



Targeting ATR in Cancer Medicine

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14.1 Introduction

Preserving genomic integrity is pivotal for cell survival; consequently, cells rely on a network of complex signaling pathways to facilitate faithful DNA replication and maintain genomic stability [1]. Increased proliferation rates are associated with genomic instability via the accumulation of DNA damage in the form of DNA double strand breaks (DSBs) or DNA single strand breaks (SSBs) caused by a variety of events, including replication stress induced by stalling of replication

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T. A. Yap and G. I. Shapiro (eds.), *Targeting the DNA Damage Response for Cancer Therapy*, Cancer Treatment and Research 186,

https://doi.org/10.1007/978-3-031-30065-3_14

forks [2–4]. These events distort genetic material due to subsequent fusion of DSBs and shortening of telomeres, which can result in translocations, gene amplification, and gene mutations [5–7].

When DNA damage or replication stress is sensed, cells are prevented entry into mitosis by activating DNA Damage Response (DDR) pathways at varying phases within the cell cycle [8]. DDR signaling pathways orchestrate tightly regulated kinase cascades to resolve DNA damage and replication stress by pausing the cell cycle and initiating repair [9, 10]. Ataxia telangiectasia and Rad3-related protein (ATR) is a key PI3K-related kinase within the DDR that senses replication stress and regulates checkpoints within the cell cycle's synthesis (S) and gap 2/mitosis (G2/M) phases to preserve genomic stability [1, 10–12].

Notably, replication stress and genomic instability are hallmarks of cancer cells, making them dependent on protective DDR pathways for survival [10, 12–14]. As such, targeting ATR in cancer medicine is an attractive therapeutic approach to circumvent cancer cell survival by exploiting their dependence on ATR-driven processes.

14.2 ATR Acts as a Gatekeeper of DNA Damage Repair

Endogenous and exogenous sources of DNA damage lead to a wide variety of adducts including DSBs, SSBs, base damage, bulky adducts and base mismatches [1]. Cells have evolved complex DNA repair mechanisms designed to specifically repair these types of damage and maintain genome stability. Repair of DNA DSBs is of particular importance as it is estimated that a single unrepaired DNA DSB can initiate cell death, highlighting the critical role of DDR pathways in cell survival [16]. DSBs are repaired by a number of mechanisms: the best characterized being the error-free homologous recombination repair (HRR) pathway, the highly efficient -but error prone- nonhomologous end joining (NHEJ) pathway, and the error prone microhomology mediated end-joining pathway (regarded as a backup pathway to HRR) [15].

Repair by HRR is initiated by an upstream activator of DDR, the ataxia telangiectasia-mutated (*ATM*) PI3K-related kinase, which upon sensing DSBs triggers a cascade of events that include cell cycle arrest, repair and apoptosis [17, 18]. During S or G2-phases, exposure of single-stranded DNA can occur as an intermediate of HRR at areas of resected DNA and also at stressed replication forks. These single-stranded DNA regions quickly become coated with the high-affinity ssDNA binding protein, replication protein A (RPA), which protects against DNA degradation. The coating of single-stranded DNA by RPA recruits ATR/ATR-interacting protein (ATRIP) complexes to sites of damage [1, 16]. Following localization to sites of damage, ATR is activated by either topoisomerase II binding protein (TopBP1) or Ewing tumour-associated antigen 1 (ETAA1) [17]. Moreover, recruitment of TopBP1 is mediated by Rad17, which loads the 9-1-1 (Rad9-Hus1-Rad1) complex onto chromatin, binds to TopBP1, and results in ATR activation [4]. Once activated, ATR proceeds to phosphorylate a series of downstream targets; however,

its activation of checkpoint kinase 1 (CHK1) is integral to its regulation of cell cycle checkpoints (Fig. 14.1) [16].

ATR commands control over the S and G2/M checkpoints by phosphorylating and activating CHK1 [1, 18]. Active CHK1 kinase in turn phosphorylates and inactivates the cell division cycle 25A/25C (CDC25A/CDC25C) phosphatase proteins, leading to their respective degradation [1]. Degradation of CDC25A thereby renders CDK2 and its associated complexes inactive by removing an inhibitory phosphorylation present on CDK2 [19]. Consequently, progression to S phase is interrupted, preventing DNA replication and promoting DNA repair [16]. ATR-mediated activation of CHK1 also interrupts the G2/M checkpoint in a Wee1-like protein kinase (WEE1) dependent manner. Active CHK1 phosphorylates and stabilizes WEE1, enhancing its activity toward CDK1 [19]. Both CDK1 and CDK2 remain in inactive states induced by WEE1's inhibitory phosphorylation [19]. Inactivation of CDC25C by CHK1 prevents the removal of the inhibitory phosphorylation on CDK1, which halts the G2/M checkpoint to allow time for post-replicative DNA repair and prevent replication of unrepaired DNA [16].

Additionally, ATR also plays a role in regulating replication forks through multiple mechanisms [1]. One mechanism involves ATR-mediated fork remodeling: ATR phosphorylates the helicase SWI/SNF-related, matrix associated, actin dependent regulator of chromatin, subfamily-A-like 1 (SMARCAL1), promoting maintenance of fork stability and fork restart in cooperation with RAD51 and zinc-finger RANBP2-type containing 3 (ZRANB3) [20]. Another known mechanism underscores the importance of the ATR-CHK1 axis in resolving replication stress during the formation of R-loops, which are RNA–DNA hybrid transcription intermediates that induce genome instability. Here, the ATR-CHK1 pathway is activated by R-loop induced reversed replication forks. Upon activation, ATR protects the genome by regulating the activity of the MUS81 endonuclease, preventing excess nucleolytic degradation of reversed forks. Active ATR also suppresses R-loop accumulation and enables replication recovery, while promoting arrest of the cell cycle at the G2/M-phase [21]. Resolution of replication stress triggers ATR to resume HRR activities by promoting fork reversal and restart, a process involving the recruitment of *BRCA2* and RAD51 to sites of damage [11, 22].

In addition to its role in HRR, ATR has further roles in DNA repair through its involvement in the inter-strand crosslink repair (ICLR) and nucleotide excision repair (NER) pathways. ICLR removes toxic inter-strand DNA crosslinks (lesions involving both strands of DNA that can result in replication fork-stalling and inhibition of transcription). The presence of ICLs activates ATR and requires ATR-mediated phosphorylation of Fanconi Anemia proteins, which are key players in mediating ICLR [23, 24]. Lastly, NER is a critical mechanism that repairs a wide variety of DNA lesions caused by chemical agents or environmental factors (particularly UV radiation) [25]. In this repair mechanism, ATR phosphorylates and stabilizes Xeroderma pigmentosa group A (XPA), thereby recruiting the protein to sites of damage during the S-phase of the cell-cycle and aiding in the activation of the NER pathway [26]. As a key player in multiple processes, ATR

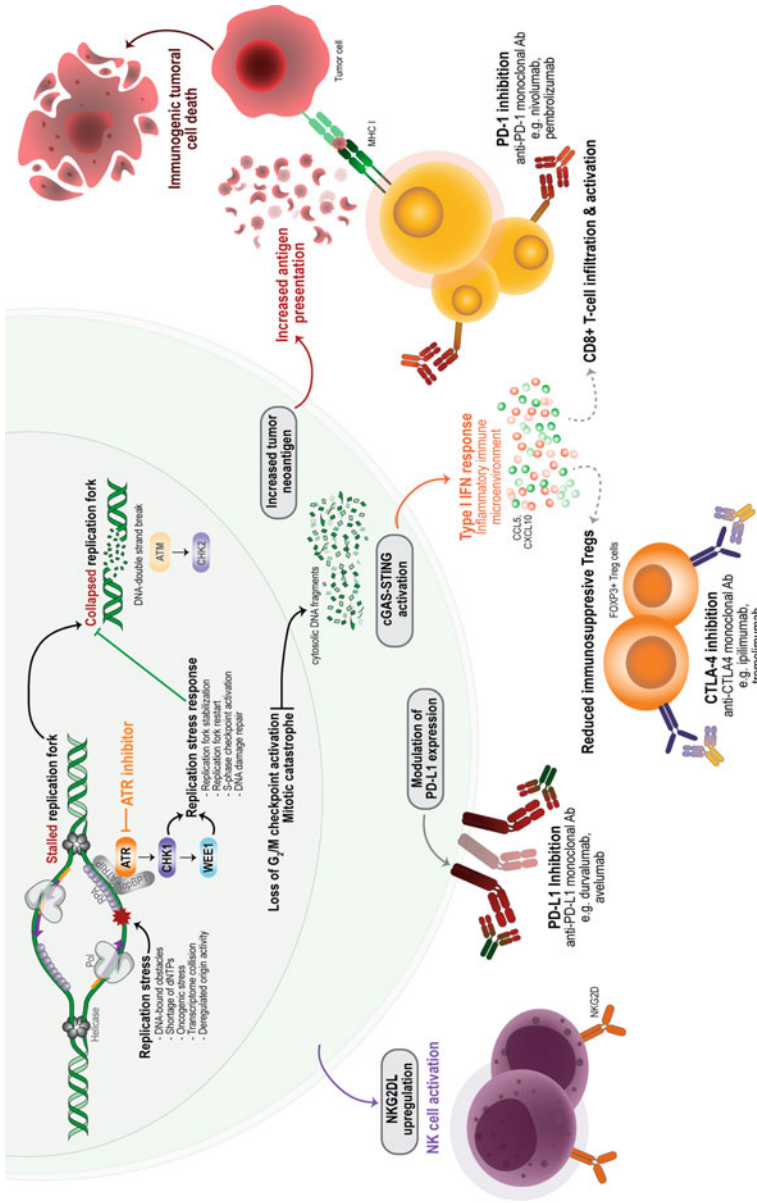


Fig. 14.1 ATR activation, inhibition and modulation of antitumor immunity. ATR is activated in response to replication stress, single-stranded DNA, and increased R-loops. ATR activation triggers a kinase cascade of CHK1 and WEE1, resulting in checkpoint activation and cell cycle arrest for DNA repair. ATR inhibition induces inappropriate mitotic entry that culminates in mitotic catastrophe and the release of DNA fragments into the cytosol, which in turn activate the cGAS-STING pathway and a type I IFN response. DDR may also be a means of increasing tumor mutational burden and therefore the generation of neoantigens

is a master regulator of DNA repair and more broadly in the replication stress response.

14.3 ATR Signaling Fosters Cancer Cell Survival

Activation of DDR in normal cells can either resolve DNA damage and/or replication stress to promote cell survival, or it can trigger programmed cell death when DNA damage cannot be removed, inhibiting tumorigenesis and preventing inheritance of DNA mutations in daughter cells [28–31]. Since DDR is often dampened in cancer cells, these cells present increased DNA damage that is tolerated due to a simultaneous amelioration of unrepaired DNA damage response. In this way, DDR is used by cancer cells as a decoy mechanism to shield against cell death and allow genomically unstable cells to traverse the cell-cycle unscathed, making cancer cells dependent on DDR for survival. That is, tolerance of replication stress is crucial for tumor viability, and oncogene-induced dysregulation of DNA replication generates high levels of replication stress in cancer cells [27–29]. Furthermore, most cancers also feature defective G1 checkpoints, largely due to *p53* signaling loss [30], rendering cancer cells dependent on S- and G2-phase checkpoints, which are ATR-regulated processes [30].

Frequent mutations in DDR genes also increase dependency on ATR signaling [28, 31]. In these situations, cancer cells rely on ATR to respond to and resolve replication stress and repair DNA damage in order to bypass cell death [27]. Given that ATR function is conserved in the vast majority of cancers, it has emerged as a favorable target in cancer medicine [32]. ATR inhibition in normal cycling cells, with intact cell-cycle checkpoints, leads to moderate cytotoxicity due to replication fork stalling and collapse; however, as cancer cells have high replication stress, they are more dependent on ATR for survival and as a result are more sensitive to its inhibition than normal cells [27, 28]. Furthermore, in-vitro data suggests that chronic use of ATR inhibitors impairs the cell's ability to repair damage by HRR while also impacting the availability of necessary HRR proteins, such as TopBP1, *BRCA1*, and *RAD51* [28]. This targeted approach has culminated in the development of many clinical studies aimed at evaluating the clinical efficacy of ATR inhibition as both a monotherapy in certain DDR defective cancer backgrounds and in combinational approaches [19, 33].

14.4 Early Development of ATR Inhibitors

The development of potent and selective ATR kinase inhibitors has been closely related to (1) the availability of well-characterized assays that permit accurate measurements of selective kinase activity, (2) the availability of structural and functional insights to guide a drug design strategy that maximizes selectivity, and (3) the development of screening tools and biomarkers that can identify suitable

patients. Notably, the first generation of small molecule ATR inhibitors struggled to find balance between potency and selectivity to reach clinical usage. For instance, while Schisandrin B, an active ingredient of the magnolia berry (*Schisandra chinensis*), inhibited ATR kinase activity at high concentrations leading to off-target effects and toxicity, the small molecule NU6087 demonstrated moderate selectivity over *ATM* homologs, but did not display selectivity over the wider kinase family [34]. With the development of a cell-screening assay that measured ATP-dependent phosphorylation of H2AX as a more accurate quantification of ATR kinase activity in experimental conditions, the small molecule ETP-46464 was selected from a library of compounds based on its increased selectivity over other ATR homologs. Although poor pharmacological properties in mice prevented ETP-46464 from advancing to clinical studies, the discovery of this compound provided proof of concept for a more reliable biomarker of replication stress that accounts for double strand breaks and has become the standard marker for quantifying DNA damage [35]. Based on recent advances in the development of well-characterized assays, as well as new insights in the structure–function relationship of ATR, many pharmaceutical companies have taken on the challenge to design and develop potent and selective ATP competitive inhibitors of ATR with the most advanced targeted therapies described here.

14.5 ATR Inhibitors in the Clinic

14.5.1 Berzosertib (M6620/VX-970/VE-822)

Shortly after the development of the assay that measured ATR-dependent phosphorylation of γ H2AX, a high throughput screen that combined structure–activity relationship with homology modeling led to the discovery of VE-821, a selective inhibitor with 600-fold selectivity for ATR over *ATM*, DNA-PK, mTOR, and PI3K [36]. In addition to increased selectivity, VE-821 also showed strong inhibition of CHK1 phosphorylation in cellular models of *ATM* and/or *p53* deficiency [37]. In-vitro experiments showed that VE-821 sensitized ovarian cancer cells to DNA damaging agents such as cisplatin and gemcitabine, and the effects of gemcitabine were potentiated when combined with VE-821 in pancreatic cancer cells [38]. It was also observed that VE-821 could further sensitize *BRCA1*-depleted cells to DNA damaging agents [44]. Such synergistic effects appear to be stronger with DNA-damaging agents, such as cisplatin and carboplatin, since DNA crosslinking triggers early activation of ATR and the DDR machinery. Interestingly, *p53*-deficient cancer cell lines were shown to be more sensitive to the combination of VE-821 and cisplatin than normal cell lines, and significant synergistic activity was observed in *ATM*-deficient cell lines [43]. These results were further confirmed by treating *ATM*-proficient cells with the triple combination of VE-821, cisplatin, and a highly selective *ATM* inhibitor (KU-55933) [37]. Taken together, these results suggested that cancer cells with defective *ATM* signaling are more reliant on ATR; hence, demonstrating a synthetic lethal interaction between the S-phase specific

ATR and the *ATM-p53* pathway mediating the G1 checkpoint [39]. However, it must be noted that recent studies have shown that *ATM* mutations and *p53* status are not enough to predict clinical benefit to ATR inhibition, and mutations in other DDR genes—such as *PTEN*, *XRCC1*, *BRCA1*, *BRCA2*, and *ARID1A*—may promote synthetic lethality with ATR inhibitors [40].

Based on promising pre-clinical data, VE-822, an optimized analog of VE-821 with increased potency and selectivity for ATR, became the first inhibitor to enter clinical trials labeled as VX-970, later named M6620 and berzosertib [41]. Berzosertib alone was found to sensitize multiple lung cancer cell lines to a wide variety of DNA-damaging chemotherapeutic agents (cisplatin, oxaliplatin, gemcitabine, etoposide, and the active metabolite of irinotecan, SN38), and the combination of berzosertib and cisplatin showed sustained tumor regression in non-small cell lung cancer (NSCLC) patient-derived xenograft models [42]. A recent CRISPR-Cas9 screen suggested that the ATR-CHK1 pathway has the potential for synthetic lethality in small cell lung cancer (SCLC) [43]. In that study, the combination of berzosertib with cisplatin displayed greater synergistic activity in different SCLC cell lines and primary lung fibroblasts when compared to treatment with the combination of cisplatin and etoposide. Interestingly, while SCLC cell-derived xenografts showed that the combination of berzosertib with cisplatin inhibited tumor growth, other studies showed that pediatric solid tumor xenografts treated with berzosertib and cisplatin displayed a larger event free survival relative to those treated with cisplatin monotherapy [44, 45]. Together, these studies were the first to confirm the clinical potential of berzosertib as a chemo-sensitizer of DNA damaging agents in lung cancer patients, as well as in pediatric solid tumors, setting the stage for several other studies that also showed the clinical potential of berzosertib in combination with cisplatin in other cancer types, including colon cancer, triple negative breast cancer (TNBC), and esophageal tumors, amongst others [44–47].

Berzosertib monotherapy has already advanced to a phase II clinical trial investigating antitumor activity in molecularly selected solid tumors, leiomyosarcoma and osteosarcoma (NCT03718091). Although clinical trials are currently studying the combination of berzosertib with radiotherapy, chemo-radiotherapy agents, PARP inhibitors, VEGF inhibitors, as well as with anti-PD-L1 antibodies, such as avelumab, the most common strategy for berzosertib treatment combinations in registered clinical trials appears to be with DNA damaging agents such as cisplatin, carboplatin, gemcitabine, topotecan, irinotecan, and paclitaxel, amongst others (Table 14.1).

The first-in-human trial of berzosertib in combination with cytotoxic chemotherapy agents in patients with advanced solid tumors started with a lead-in safety phase of berzosertib monotherapy, followed by three dose escalation arms aimed to determine the safety profile and recommended phase 2 dose (RP2D) of the combinations of berzosertib with (1) cisplatin, (2) gemcitabine with and without cisplatin, and (3) irinotecan. This trial also included 3 expansion cohorts to further elucidate preliminary anti-tumor activity for the combination of berzosertib and gemcitabine in NSCLC patients harboring *p53* mutations and/or loss of *ATM*

Table 14.1 Trials investigating berzosertib combinational strategies

ATR inhibitor	Phase	Combination strategy	Schedule	Study population	Safety	Efficacy	References
Berzosertib (M6620, VX-970, VE-822) Berzosertib (M6620, VX-970, VE-822)	I	Cisplatin Gemcitabine + Cisplatin Carboplatin	Lead-in monotherapy Part A: combination with gemcitabine as well as gemcitabine and cisplatin Part B: combination with cisplatin as well as cisplatin and etoposide Part C1: combination with gemcitabine in patients NSCLC Part C2: combination with cisplatin in subjects with advanced TNBC Part C3: combination with cisplatin or carboplatin in subjects with platinum-resistant advanced SCLC	Advanced solid tumors	Part A (berzosertib + gemcitabine) RP2D: 210 mg/m ² of berzosertib and 1000 mg/m ² of gemcitabine Grade ≥ 3 AE: neutropenia, increased ALT and fatigue (16% each), anemia and thrombocytopenia (10% each) Part B RP2D: 140 mg/m ² of berzosertib and 75 mg/m ² of cisplatin Grade ≥ 3 AE: neutropenia (21.7%), anemia (4.3%), and thrombocytopenia (4.3%)	Part A (berzosertib + gemcitabine) 60.4% of patients achieved SD and 8.3% PR as best response Part B (berzosertib + cisplatin) 57.7% of patients achieved SD and 15.4% PR as best response	NCT02157792 [48, 53]

(continued)

Table 14.1 (continued)

ATR inhibitor	Phase	Combination strategy	Schedule	Study population	Safety	Efficacy	References
	II	Gemcitabine	Gemcitabine 1000 mg/m ² (D1, D8) ± berzosertib 210 mg/m ² (D2, D9)—21-day cycles	Platinum-resistant ovarian cancers	Gemcitabine + berzosertib Grade ≥ 3 AE: Neutropenia (47%), thrombocytopenia (24%)	PFS: 22.9 weeks for the gemcitabine + berzosertib arm versus 14.7 weeks gemcitabine alone (HR, 0.57; 90% CI, 0.33–0.98; p = 0.044)	NCT02595892 [51]
	II	Gemcitabine	Gemcitabine 1000 mg/m ² (D1, D8) ± berzosertib 210 mg/m ² (D2, D9)—21-day cycles	Leiomyosarcomas	N/A	Active, not recruiting	NCT04807816
	I/II	Topotecan	Phase I: Topotecan (D1–5) + berzosertib (D5 or D2 and D5 at escalating doses)—21-day cycles Phase II: 210 mg/m ² of berzosertib and 1.25 mg/m ² of topotecan	SCLCs and extrapulmonary small-cell cancers	Phase I Grade ≥ 3 AE: Anemia, leukopenia, and neutropenia (19% each); lymphopenia (14%); and thrombocytopenia (10%)	Phase II Objective response rate among patients with SCLC was 36% (9/25)	NCT02487095 [54, 55]

(continued)

Table 14.1 (continued)

ATR inhibitor	Phase	Combination strategy	Schedule	Study population	Safety	Efficacy	References
	II	Topotecan	210 mg/m ² of berzosertib (D5 or D2) and 1.25 mg/m ² of topotecan (D1-D5), 21-day cycles	Relapsed platinum-resistant SCLCs	N/A	Active, not recruiting	NCT04768296
	I	Irinotecan	Irinotecan + berzosertib (D1, D15)—28-day cycles	Advanced, unresectable solid tumors	N/A	Active, not recruiting	NCT02595931
	II	Irinotecan	Irinotecan + berzosertib (D1, D15)—28-day cycles	Metastatic or unresectable gastric harboring <i>p53</i> mutations	N/A	Active, not recruiting	NCT03641313

(continued)

Table 14.1 (continued)

ATR inhibitor	Phase	Combination strategy	Schedule	Study population	Safety	Efficacy	References
	I/II	Sacituzumab govitecan	Sacituzumab govitecan (D1, D8) + berzosertib (escalating doses)—21-day cycles	SCLCs and HRD cancers resistant to PARPi. Pathogenic mutations in <i>BRCA1, BRCA2,</i> <i>ATM, BRIP1,</i> <i>BARD1, CDK12,</i> <i>CHEK1, CHEK2,</i> <i>FANCL, PALB2,</i> <i>PPP2R2A,</i> <i>RAD51B, RAD51C,</i> <i>RAD51D, or</i> <i>RAD54L</i>	N/A	Active, not recruiting	NCT04826341
	II	Carboplatin Docetaxel + Carboplatin	Berzosertib 90 mg/ m ² (D2, D9) + carboplatin (D1)—21-day cycles Docetaxel 60 mg/ m ² (D1) + carboplatin (D1)—21-day cycles	Metastatic castration-resistant prostate cancers	N/A	Active, not recruiting	NCT03517969

(continued)

Table 14.1 (continued)

ATR inhibitor	Phase	Combination strategy	Schedule	Study population	Safety	Efficacy	References
	I	Carboplatin + Gemcitabine	Carboplatin (D1) + Gemcitabine (D1, D8) + berzosertib (D2, D9)—21-day cycles	Metastatic Ovarian, Primary Peritoneal, or Fallopian Tube Cancer	N/A	Active, not recruiting	NCT02627443
	I	Veliparib + Cisplatin	Berzosertib (D2, D9) + veliparib BID (D1-3 and 8-10) + cisplatin (D1, D8)—21-day cycles	Advanced solid tumors	N/A	Active, not recruiting	NCT02723864
	I/II	Carboplatin + Gemcitabine + Pembrolizumab	Carboplatin (D1) + Gemcitabine (D1, D8) + berzosertib (D2, D9) + Pembrolizumab (D1) during 4, 21-day cycles. After cycle 4 berzosertib (D2, D9) + Pembrolizumab (D1)	Lung non-small cell squamous carcinoma	N/A	Active, not recruiting	NCT04216316

(continued)

Table 14.1 (continued)

ATR inhibitor	Phase	Combination strategy	Schedule	Study population	Safety	Efficacy	References
	I	Avelumab	Avelumab (D1, D15) + berzosertib (D1, D8, D15, D22)—28 day cycles	Advanced solid tumors	N/A	Active, not recruiting	NCT04266912
	Ib/II	Avelumab	Part A: safety run-in of carboplatin AUC 5 (D1) + avelumab 1600 mg (D1) + berzosertib 90 mg/m ² (D2)—21 day cycles	Recurrent platinum-sensitive ovarian cancers resistant to PARPi	N/A	Completed	NCT03704467

expression, the combination of berzosertib and cisplatin in TNBC patients with germline (*g*) *BRCA* wild type status, and the combination of berzosertib and cisplatin or carboplatin in patients with platinum-resistant advanced SCLC.

Recent results from two dose escalation arms of this first-in-human trial demonstrated preliminary antitumor activity for berzosertib when combined with gemcitabine and/or cisplatin [48]. That is, most patients who received berzosertib in combination with cisplatin (73.1%), and those who received berzosertib in combination with gemcitabine (68.7%) or berzosertib in combination with gemcitabine and cisplatin (71.0%) achieved disease control with partial response (PR) or stable disease (SD) as their best response per RECIST v1.1 [48]. Interestingly, all patients who received prior platinum-based chemotherapy, and had experienced disease progression, achieved PR when treated with berzosertib in combination with cisplatin. Since ATR inhibition can disrupt DNA replication fork stability and homologous recombination repair (the two major mechanisms of PARP inhibitor resistance), preliminary results from this trial suggest that berzosertib inhibition may contribute to re-sensitizing solid tumors to cisplatin [49].

The RP2D for the combination of berzosertib and cisplatin was determined as 140 mg/m² of berzosertib (administered on days 2 and 9), and 75 mg/m² of cisplatin administered every 3 weeks (Q3W) on day 1. This RP2D was generally well tolerated, and the safety profile of this combination was consistent with that of cisplatin alone. Importantly, while the human equivalent dose required for berzosertib target engagement was estimated from preclinical models to be ~60 mg/m², results from the first-in-human trial show that dosing berzosertib at 140 mg/m² induces a reduction in serine 345-phosphorylated CHK1, without evidence of PK interactions in a range of malignancies, including ovarian, breast, thyroid, and pancreatic cancers [50]. In a similar manner, the RP2D combination of berzosertib and gemcitabine, which is currently being evaluated in patients with advanced NSCLC in an expansion arm of this trial, was established as 210 mg/m² of berzosertib (administered on days 2 and 9), and 1000 mg/m² of gemcitabine, administered Q3W on days 1 and 8. Yet, the dose escalation for berzosertib in combination with both gemcitabine and cisplatin was terminated after two patients experienced febrile neutropenia or neutropenia as dose limiting toxicities (DLTs). Taken together, results from the first two arms of the first-in-human trial of berzosertib demonstrate that a tolerable safety toxicity profile is observed when berzosertib is combined with either gemcitabine or cisplatin, but not when combined with both agents [48].

The pharmacokinetic (PK) profile of a berzosertib monotherapy lead-in was determined across the dose range of 18–210 mg/m² (*n* = 30), and it was characterized by biphasic decline with a moderate-to-high clearance, a high distribution volume, and an apparent terminal half-life of approximately 17 hours [48]. While the PK characteristics of berzosertib in combination with either gemcitabine or cisplatin were consistent with the corresponding doses of berzosertib monotherapy, the collective PK data from these two arms suggest that pre-administration of cisplatin 24 hours before berzosertib administration does not affect the PK profile of berzosertib.

Another trial that is currently investigating the combination of berzosertib and gemcitabine is a multicenter, randomized, phase II study that recently published preliminary efficacy and safety data suggesting that this combination provides clinical benefit to platinum-resistant high-grade serous ovarian cancer (HGSOC) patients. At the cutoff date of publication, 70 patients had been randomly assigned to either receive treatment with the berzosertib and gemcitabine combination ($n = 34$) and achieved a median progression-free survival (PFS) of 22.9 weeks (90%, CI 17.9–72.0), or they were assigned to receive treatment with gemcitabine alone ($n = 36$) and achieved a median PFS of 14.7 weeks (90%, CI 9.7–36.7). Yet, while the combination of berzosertib and gemcitabine displayed a promising PFS with a hazard ratio of 0.57 (90%, CI 0.33–0.98), a higher objective response was observed for the group of patients who received treatment with gemcitabine alone. According to the authors, discrepancies between ORR and PFS are not uncommon in platinum-resistant ovarian cancer patients [51]. A sub-analysis of the patient population based on the length of the platinum-free interval also showed that patients who are treated with the berzosertib and gemcitabine combination, and who have had a platinum-free interval of 3 months or less, have a 30% increase in median PFS (27.7 weeks compared to 18.6 weeks in patients with intervals larger than 3 months). Since the PFS benefit observed for patients with a platinum-free interval of 3 months or less may be related to the enrichment for biomarkers of replicative stress, the authors followed up on this finding with further correlative assays [51]. Interestingly, results from follow-up studies using the same replication stress signature show that the combination of berzosertib and gemcitabine benefited more patients with tumors displaying low replication stress (RS-low) in contrast to patients with high replication stress tumors (who appeared to receive a greater benefit from the increase of replication stress caused by gemcitabine monotherapy [52]. Based on these results, it is suggested that increasing replication stress in RS-loss with gemcitabine concomitant with ATR inhibition by berzosertib is necessary for lethality [52].

Finally, a few clinical trials have published results about the preliminary efficacy and safety profile of the combination of berzosertib with topotecan in patients with lung cancers. A proof-of-concept phase I clinical trial that investigated the combination of berzosertib with topotecan in patients with platinum-resistant small cell lung cancer (SCLC) showed that 60% (3/5) of the patients treated achieved a PR or prolonged SD lasting ≥ 6 months, and the combination seemed to be well tolerated with no additive toxicity observed [54]. Yet, shortly after the interim results of the DDRiver SCLC 250 phase II trial investigating the combination of berzosertib with topotecan in platinum-resistant SCLC patients reported an objective response rate of 36% (9/25) and a median duration of response of 6.4 months, the trial was discontinued based on a low probability of meeting the primary objective [55, 56]. Further results from ongoing clinical trials are needed to demonstrate whether treating patients with advanced cancers, whose tumors are undergoing high replicative stress, with the combination of berzosertib and DNA-damaging chemotherapeutic agents may potentially help overcome platinum and/or PARP inhibitor resistance.

14.5.2 Ceralasertib (AZD6738)

Ceralasertib is a potent and selective ATR inhibitor with a promising preclinical data package showing efficacy in DDR-deficient settings [57]. Early preclinical studies showed that ceralasertib increases γ H2AX phosphorylation, while inhibiting phosphorylation downstream of CHK1 in a variety of *ATM*-deficient cell lines and inducing accumulation of unrepaired DNA damage and cell death in *ATM/p53*-deficient leukemia cells [58, 59]. Recently, a growth inhibition assay assessing the sensitivity of 276 cancer cell lines to ceralasertib reported that cell lines harboring CCNE1 amplification or *ARD1A*, *ATRX*, and *SETD2* mutations were associated with sensitivity. At first sight, cancer cell lines harboring *ATM* mutations were not associated with sensitivity; yet, upon stratifying the cancer cells based on *ATM* expression levels, it was shown that complete absence of *ATM* function is significantly associated with sensitivity to ceralasertib [60]. This finding, along with previous observations of antitumor responses from patients harboring *ATM* loss-of-function, supports the idea that patient selection for ATR inhibitors should consider biallelic deleterious mutations and *ATM*-null expression [61].

Preliminary results from the dose escalation and expansion monotherapy arms of the PATRIOT phase I clinical trial reported that ceralasertib was better tolerated when administered at an intermittent schedule of 2-weeks-on/2-weeks-off because only 20% of the patients experienced grade ≥ 3 treatment related adverse events (TRAEs) compared to 67% of patients when ceralasertib was administered in a continuous schedule (NCT02223923). Although it was previously shown that ceralasertib monotherapy in-vivo only induces significant tumor control/stasis and that the synergistic effects resulting in tumor regression are pronounced when ceralasertib is combined with DNA damaging agents or certain targeted small molecules, preliminary antitumor activity of the ceralasertib monotherapy arms of the PATRIOT trial show that 7% of patients achieved PR and 48% of them achieved SD as best overall response [60, 62, 63].

In-vivo, the combination of ceralasertib and cisplatin induced significant tumor reduction in HER2-positive breast cancer cells, as well as tumor regression in *ATM*-deficient lung cancer xenograft models and synergistic effects in *ATM*-deficient NSCLC cell lines [64, 65]. Results from a phase I trial investigating the combination of ceralasertib and carboplatin in advanced solid tumor patients reported that 2 (6%) of patients with low *ATM* or SLFN11 expression achieved PR as best response by RECIST v1.1, while 53% patients (including two unconfirmed PRs) achieved SD for ≥ 35 days (NCT02264678) [66]. Although no association between *ATM* and SLFN11 expression level and antitumor activity was reported, likely due to the sample size, these findings support the notion that further investigations on the interaction between ATR and loss of *ATM* function are needed. In contrast, a phase I clinical trial investigating the safety and antitumor activity of the ceralasertib and paclitaxel combination in advanced solid tumors (enriched for melanoma patients) reported one patient achieved complete response (CR), while 21% achieved PR and 32% achieved SD. Even though the ORR for the entire population was 22.6% (95% CI, 12.5–35.5), an ORR of 33.3% (95% CI, 10.8–51.8)

was reported for the subset of melanoma patients resistant to PD1/L1 treatment [67].

Although both phase I trials studying the combinations of ceralasertib with chemotherapy agents reported that the combinational strategies are safe and well tolerated, thrombocytopenia, neutropenia and anemia were reported as the most common grade ≥ 3 TRAEs, with schedule limiting consequences observed with the combination of ceralasertib and carboplatin. Since toxicity may be one of the major challenges in the implementation of ATR inhibitor combinations with DNA damaging agents and other targeted small molecules, the success of clinical trials investigating ceralasertib combinations depends on the optimization of the dose scheduling sequences and targeted genetic tumor aberrations. For instance, recent in-vivo studies suggest that to achieve tumor regressions, concurrent dosing for the ceralasertib and irinotecan combination should be extended at least one day, while a few days of ceralasertib dosing should be included after concurrent dosing with carboplatin [60]. In a similar fashion, the ATRiUM phase I clinical trial is investigating the safety and antitumor activity of ceralasertib with either intermittent or continuous gemcitabine dosing in advanced solid tumors, particularly in patients with advanced pancreatic ductal adenocarcinoma with *ATM*-loss-of-function [68, 69]. In all, results from an ongoing phase II trial investigating ceralasertib monotherapy in advanced solid tumors (enriching for mCRPC with low *ATM* expression), as well as results from the remaining arms of the PATRIOT phase I clinical trial and the ATRiUM phase I are required to further assess the clinical efficacy of ceralasertib monotherapy and in combination with chemotherapy agents in a molecularly targeted population.

Although synergistic effects in-vivo were observed when combining ceralasertib with either PARP or WEE1 inhibitors, only the PARP inhibitors and ceralasertib combination has successfully reached phase II clinical trials. Out of the six clinical trials that are currently investigating the combination of ceralasertib and olaparib in the advanced cancer setting, one phase I study reported on safety and preliminary antitumor efficacy, as well as established the RP2D of the ceralasertib and olaparib combination in patients with advanced solid tumors, and two Phase II trials have presented contrasting preliminary results based on patient selection (Table 14.2) [63, 70, 71]. Briefly, results from one of the first modular phase I clinical trials to test the combination of ceralasertib and olaparib established a concurrent RP2D of ceralasertib at 160 mg QD on days 1–7 and olaparib at 300 mg twice daily (BID) on days 1–28, with thrombocytopenia and neutropenia defined as dose limiting toxicities [72]. Within the module that tested the dose escalation of ceralasertib and olaparib, antitumor responses were observed in patients with advanced breast, ovarian, prostate, pancreatic, and ampullary cancer. Interestingly, while some of the responding tumors had *BRCA1/2* mutations, antitumor responses were independent of *ATM* status [72]. Such results are in accordance with recent preclinical studies suggesting that the combination of ceralasertib and olaparib in a concurrent schedule induces tumor regression in TNBC *BRCA*-wild type and *BRCA2*-mutated xenograft models [60], and the development of a phase II clinical trial currently

recruiting patients to investigate the combination of cerlasertib and olaparib in advanced germline *BRCA* mutated breast cancer (NCT04090567).

Although differences in study design preclude us from direct comparisons, preliminary antitumor activity from two phase II clinical trials investigating the combination of cerlasertib and olaparib seemed to be influenced by the selection of targeted genetic tumor aberrations. That is, the phase II clinical trial investigating the clinical benefit of this combination in patients with advanced solid tumors with or without ARID1A-deficiency (defined as lack of expression of BAF250a by IHC) reported an ORR of 20% for patients with ARID1A-deficiency, including two patients that achieved sustained CRs, while no objective responses were observed in the cohort of patients with active ARID1A function [73]. In contrast, the phase II clinical trial investigating signals of activity of the cerlasertib and olaparib combination in patients with HGSOC reported no partial or complete responses in a PARP naïve, genetically unselected, platinum-resistant cohort of 12 patients. Nonetheless, 75% of the patients in that trial achieved SD as best overall response by RECIST v1.1 and 27% of the patients achieved $\geq 50\%$ decrease in CA-125, most of them harboring tumors with somatic *BRCA1* mutations [74].

As mentioned by investigators of the HGSOC phase II trial, it is likely that more responses may have been achieved by focusing the patient population to ovarian cancer patients harboring tumors with *BRCA1/2* mutations and/or CCNE1 copy number amplification [74]. Taking it all together, the cerlasertib and olaparib combination appears to be well tolerated, but it is necessary to continue optimizing patient selection strategies based on the selection of genetic aberrations that induce synthetic lethality in different types of cancer types. Such concept seems to be reflected in recent preliminary results from the HUDSON trial, a phase II multidrug, biomarker selected umbrella study investigating the combination of multiple targeted small molecules with durvalumab, including cerlasertib for NSCLC patients who progressed after anti-PD-1/PD-L1 and platinum therapy (NCT03334617). Although no correlation was found between *ATM* biomarker status and clinical responses by RECIST 1.1, the HUDSON trial reported an improved ORR (11.1%) and longer PFS (7.43 months) for patients whose tumors harbored *ATM* mutations or low protein expression -when compared to an ORR of 8.3% and PFS of 4.96 months for NSCLC patients with acquired resistance to prior immunotherapy, regardless of *ATM* status [75].

By comparing gene expression profiles in paired blood samples from patients with controlled disease and patients whose disease progressed with the cerlasertib monotherapy run-in, the HUDSON trial showed increases in an antigen presentation gene signature and decreases in exhausted T-cell and NK-cell signatures, supporting a model in which cerlasertib also has an active role in the immune activation caused by the combination of cerlasertib with durvalumab [77]. These findings are also in agreement with results from a phase II clinical trial investigating the clinical activity of the cerlasertib and durvalumab combination in advanced gastric cancer patients, which reported (1) significantly longer PFS for patients whose tumors harbored *ATM*-deficiency and/or HRD-deficiency when compared to patients with active *ATM* function and HRD-proficient (5.60 months

Table 14.2 Trials investigating ceralasertib combinational strategies

ATR inhibitor	Phase	Combination strategy	Schedule	Study population	Safety	Efficacy	References
Ceralasertib (AZD6738)	I	Monotherapy	Dose escalation and dose expansion	Advanced solid tumors	Grade ≥ 3 TRAEs in 67%	21% of patients in dose escalation and 25% in dose expansion had SD ≥ 16 weeks (n = 46) 3 PR (7%) 22 SD (48%)	NCT02223923 [62]
Ceralasertib (AZD6738)	II	Monotherapy	Ceralasertib monotherapy, 28-day cycles	Advanced solid tumors and mCRPC; target 60% with low ATM expression	N/A	Recruitment ongoing	NCT04564027
Ceralasertib (AZD6738)	I	Carboplatin	Dose escalation (ceralasertib 20 mg -60 mg) sequential and concurrent dosing	Advanced solid tumors	RP2D ceralasertib 40 mg QD (D1-D2) + carboplatin AUC5 Q3W, q21d Toxicities of grade ≥ 3 were neutropenia, anemia, and thrombocytopenia	2 PR (6%) 18 SD (53%)	NCT02264678 [66]
Ceralasertib (AZD6738)	I	Paclitaxel	Paclitaxel 80 mg/m ² (D1, D8, D15) + ceralasertib at escalating doses 40 mg OD – 240 mg BID, 28-day cycles	Advanced solid tumors	RP2D ceralasertib 240 mg BID (D1–14) + paclitaxel at 80 mg/m ² (D1, 8, 15), q28w Toxicities of grade ≥ 3 were neutropenia, anemia, and thrombocytopenia	ORR 25.6% (n = 57) 1 CR (1.9%) 12 PR (21.1%) 18 SD (31.6%)	NCT02630199 [67]

(continued)

Table 14.2 (continued)

ATR inhibitor	Phase	Combination strategy	Schedule	Study population	Safety	Efficacy	References
	I	Gemcitabine	Gemcitabine at escalating doses 500–1000 mg/m ² (D3, D10, D17) + ceralasertib at escalating doses 40–120 mg (D1–21), 28-day cycles Gemcitabine at escalating doses 500–1000 mg/m ² (D3, D10, D17) + ceralasertib at escalating doses 40–120 mg, for up to 12 days, 28-day cycles	Advanced solid tumors	N/A	Recruitment ongoing	NCT03669601
	I	Trastuzumab deruxtecan	Trastuzumab deruxtecan (D1) + ceralasertib BD (D1–7)	Advanced solid tumors with HER2 expression	N/A	Recruitment ongoing	NCT04704661

(continued)

Table 14.2 (continued)

ATR inhibitor	Phase	Combination strategy	Schedule	Study population	Safety	Efficacy	References
	I	Carboplatin Olaparib Durvalumab	Dose escalation and dose expansion	Advanced solid tumors: Head & Neck, SCC, <i>ATM</i> proficient and deficient NSCLC, Gastric, Breast and Ovarian Cancer	RP2D for olaparib combination: ceralasertib 160 mg QD (D1-D7) + olaparib 300 mg BID (D1-D28) Olaparib combination: DLT thrombocytopenia and neutropenia. Toxicity, including grade ≥ 3 : thrombocytopenia, anemia, neutropenia, fatigue, decreased appetite, nausea, vomiting, constipation, diarrhea and cough Durvalumab combination: DLT thrombocytopenia Toxicity, including grade ≥ 3 : anemia, fatigue, nausea, decreased appetite, cough, vomiting, dizziness, pruritus, constipation, diarrhea, musculoskeletal chest pain and dyspnea	Tumor activity for olaparib combo: 1 CR (2.5%) 5 PR (12.8%) Independent of <i>ATM</i> status Tumor activity for durvalumab combo: 1 CR (4.8%) 2 PR (9.5%) Independent of PD-L1 expression	NCT02264678 [72]

(continued)

Table 14.2 (continued)

ATR inhibitor	Phase	Combination strategy	Schedule	Study population	Safety	Efficacy	References
	II	Olaparib	Olaparib BID (D1-D28) + Ceralasertib QD(D1-D7), 28-day cycles	Advanced metastatic <i>gBRCAm</i> breast cancer	N/A	Recruitment ongoing	NCT04090567
	II	Olaparib	Ceralasertib monotherapy Olaparib 300 mg BID (D1-D28) + Ceralasertib 160 mg QD(D1-D7), 28-day cycles	Advanced pancreatic, clear cell renal and urothelial carcinoma with <i>ARD1A</i> and <i>ATM</i> mutations	N/A	Recruitment ongoing	NCT03682289 (73)
	II	Olaparib	Olaparib 300 mg BID (D1-D28) + Ceralasertib 160 mg QD (D1-D7), 28-day cycles	Recurrent HGSOc, primary peritoneal, or fallopian cancer	Toxicity, including grade ≥ 3 : nausea, fatigue, anorexia and anemia	Recruitment ongoing No objective responses in the platinum resistance HGSOc cohort 9 SD (75%)	NCT03462342 [74]
	II	Olaparib	Olaparib BID (D1-D28) + Ceralasertib QD (D1-D7), 28-day cycles	<i>IDH</i> mutant cholangiocarcinoma or solid tumors	N/A	Recruitment ongoing	NCT03878095

(continued)

Table 14.2 (continued)

ATR inhibitor	Phase	Combination strategy	Schedule	Study population	Safety	Efficacy	References
	II	Olaparib	Olaparib BID (D1-D28) + Ceralasertib QD (D1-D7), 28-day cycles	Recurrent osteosarcoma	N/A	Recruitment ongoing	NCT04417062
	II	Durvalumab	Ceralasertib 240 mg BID (D15-D28) + Durvalumab IV at 1500 mg Q4W, 28-day cycles	Advanced biliary tract cancers with prior immunotherapy	N/A	Recruitment ongoing	NCT04298008
	II	Durvalumab	Ceralasertib 240 mg BID (D15-D28) + Durvalumab IV at 1500 mg Q4W, 28-day cycles	Advanced gastric cancer	AEs manageable with dose modification	ORR overall: 22.6% PFS: 3 months OS: 6.7 months	NCT03780608 [76]

(continued)

Table 14.2 (continued)

ATR inhibitor	Phase	Combination strategy	Schedule	Study population	Safety	Efficacy	References
	II	Durvalumab	Ceralasertib 240 mg BID (D1-D7, D22-D28) + Durvalumab IV at 1500 mg Q4W, 28-day cycles Ceralasertib 240 mg BID (D15-D28) + Durvalumab IV at 1500 mg Q4W, 28-day cycles Ceralasertib 240 or 160 mg BID (D22-D28) + Durvalumab IV at 1500 mg Q4W, 28-day cycles	NSCLC who progressed on anti-PD-1/PD-L1, with and without <i>ATM</i> -loss-of-function or <i>ATM</i> mutations	N/A	ORR overall: 8.7–11.1% PFS _{6months} : 37.0–53.8% OS _{6months} : 74.8–77.3% For population with <i>ATM</i> aberrations ORR: 13.3% PFS _{6months} : 61.2% OS _{6months} : 100%	NCT03334617 [75]
	II	Durvalumab	Ceralasertib 240 mg BID (D1-D7 cycle 1 and D22-D28 subsequent cycles) + durvalumab IV at 1500 mg Q4W, 28-day cycles versus docetaxel standard of care	NSCLC who progressed on anti-PD-1/PD-L1	N/A	Recruitment ongoing	NCT03833440

versus 1.65 months, HR 0.13., 95% CI 0.045–0.39, $p < 0.001$), as well as, (2) upregulation of the innate immune response, (3) activation of intratumoral lymphocytes, and (4) increase of tumor reactive CD8+ T-cells in patients who responded to treatment [76].

Finally, a phase III clinical trial (LATIFY, NCT05450692) will compare the clinical benefit of the ceralasertib and durvalumab combination versus docetaxel monotherapy in NSCLC patients who progressed after anti-PD-1/PD-L1 and platinum therapy. This is based on the finding that NSCLC patients with primary resistance to immunotherapy only responded to the combination of ceralasertib and durvalumab in the HUDSON trial [75].

14.5.3 Elimusertib (BAY1895344)

By evaluating the molecular interactions of available ATR inhibitors within the binding pocket of an ATR homology model created using the crystal structure of a PI3K kinase and performing a high-throughput screen, Bayer identified a lead compound that was further optimized to reduce potential off-target toxicity [78]. BAY1895344, also called elimusertib, is a potent and selective ATR inhibitor shown to increase γ H2AX phosphorylation in HT-29 cells and inhibit cell proliferation in a variety of cancer cell lines, including different lymphoma cells and cell lines harboring mutations that affect the *ATM* pathway. Elimusertib induced stronger antitumor activity than ceralasertib and berzosertib in a lymphoma cell line-derived xenograft (CDX) model, with antitumor activity also observed in ovarian, prostate and colorectal CDX models harboring DDR defects [40]. In addition, elimusertib treatment inhibited neuroblastoma cell growth and induced strong tumor growth inhibition in neuroblastoma xenograft and ALK-driven genetically modified mice models [79]. Interestingly, RNA-seq data from mice who achieved tumor size decrease after elimusertib treatment revealed expression of inflammatory response and immune tumor infiltration, suggesting that ATR inhibition by elimusertib positively impacts the tumoral immune response [79].

Synergistic antitumor efficacy for the combination of elimusertib and DNA-damaging treatments was observed in colorectal cancer cells treated with elimusertib and cisplatin, as well as in colorectal xenograft models treated with elimusertib and radiation therapy [40]. In contrast, antagonistic interactions were observed with the combination of elimusertib and docetaxel [40].

Treatment with elimusertib and olaparib displayed strong antitumor efficacy and a tolerable profile in a TNBC xenograft model and delayed tumor growth in a PARP inhibitor resistant prostate cancer xenograft model [40]. In a similar manner, synergistic antitumor activity was also observed with sequential dosing of anti-PD-1/PD-L1 antibodies and elimusertib in immunocompromised and lymphoma mice models [40]. Taken together, preclinical studies suggest that combining elimusertib with certain DNA damaging agents, as well as with DDR and checkpoint inhibitors, may result in synergistic antitumor activity when compared to the respective single-agent treatments.

Further studies are currently being conducted to determine the precise combination schedules that are safe and well-tolerated in humans. For instance, results from the dose escalation of the first-in-human trial of elimusertib in patients with advanced solid tumors determined that intermittent dosing of 40 mg BID 3 days on/4 days off is the maximum tolerated dose (MTD) of single-agent elimusertib, with pharmacodynamic data showing on-treatment tumor increases in γ H2AX levels (NCT03188965) [80]. The most frequently observed adverse events (AE) in the dose escalation was grade 3 anemia, likely due to limited differentiation and expansion of erythrocyte precursors that are sensitive to replication stress [81]. Based on the safety results from the first elimusertib monotherapy trial, combinational strategies with chemotherapy agents may induce overlapping hematologic toxicity and dose escalations should be approached with caution.

Nonetheless, this trial provided proof-of-concept for the clinical antitumor activity of elimusertib: 4 patients achieved PRs, while 8 achieved SD with a median duration of response of 11.25 months and resulting in 69% disease control rate in patients treated at MTD or above [80]. More importantly, 3 of the 4 patients that achieved PRs had tumors with low *ATM* expression by IHC, with two of them harboring deleterious *ATM* mutations. Albeit a small sample size, an ORR of 33.3% was reported for the subgroup of patients with *ATM* protein loss, and an ORR of 37.5% was calculated for the subgroup of patients harboring *ATM* deleterious mutations [80]. Within the responders for this trial, the investigators noted one heavily pretreated ovarian cancer patient who had received 9 chemotherapy lines, as well as prior PARP inhibitor and immunotherapy, achieved SD for more than year [80]. The clinical benefit observed for this PARP-resistant ovarian cancer patient harboring a *BRCA1* deleterious mutation seem to suggest that PARP inhibitor resistance may be mediated by protection of the DNA replication fork by ATR, opening the possibility of expanding ATR inhibitor treatments to PARP inhibitor-resistant patient population and providing clinical rationale for a phase I clinical trial that investigates the combination of elimusertib and niraparib in patients with advanced ovarian cancer and other solid tumors (Table 14.3, NCT04267939) [80].

14.5.4 Gartisertib (M4344/VX-803)

As an ATP-competitive inhibitor, gartisertib is a selective ATR inhibitor with 100-fold selectivity over a wide range of kinases and strong potency demonstrated by suppression of ATR-driven checkpoint kinase-1 (CHK1) phosphorylation in a prostate cancer cell line, as well as by induction of γ H2AX phosphorylation in a small-cell lung cancer cell line [82]. Remarkably, sensitivity assays and gene expression analysis of a variety of cancer lines showed that cancer cells with higher replication stress and high neuroendocrine expression signatures are highly sensitive to gartisertib treatment, suggesting that those genomic signatures may be useful for patient selection and as biomarkers of response [82].

Table 14.3 Trials investigating elimusertib combinational strategies

ATR inhibitor	Phase	Combination strategy	Schedule	Study population	Safety	Efficacy	References
Elimusertib (BAY1895344)	I/IIb	Monotherapy	Dose escalation and dose expansion	Advanced solid tumors and lymphoma, enriching for <i>ATM</i> mutations and loss-of-function	MTD 40 mg twice daily 3 days on/4 days off Grade ≥ 4 TRAEs neutropenia (33% in 80 mg cohort, 17% in 60 mg cohort), thrombocytopenia (17% in 80 mg cohort)	ORR: 30.8% in patients treated with ≥ 40 mg BID 4 PR (19%) 8 SD (38%)	NCT03188965 [80]
	I/II	Monotherapy	Dose escalation and dose expansion	Advanced solid tumors; sarcoma and lymphoma	N/A	Recruitment ongoing	NCT05071209
	I	FOLFIRI	Dose escalation and dose expansion	Advanced gastrointestinal cancers	N/A	Recruitment ongoing	NCT04535401
	I	Gemcitabine	Dose escalation and dose expansion	Advanced solid tumors; pancreatic and ovarian	N/A	Recruitment ongoing	NCT04616534
	I	Cisplatin Cisplatin + Gemcitabine	Dose escalation and dose expansion	Advanced solid tumors, enriching for urothelial cancer	N/A	Recruitment ongoing	NCT04491942
	I	Irinotecan Topotecan	Dose escalation and dose expansion	Advanced SCLC, neuroendocrine and pancreatic cancer	N/A	Recruitment ongoing	NCT04514497

(continued)

Table 14.3 (continued)

ATR inhibitor	Phase	Combination strategy	Schedule	Study population	Safety	Efficacy	References
	I	Niraparib	Dose escalation and dose expansion	Ovarian cancer and advanced solid tumors (excluding prostate)	N/A	Recruitment ongoing	NCT04267939
	Ib/II	Pembrolizumab	Dose escalation and dose expansion	Advanced solid tumors with DDR deficiency	N/A	Recruitment ongoing	NCT04095273
	I	Pembrolizumab + Radiation	Dose escalation and dose expansion	Recurrent head and neck	N/A	Recruitment ongoing	NCT04576091

As a single-agent, gartisertib was shown to suppress proliferation in prostate cancer cells at a lower concentration and at a higher rate than berzosertib and ceralasertib, and it was shown to induce tumor stasis and tumor regression in ALT mice models [83, 84]. A variety of preclinical models also demonstrated synergistic effects of different gartisertib combination strategies. For instance, the combination of gartisertib and TOP1 inhibitors showed synergistic antitumor activity in several small-cell lung cancer cell lines and cell-derived mouse xenografts, as well as in prostate cancer patient-derived tumor organoids [85]. In addition, combining gartisertib with DNA damaging agents such as gemcitabine and cisplatin, as well as with PARP inhibitors such as talazoparib, displayed synergy at noncytotoxic concentrations in a small-cell lung cancer cell line.

14.5.5 Camonsertib (RP-3500)

Camonsertib, developed by Repare Therapeutics and recently licensed to Roche, is a highly selective ATR inhibitor that demonstrated potent single-agent efficacy by a dose-dependent inhibition of CHK1 phosphorylation and induction of γ H2AX, DNA-PK and KAP1 phosphorylation in-vivo [86]. Camonsertib monotherapy induced significant tumor growth inhibition in an *ATM*-deficient colorectal xenograft model and also induced complete tumor regression in a gastric xenograft model [86]. Unlike other ATR inhibitors, tumor growth inhibition with minimal hematological adverse effects was observed with intermittent camonsertib treatment in *ATM*-deficient mouse models [86]. In line with preclinical data suggesting that intermittent camonsertib dosing schedules with dose holidays of at least 4 consecutive days allow for reticulocyte regeneration to avoid hematological toxicities in-vivo, recent preliminary data from the TRESR phase I/IIa clinical trial investigating the safety and preliminary efficacy of camonsertib showed a significant reduction of grade 3 anemia in advanced cancer patients (NCT04497116) [87]. In this study, 14.5% of patients treated with intermittent camonsertib dosing experienced grade 3 anemia, compared with 65.7% of patients who experienced grade 3 anemia after intermittent elimusertib treatment [87, 88]. Preliminary data from the TRESR trial also showed clinical activity across different tumor types, with meaningful clinical benefit in 49% of evaluable patients and an ORR of 25% [87]. Aligned with preclinical data, clinical activity was observed in CRPC patients whose tumors harbored *ATM* and *CDK12* mutations, ovarian cancer with *BRCA1* and *RAD51C* mutations, as well as breast cancer, melanoma, and HNSCC patients with tumors harboring *BRCA1* and *BRCA2* mutations. Notably, 37 patients whose tumors harbored relevant genomic mutations achieved molecular responses in ctDNA, suggesting that ctDNA responses may predict clinical benefit [87].

Intermittent concomitant rather than sequential administration of camonsertib and PARP inhibitors in different *ATM* and *BRCA1* deficient models led to stronger synergistic antitumor activity without increases in hematological toxicity [86]. Along with the additional modules of the TRESR clinical trial that are currently investigating the combination of camonsertib and talazoparib, the ATTACC phase

I/IIa clinical trial investigating the safety and preliminary efficacy of camonsertib in combination with either olaparib or niraparib is currently recruiting patients (NCT04972110).

14.5.6 M1774

Building on learnings from berzosertib, Merck KGaA developed M1774 as a potent and selective ATR inhibitor that has demonstrated antitumor activity in PDX models. The modular DDRiver Solid Tumors 301 clinical trial is currently investigating the safety and tolerability and preliminary efficacy of M1774 in patients with advanced solid tumors harboring selected mutations, including deleterious mutations in *ATM*, *ARID1A*, *ATRX* and/or *DAXX* (NCT04170153) [89]. Recent results from the dose escalation of this trial suggested an MTD of 180 mg QD continuous dosing and a recommended dose for expansion of 180 mg 2 weeks on/1 week off, with modulation of γ H2AX in peripheral blood mononuclear cells achieved in doses starting at 130 mg QD [89]. While the DDRiver 301 trial is currently recruiting patients for two dose expansion modules in biomarker selected cohorts and food effects cohort, it is also recruiting patients in a module investigating the safety and tolerability of the combination of M1774 and niraparib. In addition, a recent clinical trial investigating the safety and tolerability of M1774 in combination with a DDR inhibitor or an immune checkpoint inhibitor has recently started to recruit patients (NCT05396833).

14.5.7 ART0380

ART0380, licensed by Artios Pharma Ltd from The University of Texas MD Anderson Cancer Center and ShangPharma Innovation, demonstrated target engagement by γ H2AX and pKAP1 modulation in-vivo and, is currently being investigated as monotherapy and in combination with gemcitabine or irinotecan in a modular phase I/IIa clinical trial for advanced solid tumor patients (NCT04657068). In order to measure target engagement, Artios has developed an assay in normal peripheral blood mononuclear cells (PMBCs) and in circulating tumor cells (CTCs) [90]. Although interim results for this trial have not been presented thus far, Artios recently mentioned in a press release that upon treatment with single-agent ART0380, modulation of γ H2AX in patient blood samples is observed at a larger magnitude in CTCs than in PMBCs, and that based on preliminary results from the dose escalation phase of the trial, ART0380 has a safe and tolerable profile. Therefore, the intermittent dose escalation ART0380 has progressed to the dose expansion phase in patients with *ATM*-deficient tumors.

14.6 ATR and PARP Inhibitor Combination Strategies

Synthetic lethal strategies for cancer treatment, where cell death is induced by targeting proteins or pathways that are redundant in normal cells but not cancer cells, are showing clinical promise. Inhibitors of PARP1 (Poly (ADP)-ribose polymerase 1, a key DDR enzyme) are prime examples of anti-cancer therapeutics capable of harnessing the synthetic lethal mechanism and have revolutionized the field of cancer therapeutics. Seminal work led by multiple teams in the early 2000s identified HR-deficient *BRCA1/2*-mutated cancers as selective targets for PARP inhibitor-induced lethality [91–93]. Today, several PARP inhibitors are FDA-approved for the treatment of *BRCA1/2*-mutated cancers, including in multiple settings of ovarian cancer, metastatic breast cancer, pancreatic cancer, and advanced castration-resistant prostate cancer (CRPC) [94–97]. Unfortunately, PARP inhibitor resistance is ubiquitous in the clinic. Acquired PARP inhibitor resistance can occur following prolonged treatment, whereas primary PARP inhibitor resistance is observed in many patients with *BRCA1/2*-mutated cancers and fail to respond at treatment initiation [98]. One strategy to overcome PARP inhibitor resistance is to develop rational combination treatments to sensitize cells to PARP inhibitors.

Growing evidence suggests that ATR inhibition may help to overcome PARP inhibitor resistance [99, 100]. The *ATR* gene was identified as a mediator of PARP inhibitor sensitivity in a synthetic lethal siRNA screen [92]. DNA DSBs that are produced following exposure to PARP inhibitors renders cells dependent on ATR for DNA repair [8]. As such, exposure to an ATR inhibitor disables ATR-mediated repair pathways and promotes cell death. Additionally, a known mechanism of PARP inhibitor resistance involves restored replication fork stabilization that may involve ATR, as well as other DDR proteins, such as CHK1 and WEE1 [99]. Preclinical studies have demonstrated that ATR inhibition leads to replication fork collapse that produces irreparable DNA DSBs [101, 102]. Building on this, the rationale for the combination of PARP and ATR inhibitors was demonstrated in another preclinical study in which PARP inhibitor resistant cells exhibited enhanced sensitivity in response to dual ATR and PARP inhibition in ovarian cancer patient-derived xenograft (PDX) models [103]. Furthermore, there are multiple ongoing clinical trials currently evaluating this drug combination, with at least 10 active studies taking place world-wide at the time of publication (Table 14.4).

With an expansive landscape of trials evaluating ATR and PARP inhibitor combinations, it is important to understand the tolerability and clinical efficacy of this approach. Overlapping toxicities stemming from combined ATR and PARP inhibition may be an issue for this drug combination. One example of such toxicity was reported in a dose-finding phase I trial in which ceralasertib was combined with the olaparib PARP inhibitor, which resulted in dose limiting toxicities (DLTs) in the form of thrombocytopenia and neutropenia that restricted continuous concurrent dosing of these agents [104].

Nonetheless, promising clinical activity produced by this drug combination was reported in a separate phase II trials evaluating a cohort of patients with recurrent

Table 14.4 Ongoing clinical trials combining ATR and PARP inhibitors

ATR inhibitor	PARP inhibitor	Trial phase	Status	Indication	References
RP-3500	Niraparib or olaparib	I and II	Recruiting	Advanced solid tumor	NCT04972110
RP-3500	Talazoparib	I and II	Recruiting	Advanced solid tumor	NCT04497116
Elimusertib	Niraparib	I	Recruiting	Advanced solid tumors (excluding prostate cancer) Ovarian cancer	NCT04267939
IMP9064	Senaparib	I	Recruiting	Solid tumor Advanced solid tumor	NCT05269316
M1774	Niraparib	I	Recruiting	Metastatic or locally advanced unresectable solid tumors	NCT04170153
Ceralasertib	Olaparib	II	Recruiting	High grade serous carcinoma	NCT03462342
		II	Recruiting	Gynecological cancers	NCT04065269
		II	Active, not recruiting	Prostate cancer	NCT03787680
		II	Recruiting	Clear cell renal cell carcinoma Locally advanced pancreatic cancer Locally advanced malignant solid neoplasm Metastatic renal cell carcinoma Metastatic urothelial carcinoma Metastatic pancreatic cancer Stage III pancreatic cancer Stage III renal cell cancer Stage IV pancreatic cancer Stage IV renal cell cancer	NCT03682289
		II	Recruiting	Anatomic stage IV breast cancer Metastatic triple negative breast carcinoma	NCT03801369

ovarian cancer who had progressed on prior PARP inhibitor treatment [105]. In a cohort of thirteen patients, the reported objective response rate (ORR) was 46% across six patients who had achieved radiologic PR [105]. Of these patients, 69% had germline *BRCA* mutations, 23% had somatic *BRCA* mutations, and 8% had other homologous recombination deficient mutations [105]. Although no patient discontinued treatment due to toxicity, reported adverse events included thrombocytopenia, anemia, and neutropenia, with dose reductions reported for both ceralasertib and olaparib [105]. Interestingly, this same study reported no objective responses in a cohort of PARP inhibitor naïve patients with platinum-resistant ovarian cancer [106]. Enrichment of therapeutic responses in the cohort of patients with past PARP inhibitor exposure further supports the notion that combined ATR and PARP inhibitor strategies may be key to overcome PARP inhibitor resistance in the clinic [106].

14.7 ATR and Immune-Checkpoint Inhibitor Combination Strategies

An emerging body of preclinical and clinical evidence supports the immunomodulatory role of ATR inhibitors in the tumor microenvironment. For instance, a recent single DNA fiber analysis after ATR inhibition showed induction of chromatin bridge formation and chromosome lagging, which in turn accelerated mitotic entry and further activated the cyclic GMP-AMP synthase-stimulator of interferon genes (cGAS-STING) tumor sensing axis [107]. Since genotoxic stress also induces the release of cytosolic DNA fragments that activate the cGAS-STING pathway, the combination of ATR inhibition with chemotherapeutics seems a rational combination to activate the innate immune response [111, 112].

It was also recently shown that treating prostate cancer cell lines with elimusertib induced S-phase DNA damage, activation of cGAS-STING signaling, as well as upregulation of *CCL5* (chemokine ligand 5) and *CXCL10* (C-X-C motif chemokine ligand 10) expression that culminated in activation of innate immunity [108, 109]. This is further supported by the increase in activated cGAS-STING and TBK1 levels, CD8+ T-cell infiltration, reduction of regulatory T-cell infiltration, and T-cell exhaustion observed in immunocompetent hepatocellular carcinoma mouse models treated with the triple combination of radiation, followed by ceralasertib and PD-L1 inhibition [110]. In addition, shortly after treatment with ceralasertib there was a modest increase in the intratumoral concentration of IFN- γ and proliferating CD8+ T-cells that was accompanied by a reduction of the PD-L1 tumor upregulation induced by radiation. At later time points, the combination of ceralasertib and radiation induced an increase in infiltrating CD8+ T-cells, as well as production of IFN- γ and tumor necrosis factor α [110]. Similar results were obtained by studying the combination of ceralasertib and radiation on immunocompetent mouse models of HPV-driven cancer, where a signature of type I and II IFN gene expression and modulation of cytokine gene expression (including *CCL3*

and *CXCL10*) were associated with treatment. Interestingly, increased antigen presentation and levels of major histocompatibility complex class I were also observed in vivo with the combination of ceralasertib and radiation [111]. Taken together, results from multiple preclinical studies suggest that the combination of radiation and ATR inhibitors stimulates IFN response and triggers antigen presentation.

ATR inhibition has also been shown to suppress upregulation of the natural killer group 2D (NKG2D) cell surface ligand that binds to activated CD8+ T-cells to trigger pro-inflammatory cytokine production [112]. It has also been suggested that *ATM*/*ATR*/*CHK1* signaling upregulation leads to transcriptional activation of PD-L1 via the signal transducer and activators of transcription STAT1 and STAT3 and the IFN regulatory factor (IRF1) pathway [113]. In fact, an increase in PD-L1 expression, accompanied by increased infiltrating macrophages and reduced infiltrating CD3+ T-cells, was observed in ATR deficient melanoma models (Fig. 14.1) [114]. Remarkably, such preclinical data is supported by results from the phase I clinical trial investigating elimusertib monotherapy, where paired tumor samples from patients with PD-L1 positive tumors revealed upregulation of PD-L1 [80]. Interestingly, patients with metastatic melanoma that were previously resistant to PD-L1 inhibitors achieved durable responses when treated with the combination of ceralasertib and paclitaxel [115]. In this combinational trial, interleukin-12 fluctuations were also observed in patients that received clinical benefit suggesting activation of the innate immune response [115].

14.8 Candidate Biomarkers of ATR Sensitization

Therapeutic biomarkers are used as indicators of disease prognosis and predictive measures of treatment response [116]. Emerging data from various preclinical and clinical studies that evaluating ATR inhibitors as monotherapy or in combination strategies have identified candidate predictive biomarkers that may indicate sensitivity to ATR inhibition. Here, we summarize key genetic biomarkers and discuss their role in defining target patient populations that may respond best to ATR inhibitors.

ATM is a DDR kinase that senses and repairs dsDNA breaks and whose mutation may confer dependency on the ATR-CHK1 axis, offering an exploitable target for ATR inhibitors [117]. Although *ATM* is frequently mutated in cancer, the functional impact of many *ATM* variants is not well established [118]. Furthermore, there is significant overlap between *ATM* and ATR signaling pathways, as supported by various preclinical and clinical studies evaluating various cancer types including hematological and solid tumors [8, 12, 119]. Clinical responses have been reported from phase I studies of ATR inhibitors specifically in patients with *ATM* aberrations, including *ATM* deleterious mutations or protein loss [12, 16, 120]. Although *ATM* is frequently mutated in cancer, the functional impact of many *ATM* variants is not well established [118]. However, a large proportion of *ATM* mutations derive from missense variants, which can lead to a reduction in

ATM protein expression levels [31]. This highlights the potential use of immunohistochemistry (IHC) analysis as a clinical tool to probe *ATM* expression levels and identify those who could benefit from ATR inhibition [31]. Pilie et al., further demonstrated the utility of IHC to understand *ATM* mutation annotations reported as variants of unknown significance (VUS), in which IHC analysis reported loss of protein in up to 25% of *ATM* VUS mutations, thus clarifying their functional impact [118]. This study also identified *ATM* loss of protein in patient tumor samples without identified *ATM* mutations, which points to the involvement of other mechanisms such as epigenetic or post-translational loss [118].

Another widely evaluated biomarker of ATR inhibitor sensitivity is *p53*, which plays a prominent role in G1 checkpoint control and whose loss comprises a high proportion of cancer cases [31]. Although there is preclinical data to support *p53*'s role as a predictive biomarker, the data remains inconsistent. For instance, Toledo et al., showed that cells with defective *p53* had augmented replication stress in response to ATR inhibitors compared to cells with wildtype *p53* [35]. A similar finding was reported in Kwok et al., in which treatment with the ATR inhibitor AZD6738 resulted in selective toxicity in *p53* defective xenografts and cell lines [121]. In contrast, another study showed no increase in sensitivity to single agent ATR inhibition with VE-821 in *p53* mutant cell lines compared to matched wildtype *p53* cells [122]. Although Dillion et al., reported radio-sensitization by AZD6738 to single radiation fractions in a panel of cell lines, the narrow sensitivity range to AZD6738 was independent of *p53* status [123]. Cumulatively, despite strong rationale supporting the use of ATR inhibitors to treat *p53* deficient tumors, the conflicting data suggests further studies are necessary to assess its utility as a predictive biomarker of response. Although data is still pending, multiple clinical trials are underway to evaluate ATR inhibitors as monotherapy or in combination strategies in patients with solid tumors harboring *TP53* mutations [48, 124].

A link between ATR sensitivity and deficiency of the BAF complex component AT-Rich Interactive Domain-containing protein 1A (*ARID1A*) was established in a large-scale genetic screen reported in Williamson et al. [125]. In this study, both in-vitro and in-vivo models were used to demonstrate wide-ranged genomic instability and cell death in *ARID1A* mutant cancer cell lines and tumors in response to ATR inhibition [125]. The clinical significance of this finding is highlighted by the fact that up to 7% of all cancers are associated with *ARID1A* loss and the frequency of loss is increased in certain cancers, for example, *ARID1A* loss is reported in up to 50% of clear cell ovarian carcinoma cases [126]. Further support for ATR inhibition in the setting of *ARID1A* loss was demonstrated in Tsai et al., in which an accumulation of R-loop formation was identified as a driver of replication stress in an ovarian cancer line with *ARID1A* knockout [126]. Translation of these data to the clinical setting has also produced compelling results. Antitumor activity was observed in patients with *ARID1A*-deficient solid tumors treated with the single-agent ATR inhibitor ceralasertib, including two patients that achieved RECIST-confirmed complete responses [127]. In addition, treatment with M6620 monotherapy resulted in a RECIST-confirmed complete response after 16 cycles

in a patient with metastatic colorectal cancer with *ARID1A* mutation and IHC confirmed loss of both *ARID1A* and *ATM*, with a reported progression free survival of 29 months at their last assessment [128]. The use of cell-free DNA (cfDNA) as an indicator of treatment response was also evaluated in this study, which revealed declining levels of allele frequencies for *ARID1A*, among other identified mutations, to undetectable levels after 9 cycles of treatment with M6620 compared to baseline [128].

Targeting deficiencies in homologous recombination DNA repair (HRR) also offers a potential opportunity for ATR inhibition [129, 130]. For example, Krajewska et al., demonstrated sensitivity of the breast cancer cell line MCF-7 to ATR inhibitors upon inactivation of *RAD51* in the HRR pathway [129]. Other studies have since further elucidated the major role ATR plays in regulating homologous recombination processes. For instance, Kim et al., showed that increased ATR signaling promotes the capacity of HRR in cancer cells by regulating the abundance of homologous recombination factors [28]. In support of this, a phase I trial of the Repare ATR inhibitor RP-3500 monotherapy observed multiple clinical responses in ovarian cancer patients with PARP-inhibitor resistant cancers that harbored actionable *BRCA1* and *RAD51C* mutations [87, 117]. Other responses described in this study included patients with homologous recombination deficiency (HRD), with molecular alterations in *ATM*, *BRCA2* and *RAD51B/C* [87]. ATR inhibition in HRD-cancers is largely under clinical investigation via multiple trials that are actively recruiting patients with deleterious mutations in HRR genes.

ATR deficiency has also been shown to confer a strong synthetic lethal response with many other DDR genes as well as with inducers of DNA replication stress [29]. For example, ATR inhibition is synthetic lethal in cells with genetic defects in genes such as *APOBEC3A* and *B* as well as with overexpression of *cyclin E1* (*CCNE1*) and with *c-MYC* amplifications [28, 131–133]. In addition to those mentioned above, molecular defects in DDR genes such as *ERCC1*, *XRCC1*, *CHK1*, and *FANCD2*, and even accumulation of R-loops, all have been shown to produce a synthetic lethal effect in response to ATR inhibition [8, 28, 29]. With so many potential synthetic lethal partners possible, results from ongoing preclinical and clinical studies will be instrumental in identifying biomarkers and factors associated with therapeutic response as ATR inhibitors appear poised to enter the clinic in the coming years.

14.9 Concluding Remarks

This chapter provides a rationale for targeting ATR and summarizes the current landscape of ATR inhibitors in clinical evaluation. As a key component of the DDR, ATR is a promising druggable target that is being widely evaluated in phase I, II and III clinical trials as monotherapy and in combinations with other agents, including DNA repair inhibitors, chemo- and radiotherapy, and immunotherapy. Regardless of the approach taken, ongoing clinical studies must

address optimization of the therapeutic window for this drug class. A predominantly reported toxicity across ATR inhibitors trials is myelosuppression, which is a mechanism-based toxicity that ultimately limits the therapeutic window in both monotherapy and combination approaches [134]. This carries key implications particularly for combination strategies due to potentiating of overlapping toxicities that may deepen myelosuppression and reduce drug tolerability. Proposed rational combination strategies should limit overlapping toxicity, which may be achieved by coordinating intermittent dosing schedules to facilitate tissue recovery. Another prevalent challenge is refining the target patient population most likely to benefit from ATR inhibition. Molecular technology advances and companion diagnostics have opened the door to precision oncology and the opportunity to offer personalized treatment strategies to patients [135]. Today, many clinical studies are designed on the basis of mutational status, which has led to the approval of several tumor-agnostic drugs [136]. Interestingly, many ongoing ATR inhibitor trials are recruiting patients based on molecular alteration rather than relying solely on tumor-type. A spectrum of molecular alterations have already been identified as potential predictive biomarkers that may sensitize to ATR inhibition; however, to be clinically efficacious, the biomarkers must be sensitive and easy to measure to allow for successful integration into the clinic. In closing, although several ATR inhibitors in development are poised to address a clinically unmet need, no ATR inhibitor has received FDA-approval for cancer indications thus far. We eagerly await the results from ongoing clinical studies as FDA-approval of ATR inhibitors lies close in sight.

Acknowledgements Timothy A. Yap is supported by MD Anderson Cancer Center Support grant (NIH/NCI P30 CA016672), The National Cancer Institute (NIH/NCI R01-CA255074), the US Department of Defense Ovarian Cancer Research Program (OC200482), and the V Foundation Clinical Scholar Program (VC2020-001). Helen M.R. Robinson, Graeme C.M. Smith are employed at Artios Pharma.

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