapeutic agents increase RS and synergize with ATRis. The first reported phase I study of ATRi demonstrated that combined berzosertib with topotecan was partially effective in platinum-refractory SCLC.[41,42] Other phase I studies of berzosertib in combination with chemotherapy, including gemcitabine and cisplatin, also showed preliminary efficacy signs.[43–46] In phase I studies of ceralasertib in combination with carboplatin or paclitaxel, patients with low ATM or mutated *ATM* had complete or partial responses.[47,48] Notably, the first randomized phase II study of ATRi was evaluated based on its use in combination with gemcitabine versus gemcitabine alone in platinum-resistant HGSOC.[49] The median progression-free survival in all patients was 22.9 weeks (90% CI: 17.9–72.0) for the combination group versus 14.7 weeks (90% CI: 9.7–36.7) for the monotherapy group ($p = 0.044$). Interestingly, this benefit of the combination group was seen in patients with a platinum-free interval of 3 months or less compared with those with more than 3 months to less than 6 months. While these preliminary data from early phase studies for ATRis in combination with DNA-damaging chemotherapy showed antitumor activity, the chemotherapy combinations were associated with higher rates of adverse events.[39] Further late-stage clinical evaluation of the chemotherapy combinations is warranted and the identification of predictive biomarkers of response is the critical next step.

3.2 | PARPi combinations

In the past decade, PARP inhibitors (PARPis) have been clinically used to treat tumors with defects in HR, such as BRCA1/2.[50] PARPis generate DNA lesions through the following two general actions: catalytic inhibition of PARP1 and trapping PARP1 on damaged DNA; these actions render synthetic lethality with HR deficiency. Recently, it has also been proposed that the accumulation of ssDNA gaps behind replication forks is the primary genotoxic lesion enhancing PARPi sensitivity. PARPi induces ssDNA gaps on the leading strand behind replication forks via PrimPol-mediated repriming[33,51,52] and on the lagging strand via defects in Okazaki fragment processing.[53] PARPi-induced ssDNA gaps generated in the first S phase persist in the second S phase, where BRCA1/2-deficient cells fail to activate ATR and suppress origin firing, resulting in increased fork collapse. In addition, ATRi combined with PARPi abrogated the PARPi-induced G2/M checkpoint and synergistically increased chromosome aberrations.[54,55] In fact, several preclinical studies have described the synergistic therapeutic efficacy of ATRis with PARPis in tumor cells harboring BRCA1/2, ATM, and RNASEH2 deficiency and alternative lengthening of the telomere system.[54–57] Furthermore, ATRi combined with PARPi overcomes PARPi- or platinum-resistant ovarian cancer and PARPi-resistant BRCA-deficient cancer models.[55,58,59] Therefore, these preclinical data support the clinical development of ATRis in combination with PARPis. In reported phase II studies of ceralasertib in combination with olaparib, one study in 12 patients

TABLE 1 Selected ongoing and completed clinical trials with ATRis.

ATR inhibitor	Interventions	Cancers	Phase	Study start	Status	NCT number	Result reported
Monotherapy							
Berzosertib	Alone	Advanced solid tumor, leiomyosarcoma, osteosarcoma	II	2019	Completed	NCT03718091	
Ceralasertib	Alone	Relapsed/refractory CLL, PLL or B-cell lymphoma	I	2013	Completed	NCT01955668	
	Alone	Head and neck squamous cell carcinoma	I	2017	Completed	NCT03022409	
	Alone	Metastatic TNBC	II	2018	Recruiting	NCT03801369	
	Alone	Neoadjuvant chemotherapy-resistant residual TNBC	II	2019	Recruiting	NCT03740893	
	Alone	Progressive MDS or CMML	I	2019	Recruiting	NCT03770429	
	Alone	Advanced solid tumor	II	2020	Recruiting	NCT04564027	
Elimusertib	Alone	Advanced solid tumor and lymphomas	I	2017	Recruiting	NCT03188965	[40]
	Alone	Relapsed or refractory solid tumor	I/II	2021	Recruiting	NCT05071209	
IMP9064	Alone	Advanced solid tumor	I	2022	Recruiting	NCT05269316	
ATG-018	Alone	Advanced solid tumor and hematological malignancy	I	2022	Recruiting	NCT05338346	
ATRN-119	Alone	Advanced solid tumor	I/II	2023	Recruiting	NCT04905914	
ART0380	Alone	Advanced solid tumor	II	2023	Not yet recruiting	NCT05798611	
Chemotherapy combinations							
Berzosertib	Irinotecan, Gemcitabine, Cisplatin, Cisplatin + Gemcitabine or Etoposide or Carboplatin	Advanced solid tumor	I	2012	Completed	NCT02157792	[39,43–46]
	Topotecan	Small-cell cancer	I/II	2015	Active, not recruiting	NCT02487095	[41,42]
	Cisplatin + Gemcitabine	Metastatic urothelial cancer	II	2016	Active, not recruiting	NCT02567409	
	Cisplatin + Radiotherapy	Advanced head and neck cancer	I	2016	Active, not recruiting	NCT02567422	
	Gemcitabine	Platinum-resistant recurrent ovarian or primary peritoneal fallopian tube cancer	II	2016	Active, not recruiting	NCT02595892	[49]
	Irinotecan	Advanced solid tumor	I	2016	Active, not recruiting	NCT02595931	
	Carboplatin + Gemcitabine	Recurrent platinum-sensitive epithelial ovarian, peritoneal, and fallopian tube cancer	I	2016	Active, not recruiting	NCT02627443	
	Cisplatin + Capecitabine + Radiotherapy	Esophageal cancer and other solid tumors	I	2018	Completed	NCT03641547	

(Continues)

TABLE 1 (Continued)

ATR inhibitor	Interventions	Cancers	Phase	Study start	Status	NCT number	Result reported
	Alone, Carboplatin + Paclitaxel	Advanced solid tumor	I	2018	Active, not recruiting	NCT03309150	
	Carboplatin	Metastatic castration-resistant prostate cancer	II	2018	Active, not recruiting	NCT03517969	
	Topotecan	Relapsed small-cell lung cancer	II	2019	Active, not recruiting	NCT03896503	
	Irinotecan	Progressive TP53 mutant gastric and gastroesophageal junction cancer	II	2020	Active, not recruiting	NCT03641313	
	Topotecan	Relapsed platinum-resistant SCLC	II	2021	Active, not recruiting	NCT04768296	
	Lurbinectedin	Small-cell cancer and high-grade neuroendocrine cancer	I/II	2021	Recruiting	NCT04802174	
	Sacituzumab govitecan	SCLC, EP-SCNC and HR-deficient cancer resistant to PARP Inhibitors	I/II	2021	Recruiting	NCT04826341	
	Topotecan	Advanced solid tumor	I	2022	Recruiting	NCT05246111	
Gartisertib	Alone, Carboplatin	Advanced solid tumor	I	2015	Completed	NCT02278250	
Tuvusertib	Temozolomide	Advanced solid tumor, hematopoietic and lymphoid tumor	I/II	2023	Not yet recruiting	NCT05691491	
Ceralasertib	Paclitaxel	Refractory cancer	I	2015	Completed	NCT02630199	[48]
	Gemcitabine	Advanced solid tumor	I	2019	Recruiting	NCT03669601	
	Trastuzumab deruxtecan	HER2-positive advanced solid tumor	I	2021	Recruiting	NCT04704661	
Elimusertib	Cisplatin, Cisplatin + Gemcitabine	Advanced solid tumor	I	2021	Recruiting	NCT04491942	
	Irinotecan, Topotecan	Advanced solid tumor	I	2021	Recruiting	NCT04514497	
	FOLFIRI	Metastatic colorectal and gastric/gastroesophageal cancer	I	2021	Recruiting	NCT04535401	
	Gemcitabine	Advanced solid tumor	I	2021	Recruiting	NCT04616534	
ART0380	Alone, Gemcitabine, Irinotecan	Advanced or metastatic solid tumor	I/II	2021	Recruiting	NCT04657068	
PARPi combinations							
Berzosertib	Veliparib + Cisplatin	Refractory solid tumor	I	2017	Completed	NCT02723864	
Gartisertib	Niraparib	PARPi-resistant recurrent ovarian cancer	I	2023	Not yet recruiting	NCT04149145	
Tuvusertib	Alone, Niraparib	Advanced solid tumor	I	2019	Recruiting	NCT04170153	

TABLE 1 (Continued)

ATR inhibitor	Interventions	Cancers	Phase	Study start	Status	NCT number	Result reported
Ceralasertib	Olaparib	Advanced solid tumor	II	2015	Terminated	NCT02576444	[61]
	Olaparib	Platinum-refractory extensive-stage SCLC	II	2016	Active, not recruiting	NCT02937818	
	Olaparib	Advanced breast cancer	II	2016	Recruiting	NCT03182634	
	Olaparib	Advanced TNBC	II	2018	Active, not recruiting	NCT03330847	
	Olaparib	Recurrent ovarian cancer	II	2018	Recruiting	NCT03462342	[60]
	Olaparib	Recurrent ovarian, primary peritoneal, or fallopian tube cancer	II	2018	Recruiting	NCT03579316	
	Olaparib	Relapsed SCLC	II	2018	Completed	NCT03428607	
	Olaparib	Prostate cancer	II	2019	Active, not recruiting	NCT03787680	
	Olaparib	IDH1 and IDH2 mutant tumor	II	2019	Recruiting	NCT03878095	
	Alone, Olaparib	Gynecological cancer with ARID1A loss or no loss	II	2019	Recruiting	NCT04065269	
	Olaparib	Advanced or metastatic germline BRCA mutated breast cancer	II	2020	Recruiting	NCT04090567	
	Olaparib	Recurrent osteosarcoma	II	2020	Recruiting	NCT04417062	
Elimusertib	Niraparib	Advanced solid tumor	I	2020	Recruiting	NCT04267939	
Camonsertib	Alone, Talazoparib, Gemcitabine	Advanced solid tumor	I/II	2020	Recruiting	NCT04497116	
	Niraparib, Olaparib	Advanced solid tumor	I/II	2021	Recruiting	NCT04972110	
	Olaparib	DDR-deficient relapsed/refractory CLL	I/II	2022	Recruiting	NCT05405309	
Immunotherapy combinations							
Berzosertib	Avelumab + Carboplatin	PARPi-resistant recurrent ovarian, primary peritoneal, or fallopian tube cancer	I	2019	Completed	NCT03704467	
	Carboplatin + Gemcitabine + Pembrolizumab	Advanced NSCLC	I/II	2020	Recruiting	NCT04216316	
	Avelumab	DDR-deficient metastatic or unresectable solid tumor	I/II	2020	Recruiting	NCT04266912	
Tuvusertib	M4076, Avelumab	Advanced solid tumor	I	2022	Recruiting	NCT05396833	

(Continues)

TABLE 1 (Continued)

ATR inhibitor	Interventions	Cancers	Phase	Study start	Status	NCT number	Result reported
Ceralasertib	Alone, Carboplatin, Olaparib, AZD5305, Durvalumab	Advanced solid tumor	I/II	2014	Recruiting	NCT02264678	[47]
	Durvalumab	NSCLC	II	2015	Active, not recruiting	NCT02664935	
	Alone, Durvalumab	NSCLC	II	2017	Recruiting	NCT03334617	
	Durvalumab	Advanced solid tumor	I	2018	Recruiting	NCT05514132	
	Alone, Olaparib, Durvalumab	Advanced solid tumor	II	2019	Recruiting	NCT03682289	
	Durvalumab	Gastric cancer and melanoma	II	2019	Active, not recruiting	NCT03780608	[67,68]
	Durvalumab	NSCLC	II	2019	Recruiting	NCT03833440	
	Durvalumab	Refractory biliary tract cancer	II	2020	Recruiting	NCT04298008	
	Durvalumab, Olaparib	Advanced biliary tract cancer	II	2020	Recruiting	NCT04298021	
	Durvalumab	Relapsed SCLC	II	2020	Active, not recruiting	NCT04361825	
	Cisplatin or Carboplatin + Etoposide + Durvalumab	Extensive-stage SCLC	II	2021	Recruiting	NCT04699838	
	Durvalumab + Nab-paclitaxel	Advanced TNBC	II	2022	Recruiting	NCT05582538	
Elimusertib	Pembrolizumab	Advanced solid tumor	I	2019	Active, not recruiting	NCT04095273	
	Pembrolizumab + Radiotherapy	Recurrent head and neck cancer	I	2021	Recruiting	NCT04576091	
Other combinations							
Berzosertib	Whole-brain radiotherapy	Brain metastases from lung cancer	I	2016	Active, not recruiting	NCT02589522	
	Radiotherapy	Chemotherapy-resistant triple-negative and ER/PR-positive, HER2-negative breast cancer	I	2019	Recruiting	NCT04052555	
Tuvusertib	Peposertib	Advanced solid tumor	I	2023	Not yet recruiting	NCT05687136	
Ceralasertib	Acalabrutinib	Relapsed/refractory aggressive non-Hodgkin's lymphoma	I	2018	Completed	NCT03527147	
	Alone, Acalabrutinib	Relapsed or refractory CLL	I	2020	Active, not recruiting	NCT03328273	
Camonsertib	RP-6306	Advanced solid tumor	I	2021	Recruiting	NCT04855656	
	Radiotherapy	Metastatic cancer with ATM mutation	I/II	2022	Recruiting	NCT05566574	

Note: These clinical trial data are from ClinicalTrials.gov (April 2023). Ongoing clinical trials include the status active, not recruiting, recruiting, and not yet recruiting.

with platinum-resistant high-grade ovarian cancer observed no objective response.[60] In another study, two out of five patients with tumors harboring the *ATM* mutation achieved complete response or stable disease. Of seven patients with PARP inhibitor-resistant HGSOC, one achieved a partial response and five had stable disease.[61] Further testing of larger populations and randomized trials are warranted.

3.3 | Immunotherapy combinations

Other proposed combination drugs with ATRis are immune checkpoint inhibitors (ICIs), such as monoclonal antibodies targeting PD-1/PD-L1. Accumulating evidence indicates that, upon RS, DNA fragments might be released from the nucleus into the cytosol, causing cGAS–STING pathway activation,[62] leading to T-cell priming and recruitment and boosting the efficacy of immunotherapies.[63] Moreover, ATRi downregulates PD-L1 expression by activating the CDK1–SPOP pathway and sensitizes tumor cells to T-cell-mediated killing.[64,65] These results provide a rationale for combination therapy with ATRis and ICIs. Preclinical studies showed beneficial results by combination therapy with ATRi and ICI and by triple therapy with ATRi, ICI, and radiation.[65,66] In phase II studies with ceralasertib in combination with durvalumab, 9 of 30 patients with gastric cancer and 7 of 31 patients with melanoma achieved partial responses.[67,68]

Overall, ATRis exhibit preliminary antitumor activities in these clinical studies when RS is increased by genetic alterations or by drug combinations, but many ongoing clinical studies are still awaiting results. Whereas *ATM* loss seems to be a useful biomarker for predicting vulnerability to ATRi monotherapy and combination therapy, the identification of further predictive biomarkers is critical for the success of therapeutic approaches using ATRis.

4 | BIOMARKERS

To optimize ATRi therapy, *in vitro* and *in vivo* assays have identified several potential biomarkers associated with the response to ATRis. These biomarkers may be categorized into four groups (Table 2). The first group is associated with cell signaling pathways activated in response to RS. As fundamental models, various types of RS inducers generally increase the phosphorylated forms of ATR, Chk1, and RPA32 as markers of ATR signaling activation and γH2A.X levels as markers of DNA damage.[13] These phosphorylated forms reflect the cellular response to low or high levels of RS, indicating the utility of immunohistochemistry assays based on specific antibodies. The second group includes genetic alterations leading to RS. Activation of oncogenes such as Ras, Myc, and Cyclin E can elicit RS through different mechanisms and increase susceptibility to ATRis.[69–71] In addition, APOBEC3A/B activities impose a unique type of RS by generating abasic sites at replication forks and rendering cancer cells susceptible to ATRis.[72] The third group includes loss of function in DDR and DNA repair. Cells harboring defects in DDR and DNA repair are highly dependent on alternative DDR pathways, resulting in synthetic lethality with additional DDR inhibition because of unbearable DNA damage. Consistently, ATRis are toxic in ATM-, p53-, or HR-deficient tumor cells.[38,59,73] Deficiencies in XRCC1, ERCC1, ARID1A, and RNASEH2, which are involved in DNA repair, were also identified as predictive biomarkers of ATRi susceptibility.[74–76] BRCA1/2 deficiency, especially in the context of PARPi- or Cisplatin-resistant tumors, confers ATRi alone or ATRi and PARPi combination sensitivity.[55,59] The fourth group is biomarkers resistant to ATRis. Cells with low levels of CDC25A and UPF2 were resistant to ATRi,[77,78] suggesting the possibility of previously unknown mechanisms underlying resistance to ATRis. The clinical utility of these biomarkers is currently under active investigation (Table 1), and further validation is clearly needed to stratify patients who will respond to ATRi therapy.

As mentioned above, genome-wide CRISPR or si/shRNA screens enable a comprehensive search for predictive biomarkers of ATRis and have been used to identify some genetic biomarker candidates. One of the concerns is that these screening models have evaluated the response to ATRis under acute RS after knockout or knockdown but lack a process to become RS tolerant. Alternatively, recent studies have clarified that RS tolerance can overcome acute RS and promote cancer survival. As cells with oncogenic alterations seem to acquire RS tolerance mechanisms during the long process of tumorigenesis, cancer cells may no longer be highly dependent on the ATR response against such RS induced by acute loss of biomarkers. Therefore, a strategy to examine biomarkers after the chronic loss of candidate factors would allow for the identification of biomarkers that can account for acute RS and RS tolerance.

We previously identified a deficiency in SMARCA4, a core component of the SWI/SNF chromatin remodeling complex, as a predictive biomarker of ATRi efficacy using LADC cell line-based screening.[79] As a chronic response, SMARCA4-deficient cells increase heterochromatin formation and thereby elevate RS, rendering them dependent on ATR-mediated RS tolerance for survival. However, in the absence of SMARCA4, ATRi-induced acute RS causes severe ssDNA exposure on nascent DNA near the reversed forks around heterochromatin in a Mre11-dependent manner, leading to replication catastrophe. Thus, SMARCA4 loss synergistically confers ATRi susceptibility by increasing heterochromatin-associated RS and by allowing Mre11 to destabilize reversed forks. In agreement with our results, Gupta and colleagues also reported that SMARCA4 loss led to clinically relevant gene expression changes related to RS and prereplication functions in LADC patients and in a mouse model; these changes activated ATR signaling.[80] The dependence on ATR under replication defects provides a possible explanation for how lung cancer cells tolerate SMARCA4 loss and confer ATRi susceptibility. Although what kind of RS tolerance mechanisms are at play in SMARCA4-deficient cancer cells awaits further investigation, ATRis may target not only acute RS but also RS tolerance in SMARCA4-deficient cells. Thus, SMARCA4 deficiency can be a beneficial predictive biomarker of ATRi efficacy.

TABLE 2 Categories of biomarkers for predicting ATRi efficacy.

Biomarkers associated with cell signaling pathways activated in response to replication stress	p-ATR
	p-Chk1
	p-RPA32
	γH2A.X
Biomarkers of genetic alterations leading to replication stress	MYC overexpression
	RAS mutation
	Cyclin E overexpression
	APOBEC3A/B overexpression
	EWS translocation
	MLL translocation
Biomarkers of loss of function in DNA repair and DDR	ARID1A deficiency
	RNASEH2 deficiency
	ATM deficiency
	p53 deficiency
	ERCC1 deficiency
	XRCC1 deficiency
	PARPi- or platinum-resistant BRCA1/2 deficiency
	RAD51 deficiency
	SLFN11 deficiency
	SMARCA4 deficiency
Biomarkers resistant to ATRis	CDC25A deficiency
	UPF2 deficiency

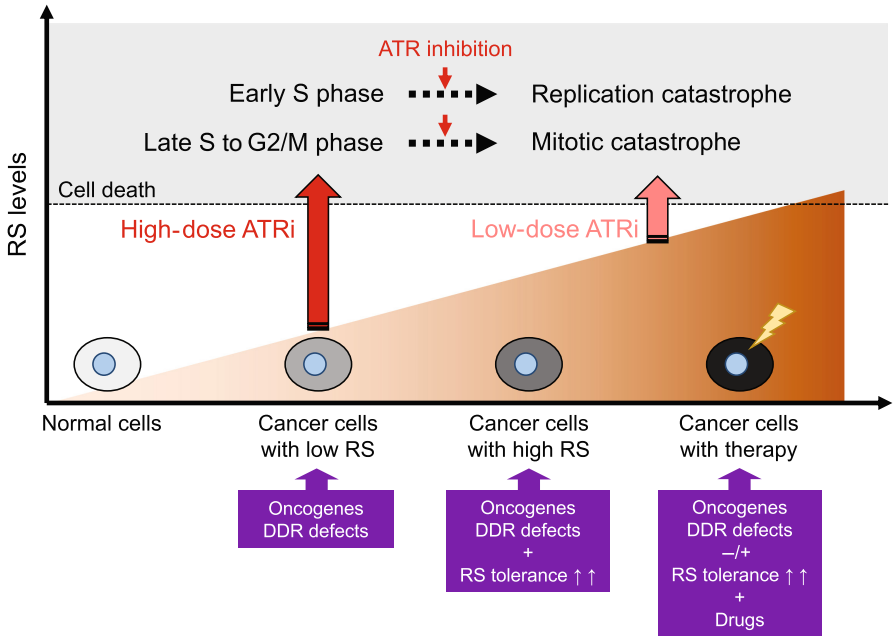

FIGURE 2 Potential models of action of ataxia telangiectasia and Rad3-related inhibitors (ATRis) depending on DNA replication stress (RS) levels. Because their RS stems from a high rate of DNA replication and/or genomic instability, cancer cells often have an increased reliance on the ATR signaling pathway compared with normal cells, conferring susceptibility to therapeutic approaches using ATRis. Cancer cells harboring oncogene activation and/or DNA damage response defects survive with low levels of RS and might require high doses of ATRis to reach the RS threshold that induces cell death. When cancer cells acquire ATR-mediated RS tolerance, they are more dependent on ATR signaling for survival. In addition, cancer cells treated with RS-inducing drugs exhibit high levels of RS that synergize with low doses of ATRis to induce cell death. Finally, RS at levels over the threshold, induced by ATRis, can lead to cell death through replication catastrophe in early S cells and mitotic catastrophe in late S to G2/M phase cells.

5 | PERSPECTIVE

ATR kinase is a master regulator in response to RS, contributing to both genomic integrity in normal cells and RS tolerance in cancer cells. Importantly, preclinical evidence has suggested that targeting ATR might be a selective strategy for cancer cells, but not normal cells, making ATR an attractive target; furthermore, the causes and levels of RS in cancer cells might be critical determinants of ATRi efficacy. Here, we provide potential models of ATRi action depending on RS levels in cells (Figure 2). Cancer cells with low RS due to factors such as activation of oncogenes or defects in DDR might require high doses of ATRis to reach the RS threshold to induce cell death. When cancer cells can adapt to RS by acquiring ATR-mediated RS tolerance mechanisms, they become more addicted to ATR signaling for survival. Therefore, RS tolerance dependency might be a new type of predictive biomarker of ATRi efficacy. Finally, cancer cells treated with chemotherapeutic agents such as cisplatin and PARPi exhibit very high RS, which may be sufficient by itself to reach the RS threshold or to synergize with low doses of ATRis. As RS causes cell cycle arrest at S and/or G2 phase in an ATR-dependent manner, ATR inhibition can induce replication catastrophe in early S phase cells, promote early mitotic entry and predominantly induce mitotic catastrophe in cells in late S phase or G2 phase. However, RS levels are determined in a highly complex manner by multiple factors and are associated with ATRi susceptibility. Therefore, the development of a method to accurately measure RS in cancer cells is expected to open further possibilities for ATRi therapy. Hopefully, this knowledge will be integrated into clinical development, and ATRis will become the new drugs of choice in the fight against cancer in the future.

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ORCID

Bunsyo Shiotani https://orcid.org/0000-0002-3583-4318

REFERENCES

1. Saxena S, Zou L. Hallmarks of DNA replication stress. *Mol Cell*. 2022;82:2298-2314.
2. Zeman MK, Cimprich KA. Causes and consequences of replication stress. *Nat Cell Biol*. 2014;16:2-9.
3. Kotsantis P, Petermann E, Boulton SJ. Mechanisms of oncogene-induced replication stress: jigsaw falling into place. *Cancer Discov*. 2018;8:537-555.
4. Macheret M, Halazonetis TD. DNA replication stress as a hallmark of cancer. *Annu Rev Pathol*. 2015;10:425-448.
5. Cimprich KA, Cortez D. ATR: an essential regulator of genome integrity. *Nat Rev Mol Cell Biol*. 2008;9:616-627.
6. Zou L, Elledge SJ. Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. *Science*. 2003;300:1542-1548.
7. Liu S, Shiotani B, Lahiri M, et al. ATR autophosphorylation as a molecular switch for checkpoint activation. *Mol Cell*. 2011;43:192-202.
8. Delacroix S, Wagner JM, Kobayashi M, Yamamoto K, Karnitz LM. The Rad9-Hus1-Rad1 (9-1-1) clamp activates checkpoint signaling via TopBP1. *Genes Dev*. 2007;21:1472-1477.
9. Kumagai A, Lee J, Yoo HY, Dunphy WG. TopBP1 activates the ATR-ATRIP complex. *Cell*. 2006;124:943-955.
10. Haahr P, Hoffmann S, Tollenaere MA, et al. Activation of the ATR kinase by the RPA-binding protein ETAA1. *Nat Cell Biol*. 2016;18:1196-1207.
11. Bass TE, Luzwick JW, Kavanaugh G, et al. ETAA1 acts at stalled replication forks to maintain genome integrity. *Nat Cell Biol*. 2016;18:1185-1195.
12. Shiotani B, Nguyen HD, Hakansson P, et al. Two distinct modes of ATR activation orchestrated by Rad17 and Nbs1. *Cell Rep*. 2013;3:1651-1662.
13. Yazinski SA, Zou L. Functions, regulation, and therapeutic implications of the ATR checkpoint pathway. *Annu Rev Genet*. 2016;50:155-173. doi:10.1146/annurev-genet-121415-121658
14. Saldivar JC, Cortez D, Cimprich KA. The essential kinase ATR: ensuring faithful duplication of a challenging genome. *Nat Rev Mol Cell Biol*. 2017;18:622-636. doi:10.1038/nrm.2017.67
15. Karlsson-Rosenthal C, Millar JB. Cdc25: mechanisms of checkpoint inhibition and recovery. *Trends Cell Biol*. 2006;16:285-292.
16. O'Connell MJ, Raleigh JM, Verkade HM, Nurse P. Chk1 is a wee1 kinase in the G2 DNA damage checkpoint inhibiting cdc2 by Y15 phosphorylation. *EMBO J*. 1997;16:545-554.
17. Saldivar JC, Hamperl S, Bocek MJ, et al. An intrinsic S/G(2) checkpoint enforced by ATR. *Science*. 2018;361:806-810.
18. Remus D, Beuron F, Tolun G, Griffith JD, Morris EP, Diffley JF. Concerted loading of Mcm2-7 double hexamers around DNA during DNA replication origin licensing. *Cell*. 2009;139:719-730.
19. Yekezare M, Gomez-Gonzalez B, Diffley JF. Controlling DNA replication origins in response to DNA damage—inhibit globally, activate locally. *J Cell Sci*. 2013;126:1297-1306.
20. Liu H, Takeda S, Kumar R, et al. Phosphorylation of MLL by ATR is required for execution of mammalian S-phase checkpoint. *Nature*. 2010;467:343-346.
21. Toledo LI, Altmeyer M, Rask MB, et al. ATR prohibits replication catastrophe by preventing global exhaustion of RPA. *Cell*. 2013;155:1088-1103.
22. Quinet A, Tirman S, Cybulla E, Meroni A, Vindigni A. To skip or not to skip: choosing repriming to tolerate DNA damage. *Mol Cell*. 2021;81:649-658.
23. Cybulla E, Vindigni A. Leveraging the replication stress response to optimize cancer therapy. *Nat Rev Cancer*. 2022;23:6-24. doi:10.1038/s41568-022-00518-6
24. Couch FB, Bansbach CE, Driscoll R, et al. ATR phosphorylates SMARCAL1 to prevent replication fork collapse. *Genes Dev*. 2013;27:1610-1623.

25. Ragland RL, Patel S, Rivard RS, et al. RNF4 and PLK1 are required for replication fork collapse in ATR-deficient cells. *Genes Dev*. 2013;27:2259-2273.
26. Kim JJ, Lee SY, Choi JH, Woo HG, Xhemalce B, Miller KM. PCAF-mediated histone acetylation promotes replication fork degradation by MRE11 and EXO1 in BRCA-deficient cells. *Mol Cell*. 2020;80:327-344 e328.
27. Sabbioneda S, Bortolomai I, Giannattasio M, Plevani P, Muzi-Falconi M. Yeast Rev1 is cell cycle regulated, phosphorylated in response to DNA damage and its binding to chromosomes is dependent upon MEC1. *DNA Repair (Amst)*. 2007;6:121-127.
28. Pages V, Santa Maria SR, Prakash L, Prakash S. Role of DNA damage-induced replication checkpoint in promoting lesion bypass by translesion synthesis in yeast. *Genes Dev*. 2009;23:1438-1449.
29. Gohler T, Sabbioneda S, Green CM, Lehmann AR. ATR-mediated phosphorylation of DNA polymerase eta is needed for efficient recovery from UV damage. *J Cell Biol*. 2011;192:219-227.
30. Murphy AK, Fitzgerald M, Ro T, et al. Phosphorylated RPA recruits PALB2 to stalled DNA replication forks to facilitate fork recovery. *J Cell Biol*. 2014;206:493-507.
31. Buisson R, Niraj J, Rodrigue A, et al. Coupling of homologous recombination and the checkpoint by ATR. *Mol Cell*. 2017;65:336-346.
32. Somyajit K, Basavaraju S, Scully R, Nagaraju G. ATM- and ATR-mediated phosphorylation of XRCC3 regulates DNA double-strand break-induced checkpoint activation and repair. *Mol Cell Biol*. 2013;33:1830-1844.
33. Quinet A, Tirman S, Jackson J, et al. PRIMPOL-mediated adaptive response suppresses replication fork reversal in BRCA-deficient cells. *Mol Cell*. 2020;77:461-474 e469.
34. Mehta KPM, Thada V, Zhao R, et al. CHK1 phosphorylates PRIMPOL to promote replication stress tolerance. *Sci Adv*. 2022;8:eabm0314.
35. Eykelenboom JK, Harte EC, Canavan L, et al. ATR activates the S-M checkpoint during unperturbed growth to ensure sufficient replication prior to mitotic onset. *Cell Rep*. 2013;5:1095-1107.
36. Kabeche L, Nguyen HD, Buisson R, Zou L. A mitosis-specific and R loop-driven ATR pathway promotes faithful chromosome segregation. *Science*. 2018;359:108-114.
37. Bass TE, Cortez D. Quantitative phosphoproteomics reveals mitotic function of the ATR activator ETAA1. *J Cell Biol*. 2019;218:1235-1249.
38. Reaper PM, Griffiths MR, Long JM, et al. Selective killing of ATM- or p53-deficient cancer cells through inhibition of ATR. *Nat Chem Biol*. 2011;7:428-430.
39. Yap TA, O'Carrigan B, Penney MS, et al. Phase I trial of first-in-class ATR inhibitor M6620 (VX-970) as monotherapy or in combination with carboplatin in patients with advanced solid tumors. *J Clin Oncol*. 2020;38:3195-3204.
40. Yap TA, Tan DSP, Terbuch A, et al. First-in-human trial of the Oral ataxia telangiectasia and RAD3-related (ATR) inhibitor BAY 1895344 in patients with advanced solid tumors. *Cancer Discov*. 2021;11:80-91.
41. Thomas A, Redon CE, Sciuto L, et al. Phase I study of ATR inhibitor M6620 in combination with Topotecan in patients with advanced solid tumors. *J Clin Oncol*. 2018;36:1594-1602.
42. Thomas A, Takahashi N, Rajapakse VN, et al. Therapeutic targeting of ATR yields durable regressions in small cell lung cancers with high replication stress. *Cancer Cell*. 2021;39:566-579 e567.
43. Middleton MR, Dean E, Evans TRJ, et al. Phase 1 study of the ATR inhibitor berzosertib (formerly M6620, VX-970) combined with gemcitabine +/− cisplatin in patients with advanced solid tumours. *Br J Cancer*. 2021;125:510-519.
44. Shapiro GI, Wesolowski R, Devoe C, et al. Phase 1 study of the ATR inhibitor berzosertib in combination with cisplatin in patients with advanced solid tumours. *Br J Cancer*. 2021;125:520-527.
45. Plummer R, Dean E, Arkenau HT, et al. A phase 1b study evaluating the safety and preliminary efficacy of berzosertib in combination with gemcitabine in patients with advanced non-small cell lung cancer. *Lung Cancer*. 2022;163:19-26.
46. Telli ML, Tolaney SM, Shapiro GI, et al. Phase 1b study of berzosertib and cisplatin in patients with advanced triple-negative breast cancer. *NPJ Breast Cancer*. 2022;8:45.
47. Yap TA, Krebs MG, Postel-Vinay S, et al. Ceralasertib (AZD6738), an Oral ATR kinase inhibitor, in combination with carboplatin in patients with advanced solid tumors: a phase I study. *Clin Cancer Res*. 2021;27:5213-5224.
48. Kim ST, Smith SA, Mortimer P, et al. Phase I study of Ceralasertib (AZD6738), a novel DNA damage repair agent, in combination with weekly paclitaxel in refractory cancer. *Clin Cancer Res*. 2021;27:4700-4709.
49. Konstantinopoulos PA, Cheng SC, Wahner Hendrickson AE, et al. Berzosertib plus gemcitabine versus gemcitabine alone in platinum-resistant high-grade serous ovarian cancer: a multicentre, open-label, randomised, phase 2 trial. *Lancet Oncol*. 2020;21:957-968.
50. Mateo J, Lord CJ, Serra V, et al. A decade of clinical development of PARP inhibitors in perspective. *Ann Oncol*. 2019;30:1437-1447.
51. Berti M, Ray Chaudhuri A, Thangavel S, et al. Human RECQ1 promotes restart of replication forks reversed by DNA topoisomerase I inhibition. *Nat Struct Mol Biol*. 2013;20:347-354.
52. Simoneau A, Xiong R, Zou L. The trans cell cycle effects of PARP inhibitors underlie their selectivity toward BRCA1/2-deficient cells. *Genes Dev*. 2021;35:1271-1289.
53. Cong K, Peng M, Kousholt AN, et al. Replication gaps are a key determinant of PARP inhibitor synthetic lethality with BRCA deficiency. *Mol Cell*. 2021;81:3128-3144 e3127.
54. Lloyd RL, Wijnhoven PWG, Ramos-Montoya A, et al. Combined PARP and ATR inhibition potentiates genome instability and cell death in ATM-deficient cancer cells. *Oncogene*. 2020;39:4869-4883.
55. Kim H, Xu H, George E, et al. Combining PARP with ATR inhibition overcomes PARP inhibitor and platinum resistance in ovarian cancer models. *Nat Commun*. 2020;11:3726.
56. Zimmermann M, Bernier C, Kaiser B, et al. Guiding ATR and PARP inhibitor combinationswith chemogenomic screens. *Cell Rep*. 2022;40:111081.
57. Roulston A, Zimmermann M, Papp R, et al. RP-3500: a novel, potent, and selective ATR inhibitor that is effective in preclinical models as a monotherapy and in combination with PARP inhibitors. *Mol Cancer Ther*. 2022;21:245-256.
58. Murai J, Feng Y, Yu GYK, et al. Resistance to PARP inhibitors by SLFN11 inactivation can be overcome by ATR inhibition. *Oncotarget*. 2016;7:76534-76550.
59. Yazinski SA, Comaills V, Buisson R, et al. ATR inhibition disrupts rewired homologous recombination and fork protection pathways in PARP inhibitor-resistant BRCA-deficient cancer cells. *Genes Dev*. 2017;31:318-332.
60. Shah PD, Wethington SL, Pagan C, et al. Combination ATR and PARP inhibitor (CAPRI): a phase 2 study of ceralasertib plus olaparib in patients with recurrent, platinum-resistant epithelial ovarian cancer. *Gynecol Oncol*. 2021;163:246-253.
61. Mahdi H, Hafez N, Doroshow D, et al. Ceralasertib-mediated ATR inhibition combined with Olaparib in advanced cancers harboring DNA damage response and repair alterations (Olaparib combinations). *JCO Precis Oncol*. 2021;5:1432-1442.
62. Técher H, Pasero P. The replication stress response on a narrow path between genomic instability and inflammation. *Front Cell Dev Biol*. 2021;9:702584.
63. Ragu S, Matos-Rodrigues G, Lopez BS. Replication stress, DNA damage, inflammatory cytokines and innate immune response. *Genes (Basel)*. 2020;11:409.

64. Sun LL, Yang RY, Li CW, et al. Inhibition of ATR downregulates PD-L1 and sensitizes tumor cells to T cell-mediated killing. *Am J Cancer Res*. 2018;8:1307-1316.
65. Tang Z, Pilie PG, Geng C, et al. ATR inhibition induces CDK1-SPOP signaling and enhances anti-PD-L1 cytotoxicity in prostate cancer. *Clin Cancer Res*. 2021;27:4898-4909.
66. Sheng H, Huang Y, Xiao Y, et al. ATR inhibitor AZD6738 enhances the antitumor activity of radiotherapy and immune checkpoint inhibitors by potentiating the tumor immune microenvironment in hepatocellular carcinoma. *J Immunother Cancer*. 2020;8:e000340.
67. Kim R, Kwon M, An M, et al. Phase II study of ceralasertib (AZD6738) in combination with durvalumab in patients with advanced/metastatic melanoma who have failed prior anti-PD-1 therapy. *Ann Oncol*. 2022;33:193-203.
68. Kwon M, Kim G, Kim R, et al. Phase II study of ceralasertib (AZD6738) in combination with durvalumab in patients with advanced gastric cancer. *J Immunother Cancer*. 2022;10:e005041.
69. Gilad O, Nabet BY, Ragland RL, et al. Combining ATR suppression with oncogenic Ras synergistically increases genomic instability, causing synthetic lethality or tumorigenesis in a dosage-dependent manner. *Cancer Res*. 2010;70:9693-9702.
70. Jones RM, Mortusewicz O, Afzal I, et al. Increased replication initiation and conflicts with transcription underlie cyclin E-induced replication stress. *Oncogene*. 2013;32:3744-3753.
71. Cottini F, Hideshima T, Suzuki R, et al. Synthetic lethal approaches exploiting DNA damage in aggressive myeloma. *Cancer Discov*. 2015;5:972-987.
72. Buisson R, Lawrence MS, Benes CH, Zou L. APOBEC3A and APOBEC3B activities render cancer cells susceptible to ATR inhibition. *Cancer Res*. 2017;77:4567-4578.
73. Krajewska M, Fehrmann RS, Schoonen PM, et al. ATR inhibition preferentially targets homologous recombination-deficient tumor cells. *Oncogene*. 2015;34:3474-3481.
74. Mohni KN, Kavanaugh GM, Cortez D. ATR pathway inhibition is synthetically lethal in cancer cells with ERCC1 deficiency. *Cancer Res*. 2014;74:2835-2845.
75. Williamson CT, Miller R, Pemberton HN, et al. ATR inhibitors as a synthetic lethal therapy for tumours deficient in ARID1A. *Nat Commun*. 2016;7:13837.
76. Wang C, Wang G, Feng X, et al. Genome-wide CRISPR screens reveal synthetic lethality of RNASEH2 deficiency and ATR inhibition. *Oncogene*. 2019;38:2451-2463.
77. Ruiz S, Mayor-Ruiz C, Lafarga V, et al. A genome-wide CRISPR screen identifies CDC25A as a determinant of sensitivity to ATR inhibitors. *Mol Cell*. 2016;62:307-313.
78. O'Leary PC, Chen H, Doruk YU, et al. Resistance to ATR inhibitors is mediated by loss of the nonsense-mediated decay factor UPF2. *Cancer Res*. 2022;82:3950-3961.
79. Kurashima K, Kashiwagi H, Shimomura I, et al. SMARCA4 deficiency-associated heterochromatin induces intrinsic DNA replication stress and susceptibility to ATR inhibition in lung adenocarcinoma. *NAR Cancer*. 2020;2:zcaa005.
80. Gupta M, Concepcion CP, Fahey CG, et al. BRG1 loss predisposes lung cancers to replicative stress and ATR dependency. *Cancer Res*. 2020;80:3841-3854.

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