CHAPTER

10

# Targeting the Ataxia Telangiectasia and Rad3 Signaling Pathway to Overcome Chemoresistance in Cancer

*Marwan Kwok*[*,**], *Tatjana Stankovic*[*]

***Institute of Cancer and Genomic Sciences, University of Birmingham, United Kingdom**
****Centre for Clinical Haematology, Queen Elizabeth Hospital Birmingham, United Kingdom**

## Abstract

The ataxia telangiectasia and Rad3-related (ATR) signaling pathway controls DNA replication initiation and cell-cycle progression and coordinates the repair of damaged replication forks. It is, therefore, an important regulator of the cellular response to DNA replication stress. Inhibition of the ATR signaling pathway results in dysregulation of replication origin initiation, leading to exacerbation of replication stress manifested by the slowing and stalling of replication forks, and enhanced susceptibility to fork breakage. Accordingly, inhibition of ATR or its downstream effector Chk1 is potently cytotoxic to tumor cells with heightened levels of endogenous replication stress, particularly in tumors with DNA damage response or cell-cycle checkpoint defects. This results in a functional addiction to the ATR pathway. Small-molecule inhibitors of ATR and Chk1 are currently under investigation. Preclinical studies of these inhibitors have demonstrated single-agent efficacy across a range of malignancies, and results from clinical trials are emerging. In addition, markers of sensitivity to ATR and Chk1 inhibitors are being identified, and novel chemotherapeutic combinations involving these agents are being developed. A number of unresolved issues notwithstanding, the ATR signaling pathway represent a promising target for cancer therapeutics.

## ABBREVIATIONS

| | |
|---|---|
| **ALT** | Alternative lengtheningof telomeres |
| **AML** | Acute myeloid leukemia |
| **ARID1A** | AT-rich interaction domain1A |
| **ATM** | Ataxia telangiectacia mutated |
| **ATR** | Ataxia telangiectacia andRad3-related |
| **ATRIP** | ATR-interacting protein |
| **BLM** | Bloom syndrome protein |
| **BRCA1** | Breast cancer type 1 susceptibility protein |
| **cdk** | Cyclin-dependent kinase |
| **chk1** | Checkpoint kinase 1 |
| **CLL** | Chronic lymphocytic leukemia |

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**DDR** DNA damageresponse
**DLBCL** Diffuse large B cell lymphoma
**DSBs** Double-stranded breaks
**FANCM** Fanconi anemia complementation group M
**GCB** Germinal center B-cell like
**HRR** Homologousrecombination repair
**NHEJ** Nonhomologous end joining
**PARP** Poly-(ADP-ribose)polymerase
**PDX** Patient-derived xenotransplantation
**PIKK** Phosphoinositide 3-kinase-relatedkinase
**RPA** Replication protein A
**RS** Replication stress
**ssDNA** Single-strandedDNA
**T-ALL** T-cell acute lymphoblastic leukemia
**TOPB1** Topoisomerase 2-bindingprotein 1
**WRN** Werner syndrome ATP-dependent helicase

## 10.1 INTRODUCTION

The DNA damage response (DDR) is essential for the maintenance of genome integrity. This is accomplished through a network of intricately connected pathways, resulting in a dichotomous outcome: either resolution of the incipient DNA damage or apoptotic cell death, depending on the nature and severity of the afflicting lesion. Tumorigenesis is associated with a pervasive corruption of one or more of these pathways, often through mutation or deletion of key DNA repair or regulatory proteins, conferring on malignant cells a survival advantage, avoidance of apoptosis, and chemoresistance [1]. However, the subversion of normal DDR mechanisms can render tumor cells susceptible to further DNA insults, the accumulation of which may become incompatible with survival and results in cell death during mitosis, a process termed mitotic catastrophe [2,3]. Moreover, the disruption of a DNA repair pathway constrains tumor cells to rely on collateral repair pathways to maintain genome stability, thereby creating a vulnerability that can be amenable to therapeutic targeting [4].

Ataxia telangiectasia and Rad3-related (ATR) is a phosphoinositide 3-kinase-related kinase (PIKK) that is the master regulator of one such DDR pathway, with wide-ranging roles in DNA replication [5]. Studies in the last decade have shed light into its function and interaction with other DDR pathways, as well as the effects of inhibiting this pathway. We herein review our current understanding of the mechanistic basis of ATR signaling and illustrate how some tumor cells are distinctively reliant on this pathway and how targeting this pathway leads to tumor cell death. We also highlight important preclinical and clinical studies on this therapeutic target, as a single agent and in combination with other chemotherapeutic agents, and discuss unresolved questions and directions for future research.

## 10.2 FUNCTIONAL ROLES OF THE ATR SIGNALING CASCADE

### 10.2.1 The Role of ATR in the Regulation of DNA Replication Initiation

Initiation of DNA replication is a stochastic event, occurring at replication origins from which replication forks arise. Replication origins are clustered and replicated together within

replication factories. An important function of ATR is its ability to protect replication forks by acting as a negative regulator of replication origin initiation. This limits the activation of new replication factories, thereby directing replication toward already active factories [6,7]. Such negative regulation of replication initiation is of paramount importance in preventing the depletion of cellular pools of nucleotides and replication proteins, which would otherwise result in genomic instability. Indeed, only a small proportion of licensed origins is active in an unperturbed cell cycle, with the rest remaining dormant [6].

### 10.2.2 The Role of the ATR Signaling Pathway in the Maintenance of Replication Fork Stability

During DNA replication when the double-stranded DNA helix unwinds, replication fork progression can slow or stall due to replication obstacles such as DNA damage. Continued unwinding of the DNA helix despite stalled DNA polymerase leads to exposure of long stretches of single-stranded DNA (ssDNA) that are prone to damage. This results in replication fork breakage. The ATR pathway functions to prevent this outcome by protecting exposed ssDNA and repairing DNA lesions at these sites. Exposed ssDNA is rapidly coated with replication protein A (RPA), which stabilizes the replication fork and protects ssDNA from deleterious degradation by nucleases [8].

The ATR signaling cascade is initiated by the independent recruitment of two complexes, namely the ATR and ATR-interacting protein (ATRIP) and the RAD9–RAD1–HUS1 (9–1–1) complex, to RPA-coated ssDNA where RPA binds ATRIP and engages with the 9–1–1 complex. Further recruitment of topoisomerase 2-binding protein 1 (TOPBP1) activates ATR by stimulating ATR kinase activity [9]. Recently, the RPA-binding protein Ewing's tumor-associated antigen 1 was also reported to have ATR stimulatory activity and ability to activate ATR independent of TOPBP1 in a parallel pathway [10–12]. Functionally active ATR in turn phosphorylates the downstream effector molecule Chk1 and other substrates such as Werner syndrome ATP-dependent helicase (WRN), Bloom syndrome protein (BLM), and SMARCAL1. A recent study using iPOND method confirmed the recruitment of these proteins to DNA replisomes upon replication fork stalling and highlighted their functional importance in preventing fork collapse and breakage during DNA replication [13].

Through these proteins, ATR promotes replication fork stability and mediates replication restart in the event of fork stalling. This is achieved through fork reversal, whereby stalled forks reverse their course and through fork remodeling resulting in the formation of four-way junctions [14,15]. Helicases such as WRN and BLM, as well as translocases such as SMARCAL1 and the Fanconi anemia complementation group M protein (FANCM) are thought to play a major role in this process [16–21]. Fork reversal, remodeling, and restart together ensure completion of DNA replication and prevent fork breakage.

Proteins downstream of ATR also serve additional functions in facilitating fork repair, through suppression of new origin firing (BLM and FANCM) and protection of replication forks from unscheduled DNA degradation (WRN) [22,23]. Furthermore, Chk1 diffuses throughout the nucleus to suppress new origin firing. Thus, the regulatory activities of ATR signaling are amplified and propagated globally in the event of replication fork stalling [6,24]. This is crucial in curtailing further fork stalls to conserve the supply of RPA for the hitherto perturbed sites and to ensure that an excess of RPA over ssDNA is maintained [25].

### 10.2.3 The Role of the ATR Signaling Pathway in Cell-Cycle Regulation

Finally, through the downstream kinase Chk1, ATR also plays a major role in controlling cell-cycle progression. Chk1 regulates cellular entry into mitosis through the G2/M checkpoint. Mechanistically, this involves phosphorylation of Cdc25 phosphatases to prevent its binding to the cyclin B1–cyclin-dependent kinase (Cdk1) complex. Cdc25 phosphatases remove inhibitory phosphorylation from Cdk1 that is a prerequisite for the activation of cyclin B1–Cdk1, which initiates mitotic entry. In the presence of stalled replication forks, abolition of cyclin B1–Cdk1 activity through Chk1-mediated phosphorylation of Cdc25 phosphatases prevents premature entry of replicatively perturbed cells into mitosis until replication stalling is rectified and genomic duplication complete [26,27].

## 10.3 THE ATR PATHWAY AS A CANCER THERAPEUTIC TARGET

### 10.3.1 Induction of Replication Stress as a Consequence of ATR Pathway Inhibition

When replication fork progression is disrupted, slowing or stalling of replication forks ensues. This results in replication stress (RS), with cells continuing to progress through cell cycle despite the presence of unreplicated DNA [28]. Low levels of RS are ubiquitous in cycling cells and are often tolerated [29]. On the other hand, excessive amounts of RS arising, for instance, from the accumulation of unrepaired DNA damage, misincorporated ribonucleotides, or from difficult-to-replicate segments of DNA within fragile sites, inevitably lead to cell death [28]. The ATR signaling pathway ameliorates RS through its role in the regulation of DNA replication initiation, replication fork stability, and cell-cycle progression, as outlined above. Conversely, inhibition of the ATR pathway is a potent inducer of RS.

A consequence of ATR pathway inhibition is excessive replication origin initiation in an unscheduled and uncontrolled manner. This occurs independent of any additional sources of RS, either endogenous or exogenous. Indeed, DNA fiber analysis showed that the number of active replication forks in cancer cells was increased when ATR is inhibited, even in the absence of DNA damaging agents or other sources of RS [18,30]. Excessive and uncontrolled origin initiation as a result of ATR inhibition depletes essential replication factors such as nucleotides and replication proteins. This leads to a decrease in the rate of replication fork elongation and stalling of replication forks [24] and, as evidenced in cancer cells, to rapid reduction in inter-origin distance indicating increased replication origins [18,31]. These observations are consistent with the induction of RS.

Excessive and uncontrolled initiation of dormant replication origins also generates increased ssDNA. Exposed ssDNA is normally coated with RPA. However, when ATR is inhibited, the excess of ssDNA exhausts the nuclear pool of RPA. Unprotected ssDNA is susceptible to fork breakage, thereby precipitating DNA damage [30]. Furthermore, ATR inhibition prevents proper regulation of SMARCAL1, the deregulated activities of which promote excessive replication fork regression and fork collapse [18]. Together, these mechanisms underpin the cytotoxic effect of ATR pathway inhibition.

### 10.3.2 Replication Stress as a Therapeutic Vulnerability in Cancer Cells

An elevated level of RS is a defining feature of malignant cells. Although the precise mechanisms underlying oncogene-induced RS remain unclear, it is plausible that the accelerated consumption of RPA resulting from unchecked proliferation mediated by oncogenes could be a contributing factor [30]. Other potential mechanisms, such as the deregulation of replication initiation as well as interference between replication and transcription, arising either directly or indirectly from oncogenic activity, have also been postulated [32,33]. Given that the high constitutive levels of cellular RS is inherent in many tumors, it follows that they are likely to be particularly sensitive to RS overload instigated by inhibition of the ATR signaling pathway.

Until recently, it has been assumed that ATR is physiologically indispensable and not amenable to therapeutic targeting [34]. Indeed, human ATR mutations are uncommon, even in cancer. Among the few cancer types where *ATR* mutations have been reported, the acquisition of these mutations has been associated with tumor progression and poor clinical outcome [35,36]. Moreover, abrogation of the *ATR* gene is embryonically lethal, whereas its deletion in adult mice results in rapid aging and stem-cell loss [37,38]. In patients with Seckel syndrome in whom ATR signaling is defective due to hypomorphic germline mutation of the *ATR* gene, growth retardation, dwarfism, microcephaly, and mental impairment are typical manifestations [39,40].

Nonetheless, although complete abolition of ATR activity likely results in toxicity to healthy tissues, suppression of ATR activity was shown to be tolerable in healthy cells but not in tumor cells. In an elegant study by Schoppy et al. [41], conditional reduction of ATR in mice to 10% of normal levels using Cre–lox recombination resulted in minimal adverse effect on healthy hematopoietic and intestinal tissues. In contrast, suppression of ATR to this level was sufficient to severely restrict the growth of fibrosarcomas driven by H-Ras and p53 loss, as well as acute myeloid anemia driven by K-Ras and the *MLL-ENL* translocation [41]. Such reduction in ATR levels likewise prevented the development of Myc-driven lymphomas and pancreatic tumors featuring high levels of RS, as demonstrated by Murga et al. [42]. Corroborating these reports, we recently showed that therapeutic doses of an ATR kinase inhibitor were well tolerated in mice, while achieving effective tumor reduction [31]. Therefore, healthy and tumor cells demonstrate differential sensitivity to ATR pathway inhibition, providing a therapeutic window for the targeting of tumor cells.

## 10.4 PREDICTIVE BIOMARKERS OF SENSITIVITY TO ATR PATHWAY INHIBITION

### 10.4.1 ATR Pathway Addiction and the Concept of Synthetic Lethality

The substantial functional redundancy within cellular DDR pathways is widely recognized, wherein more than one pathway is capable of performing the same role. Physiologically, this provides protection against disruption of normal DDR mechanisms, particularly in cancer where DDR genes are mutated with high frequency. However, when one pathway is disrupted, cells become greatly reliant on collateral pathways. Thus, where two independent

pathways regulate an essential DDR process, the absence of one pathway is compatible with cell survival, whereas the absence of both results in cell death. This gives rise to an emerging therapeutic strategy known as synthetic lethality, in which collaborating pathways are abolished to induce cytotoxicity [43,44].

From the studies highlighted above, it is apparent that tumors with heightened levels of RS, such as those driven by Myc or Ras, are exquisitely sensitive to ATR pathway inhibition. Understanding other circumstances under which tumor cells become addicted to the ATR signaling pathway is therefore essential to identifying further predictive biomarkers of sensitivity to ATR inhibition. In the following sections, we summarize current evidence for synthetic lethality of ATR pathway inhibition with various DDR defects prevalent in cancers.

### 10.4.2 Synthetic Lethality With G1/S Cell-Cycle Checkpoint Defects

The G1/S cell-cycle checkpoint is regulated by p53, a key regulatory transcription factor. In response to DNA damage, p53 transcriptionally activates certain genes and represses others to promote G1/S cell-cycle arrest [45]. The *TP53* tumor suppressor gene encoding p53 is among the most frequently mutated in cancer [46]. The loss of the G1/S checkpoint due to *TP53* loss imposes upon tumor cells a dependence on the G2/M checkpoint controlled primarily through ATR/Chk1. Consequently, ATR pathway inhibition has synthetically lethal properties in *TP53*-defective tumors, the evidence of which can be gleaned from several studies.

Nghiem et al. [47] provided one of the earliest evidence for this concept demonstrating hypersensitivity of G1/S checkpoint-deficient cells to ATR loss. In this study, the authors observed that the osteosarcoma cell line U2OS in the absence of ATR function displayed premature chromatin condensation, a feature associated with mitotic catastrophe, in response to hydroxyurea or ultraviolet radiation-induced RS. Moreover, repression of p53 function by overexpression of MDM2 markedly potentiated the lethal effect of ATR inhibition in these cells [47]. These findings were recapitulated in a later study showing that a p53-deficient colorectal cell line rendered ATR deficient by knock-in of a Seckel gene ($ATR^{s/s}$) exhibited marked sensitivity to hydroxyurea- and cisplatin-induced RS, whereas restoration of p53 function reduced its sensitivity to these agents [48].

These *in vitro* studies were complemented by experiments in murine models by Ruzankina et al. [49], who generated *ATR*-mosaic knockout models from $TP53^{-/-}$ mice. Mice with concomitant loss of both *ATR* and *TP53* displayed markedly reduced survival compared to those harboring isolated loss of *ATR* and exhibited high levels of DNA damage as evidenced by the accumulation of γH2AX-positive cells [49]. In a further study using xenotransplantation models of both p53-wild-type and p53-deficient triple-negative breast cancer, Chk1 inhibition was shown to potentiate chemotherapy-induced apoptosis in p53-mutant xenografts but not in p53-wild-type counterparts. Combining Chk1 inhibition with the topoisomerase inhibitor irinotecan resulted in suppression of tumor growth and prolonged survival in xenografts with p53 deficiency but not in wild-type xenografts [50].

In addition to *TP53* loss, cyclin E overexpression has also been reported to exacerbate the effects of ATR pathway inhibition [51]. Cyclin E promotes G1/S cell-cycle progression and hence contributes to a heightened level of cellular replication stress.

### 10.4.3 Synthetic Lethality With Double-Stranded DNA Repair Defects

Ataxia telangiectasia mutated (ATM) is a kinase that acts upstream of p53 and controls a DDR pathway critical to resolving double-stranded DNA breaks (DSBs). ATM has considerable functional redundancy with the ATR pathway [5,52]. Therefore, a second scenario in which tumor cells can become addicted to the ATR pathway is through ATM loss. Like the *TP53* gene, deletions or mutations of the *ATM* gene are also frequent in various malignancies [53]. Moreover, loss of ATM promotes tumor progression and chemoresistance similar to p53 loss [54–56].

When ATR function is inhibited, stalled replication forks with persistent ssDNA collapse, resulting in the formation of partially replicated sister chromatid fragments with DNA double-stranded ends [5,13,28]. This is a form of DSBs that requires homologous recombination repair (HRR) through the ATM pathway. When HRR is defective, these chromatid fragments accumulate, and while they can be ligated through nonhomologous end joining (NHEJ), the latter is a low-fidelity repair process that potentially gives rise to aberrant sequence deletions or chromosomal translocations. In addition, ATM also plays an important role in cell-cycle regulation through both p53-dependent and -independent mechanisms [57]. Thus, when ATR is inhibited in ATM-deficient cells, accumulation of DNA damage ensues and defective cell-cycle checkpoints due to combined loss of ATR and ATM permit unrestricted entry into mitosis, leading to mitotic catastrophe.

Consistent with this, Reaper et al. [58] showed that ATR inhibition was invariably cytotoxic to ATM or p53-defective tumor cells. Moreover, combination of ATR inhibition with RS-inducing genotoxic agents such as cisplatin and carboplatin was more profoundly synergistic in tumor cells with constitutive ATM or p53 defects, and in cells subjected to ATM pharmacological inhibition or p53 siRNA knockdown [58]. In our recently published study, we extended these observations to primary tumor samples and patient-derived xenotransplantation (PDX) models. ATR inhibition was selectively cytotoxic to primary chronic lymphocytic leukemia (CLL) cells harboring deletions or mutations of *ATM* or *TP53*, in comparison with a panel of CLL samples without these aberrations and with healthy donor peripheral blood lymphocytes. Remarkably, ATR inhibitor treatment in murine xenografts derived from CLL patients with biallelic *ATM* loss led to a reduction not only in tumor load, but also in the proportion of CLL cells with *ATM* defects, indicating specificity of ATR inhibition for these defects [31].

Several other studies have demonstrated synthetic lethality between ATR inhibition and ATM or other HRR defects. ATR inhibition was lethal in cell lines derived from patients suffering from gastric cancer with ATM deficiency, in ovarian cancer with Breast cancer type 1 susceptibility protein 1 (BRCA1) deficiency, and in Rad51-depleted cells [59–62]. Taken together, these studies demonstrate that defects in HRR-dependent DSB repair are markers of sensitivity to ATR pathway inhibition.

Fig. 10.1 summarizes the synthetically lethal interaction between ATR pathway inhibition and ATM or p53 loss.

### 10.4.4 Other Markers of Sensitivity

A number of additional DNA repair defects have been shown to confer enhanced sensitivity to ATR pathway inhibition. These defects include deficiency in the DNA repair proteins XRCC1, ERCC1, POLD1, or PRIM1 [63–65]. XRCC1 is a protein involved in base excision

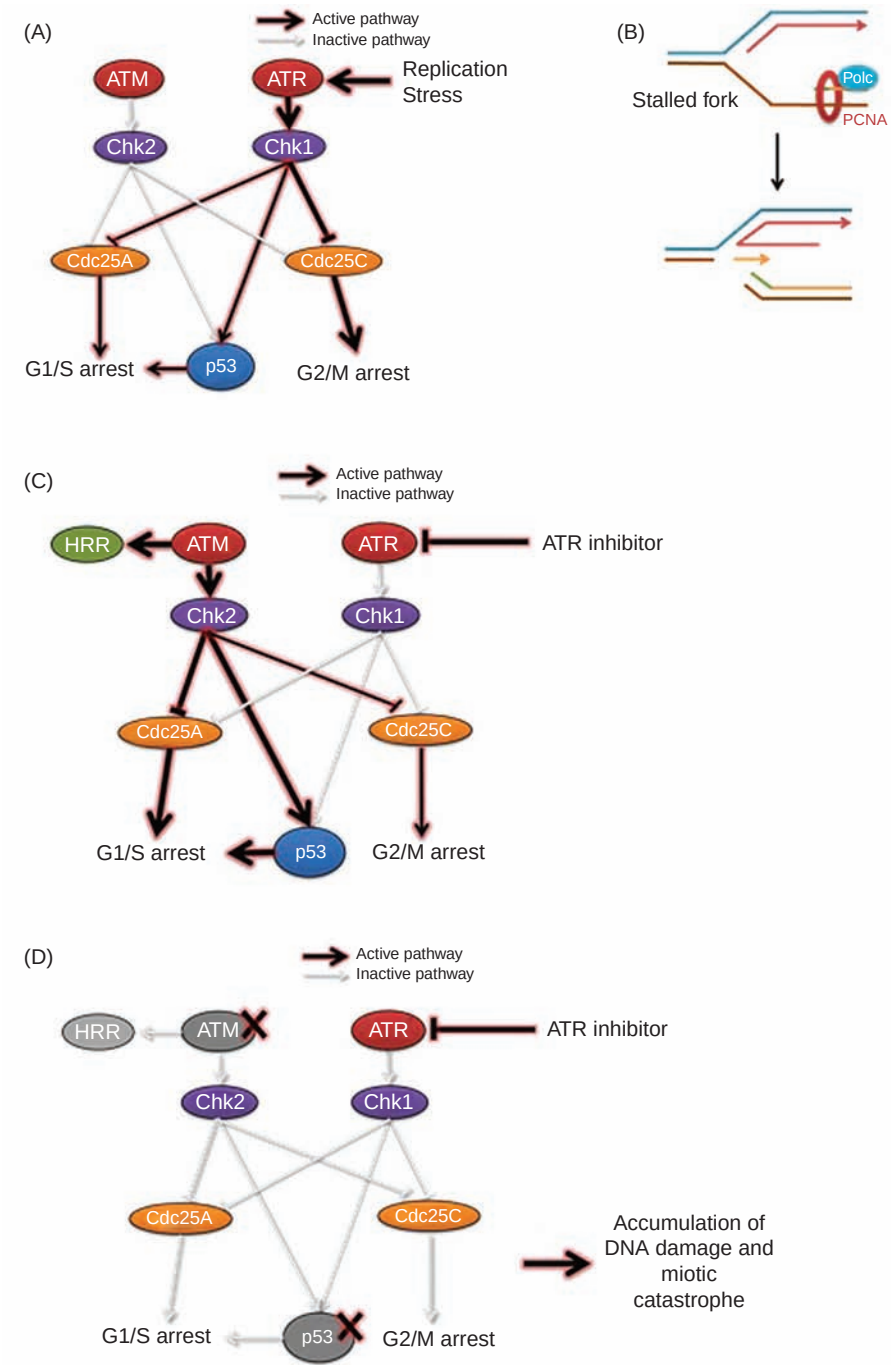


FIG. 10.1 **A model for synthetic lethality in CLL cells with ATM or p53 deficiency by inhibition of ATR.** ATM and ATR are master regulators of DDR, with ATM being activated in response to DNA double-strand breaks, and ATR in response to replication stress. (A) Activation of the ATR pathway leads to cell-cycle arrest mediated primarily

repair and the single-strand break repair pathway, whereas ERCC1 mediates the repair of several types of DNA lesions including bulky adducts, DSBs, and inter-strand cross-links and facilitates the separation of sister chromatids at fragile sites. POLD1 and PRIM1, on the other hand, are involved in DNA replication synthesis. Recently, an RNAi screen identified AT-rich interaction domain 1A (ARID1A) deficiency as an additional marker of sensitivity to ATR inhibition. Mutations in ARID1A are common in cancers and lead to disrupted topoisomerase localization and cell-cycle progression, thus imposing on cancer cells a dependence on the ATR signaling pathway [66].

Finally, a study by Flynn et al. [67] has alluded to the hypersensitivity to ATR inhibition in cancer cells reliant on a mechanism of telomere maintenance known as alternative lengthening of telomeres (ALT), whereby telomeres are elongated through recombination. The absence of ATR leads to abrogation of ALT, compromising telomere stability in ALT-dependent cancer cells, thus resulting in DNA damage, telomere loss, and selective lethality of these cells.

## 10.5 PRECLINICAL EVALUATION OF SMALL-MOLECULE INHIBITORS TARGETING THE ATR PATHWAY

### 10.5.1 Development and Evaluation of ATR Pathway Inhibitors

The design and development of selective ATR kinase inhibitors has been coupled with difficulty because of the atypical nature of PIKKs relative to other more conventional kinases. Early inhibitors of ATR were not specific, but more recently two drug companies have manufactured potent and highly specific ATR inhibitors. They are VE-821 and its analog VX-970 produced by Vertex Pharmaceuticals, as well as AZ20 and its analog AZD6738 developed by AstraZeneca [68,69]. Both compounds are ATP-competitive ATR kinase inhibitors, with AZD6738 additionally being available in an oral formulation.

In contrast, Chk1 inhibitors were accessible much earlier, and to date a number of Chk1 inhibitors have been developed. The earlier Chk1 inhibitors, such as UCN-01, however, lacked target selectivity and had poor pharmacokinetic properties, thus limiting their utility [70]. Next-generation Chk1 inhibitors, including AZD7762, MK-8776 (Sch900776), LY2603618, LY2606368, PF-00477736, V158411, and SAR020106, had considerably less off-target effects with an improved pharmacokinetic profile [71].

Numerous preclinical studies on ATR and Chk1 kinase inhibitors have been conducted on an array of solid and hematological malignancies, using a variety of experimental models. Although cell line models of human malignancies are easy to manipulate and provide an excellent basis for the initial evaluation of novel therapeutic agents, effects observed on cell lines

through the G2/M checkpoint, and repair of stalled replication forks. (B–C) Inhibition of ATR leads to collapse of stalled replication forks into partially replicated DNA fragments with double-stranded ends that are repaired through the ATM/p53 pathway. This involves cell-cycle arrest mediated primarily through the G1/S checkpoint and HRR. (D) In cells with defective ATM or p53, inhibition of ATR results in intolerable accumulation of unrepaired DNA damage. This arises from impaired HRR due to defective ATM and/or impaired cell-cycle regulation resulting from combined loss of functional ATR and ATM/p53. Abbreviations: CLL, chronic lymphocytic leukemia; ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3; HRR, homologous recombination repair. Adapted from Kwok et al., Blood, 2016, 127:582–95) [31].

are not always reflective of their effects *in vivo*. Therefore, results from studies on primary tumor material, as well as animal models, especially PDX, are invaluable additions to *in vitro* experimentation in informing the potential drug utility and effects, beneficial or otherwise, prior to their clinical deployment. In the following sections, we examine currently available *in vitro* and *in vivo* preclinical data on ATR and Chk1 inhibitors.

### 10.5.2 Single-Agent Efficacy of ATR Inhibitors in Cancer

*In vitro*, exposure of *TP53*-mutant multiple myeloma cell lines to 1 μM of VE-821 for 3 days resulted in a reduction in cell viability by >50% [72]. VE-821 monotherapy was shown to be cytotoxic also to glioma and lymphoma cell lines, to rectal carcinoma cell lines under hypoxic conditions, and to breast cancer and non-small-cell lung cancer lines with ERCC1 defect [60,63,73,74]. In colon cancer, HCT166 cells, particularly those deficient in ARID1A, responded to VE-821, VX-970, and AZ20, as reflected by marked reduction (by >90%) in viability and clonogenic survival upon treatment with ATR inhibitor doses of ≤1 μM [66]. In acute myeloid leukemia (AML), AZ20 doses of 350 nM to 1.4 μM were sufficient to reduce viability of AML cell lines by 50% ($EC_{50}$), whereas in primary AML samples this was achieved with AZ20 doses of 800 nM to 27 μM [75]. With AZD6738, single-agent efficacy was seen in cell lines of HER2-positive breast cancer and ATM-deficient gastric cancer [61,76]. Furthermore, in CLL, we demonstrated that AZD6738 treatment for 4 days led to lethality in *TP53* or *ATM* defective CLL cell lines and proliferating primary CLL samples, with an average $EC_{50}$ of 1.4 μM in cell lines and 8.5 μM in primary CLL cells [31].

*In vivo*, VX-970 suppressed tumor growth and prevented tumor establishment in mice xenografted with AR1D1A-deficient HCT116 or ovarian cancer TOV-21G cells, whereas AZ20 showed single-agent efficacy in reducing tumor infiltration and improving survival of murine models transplanted with *MLL*-rearranged AML cells [66,77]. Moreover, AZD6738 monotherapy, administered orally for 2 weeks, led to marked reduction in tumor load in PDX of biallelic *ATM*- or *TP53*-inactivated CLL, accompanied by a reduction in the proportion of CLL cells with these defects [31]. AZD6738 also countered tumor growth and induced apoptosis in a murine xenograft model of ATM-deficient gastric cancer [61].

A summary of the major preclinical studies involving ATR inhibitors in the various cancer types is presented in Table 10.1.

### 10.5.3 Single-Agent Efficacy of Chk1 Inhibitors in Cancer

*In vitro*, single-agent Chk1 inhibition with ≤1 μM AZD7762 led to >90% reduction in the viability of radioresistant breast cancer cell lines as well as viability reductions of varying magnitudes in metastatic melanoma cell lines [78,79]. With MK-8776 (Sch900776), single-agent cytotoxic effects were seen in neuroblastoma lines (median $EC_{50}$ 900 nM), in cell lines of myeloid leukemia and in BRCA-mutant ovarian cancer [80,81]. Cell lines that were sensitive to MK-8776 monotherapy were found to display aberrant Cdk2 activation in S phase upon Chk1 inhibition, leading to DSBs, possibly because these cells depend highly on constitutive suppression of Cdc25 phosphatases by Chk1 [82]. Moreover, LY2603618 exerted single-agent cytotoxicity on AML cell lines ($EC_{50}$ 0.1–1.6 μM) and patient samples ($EC_{50}$ <9 μM) as well as in osteosarcoma cell lines [83,84]. Furthermore, single-agent PF-00477736 activity was evident

**TABLE 10.1** Preclinical Studies involving ATR Inhibitors

| Malignancy | Study | Inhibitor | Experimental Model | Biomarker of Sensitivity | Treatments Potentiated by ATR Inhibitor |
|---|---|---|---|---|---|
| AML | Ma et al., 2017 [75] | AZ20 | Cell lines, primary cells | — | Cytarabine |
| | Chauduri et al., 2014 [93] | VE-821 | Cell lines | — | Wee1 inhibitor |
| Breast | Yazinski et al., 2017 [62] | AZ20, VE-821 | Cell lines, primary cells | BRCA1 deficiency | PARP1 inhibitor |
| | Kim et al., 2017 [76] | AZD6738 | Cell lines | — | Cisplatin |
| Cervical | Teng et al., 2015 [115] | ETP-46464 | Cell lines | — | Cisplatin, radiotherapy |
| CLL | Kwok et al., 2016 [31] | AZD6738 | Cell lines, primary cells, PDX | ATM, p53 deficiency | Chlorambucil, fludarabine, cyclophosphamide, bendamustine, ibrutinib |
| Colorectal | Hocke et al., 2016 [65] | VX-970, NU-6027 | Cell lines | POLD1 deficiency, PRIM1 deficiency | — |
| Endometrial | Teng et al., 2015 [115] | ETP-46464 | Cell lines | — | Cisplatin, radiotherapy |
| Esophageal | Leszczynska et al., 2016 [116] | VX-970 | Cell lines, cell-line xenografts | — | Cisplatin, carboplatin, radiotherapy |
| Gastric | Min et al., 2017 [61] | AZD6738 | Cell lines, cell-line xenografts | ATM deficiency | — |
| Lung | Vendetti et al., 2015 [90] | AZD6738 | Cell lines, cell-line xenografts | — | Cisplatin, gemcitabine, radiotherapy |
| | Hall et al., 2014 [89] | VX-970 | Cell lines, primary cells, PDX | — | Cisplatin |
| Lymphoma | Muralidharan et al., 2016 [73] | AZ20, VE-821 | Cell lines, cell-line xenografts | — | BET inhibitor |
| | Menezes et al., 2015 [117] | WO2010/073034 | Cell lines, cell-line xenografts | ATM deficiency | — |

(*Continued*)

**TABLE 10.1** Preclinical Studies Involving ATR Inhibitors (*cont.*)

| Malignancy | Study | Inhibitor | Experimental Model | Biomarker of Sensitivity | Treatments Potentiated by ATR Inhibitor |
|---|---|---|---|---|---|
| Myeloma | Cottini et al., 2015 [72] | VE-821 | Cell lines, primary cells | Myc expression, p53 deficiency | Piperlongumine |
| Ovarian | Kim et al., 2016 [81] | AZD6738 | Cell lines, PDX | — | PARP1 inhibitor |
| | Huntoon et al., 2013 [118] | VE-821 | Cell lines | — | Cisplatin, gemcitabine, PARP inhibitor |
| Pancreatic | Prevo et al., 2012 [119] | VE-821 | Cell lines, primary cells | — | Gemcitabine, radiotherapy |
| | Fokas et al., 2012 [120] | VX-970 | Cell lines, cell-line xenografts | — | Gemcitabine, radiotherapy |
| Various | Toledo et al., 2011 [51] | ETP-46464 | Cell lines | p53 deficiency, cyclin E overexpression | Hydroxyurea, Chk1 inhibitor |
| | Sultana et al., 2013 [64] | NU-6027 | Cell lines | XRCC1 deficiency | Cisplatin |
| | Repear et al., 2011 [58] | VE-821 | Cell lines | ATM, p53 deficiency | Cisplatin |
| | Pires et al., 2012 [74] | | Cell lines | — | Radiotherapy |
| | Mohni et al., 2015 [91] | | Cell lines | TLS polymerase, 53BP1 | Cisplatin |
| | Krajewska et al., 2015 [59] | | Cell lines | HRR deficient, Rad51 deficiency | — |
| | Middelton et al., 2015 [60] | | Cell lines | HR/BER defects | — |
| | Josse et al., 2014 [92] | VE-821, VX-970 | Cell lines, cell-line xenografts | — | Topoisomerase inhibitor |
| | Williamson et al., 2016 [66] | | Cell lines, cell-line xenografts | ARID1A deficiency | — |
| | Mohni et al., 2014 [63] | VE-821 | Cell lines | ERCC1 deficiency | — |

Abbreviations: AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; PDX, patient-derived xenografts; TLS, translesion synthesis.

in T-cell acute lymphoblastic leukemia (T-ALL) cell lines ($EC_{50}$ 20–109 nM) and primary cells ($EC_{50}$ <200 nM) which overexpress Chk1 [85]. PF-00477736 also exerted single-agent activity in cell lines of mantle cell lymphoma ($EC_{50}$ 0.68 nM), germinal center B-cell like (GCB) diffuse large B cell lymphoma (DLBCL; $EC_{50}$ 10.2 nM) and activated B-cell (ABC) DLBCL ($EC_{50}$ 87.3 nM) [86]. Finally, V158411 inhibited cell proliferation and induced caspase activation in breast and ovarian cancer cell lines [87].

*In vivo*, AZD6722 treatment for 3 days resulted in delay in tumor growth in MCF-7/C6 radioresistant breast cancer xenografts [78]. MK-8776 (Sch900776), on the other hand, reduced tumor volume in MDA-MB-231 breast cancer xenografts [88]. Furthermore, in a xenotransplantation model of T-ALL, 30 days of treatment with PF-00477736 yielded a significant reduction in tumor growth compared to vehicle-treated controls [85].

A selection of important preclinical studies in different cancers involving Chk1 inhibitors is presented in Table 10.2.

### 10.5.4 Chemotherapeutic Combinations Involving ATR Pathway Inhibitors

ATR and Chk1 inhibitors can be readily combined with conventional chemotherapeutic agents. Although the effectiveness of ATR/Chk1 inhibitor monotherapy is often dependent on specific tumor phenotypes, such as p53 deficiency or Myc overexpression, ATR and Chk1 inhibitors, when tested in preclinical models, synergistically enhance the effect of chemotherapy across a broad range of cancer phenotypes, DDR defective or otherwise. This can be ascribed to the potentiation of chemotherapy-induced RS by ATR/Chk1 inhibitors. The ability of ATR pathway inhibitors to resensitize chemoresistant tumor cells to conventional chemotherapeutic agents is of particular clinical significance.

Among the numerous chemotherapeutic agents that synergize with ATR/Chk1 inhibitors, cisplatin and gemcitabine are the most frequently studied. Cisplatin causes DNA breaks and crosslinks, whereas gemcitabine induces RS by decreasing the deoxyribonucleotide triphosphate required for DNA replication. ATR inhibitors broadly sensitized a panel of lung cancer cell lines, cell line xenografts, and PDX to cisplatin or gemcitabine, and ovarian cancer cell lines to cisplatin [89,90]. The sensitization to cisplatin is especially profound in tumor cells with deficiency in ATM, p53, or XRCC1, or with loss of 53BP1 or polymerase ζ involved in translesion synthesis [48,58,64,91]. Other DNA damaging agents that synergize with ATR inhibitors include cytarabine, as observed in AML cell lines and primary cells, as well as chlorambucil, bendamustine, fludarabine, and cyclophosphamide, as observed in CLL cell lines and primary cells. ATR inhibitors also sensitized tumor cells to topoisomerase inhibitors through disruption of DNA replication initiation and fork elongation processes [92].

Furthermore, ATR pathway inhibitors display synergy across a range of novel agents. Two studies, for instance, have highlighted the synergistic interaction between poly-(ADP-ribose) polymerase (PARP) inhibitors and ATR or Chk1 inhibitors in ovarian and breast cancer, respectively, using cell lines complemented by primary tumor samples or PDX [62,81]. PARP inhibitor treatment led to increased accumulation of cells in G2 phase, thus intensifying their reliance on the ATR pathway for checkpoint control and the maintenance of genome stability. ATR and/or Chk1 inhibitors have also shown synergy with inhibitors of other DNA repair molecules, such as Wee1, which contributes to the regulation of the G2/M checkpoint, and MK2, which is critical for prolonged checkpoint maintenance [79,86,93–96]. Finally,

**TABLE 10.2** Preclinical Studies Involving Chk1 Inhibitors

| Malignancy | Study | Inhibitor | Experimental Model | Biomarker of Sensitivity | Treatments Potentiated by Chk1 Inhibitor |
|---|---|---|---|---|---|
| ALL | Sarmento et al., 2015 [85] | PF477736 | Cell lines, cell-line xenografts, primary cells | — | — |
| AML | Zhao et al., 2016 [83] | LY2603618 | Cell lines, primary cells | — | Bcl-2 inhibitor |
| | Chauduri et al., 2014 [93] | MK-8776 | Cell lines | — | Wee1 inhibitor |
| | Yuan et al., 2014 [80] | SCH900766, AZD7762 | Cell lines | — | — |
| Bladder | Wang et al., 2015 [121] | Gö6976 | Cell lines | — | Gemcitabine |
| Breast | Zhang et al., 2016 [78] | AZD7762 | Cell lines, cell-line xenografts | — | — |
| | Zhou et al., 2017 [88] | MK-8776 | Cell lines, cell-line xenografts | — | Radiotherapy |
| | Ma et al., 2012 [50] | UCN-01, AZD7762 | PDX | p53 deficiency | Topoisomerase inhibitor |
| | Tang et al., 2012 [122] | | Cell lines, cell-line xenografts | — | PARP inhibitor, radiotherapy |
| | Bryant et al., 2014 [87] | V158411, PF477736, AZD7762 | Cell lines | — | Gemcitabine, cisplatin |
| Colon | Martino-Echarri et al., 2014 [123] | MK-8776, AZD7762 | Cell lines | — | 5-Fluorouracil |
| | Origanti et al., 2013 [124] | UCN-01 | Murine models, orthotropic models | p53 deficiency, p21 deficiency | Topoisomerase inhibitor |
| Head & neck | Gadhikar et al., 2013 [125] | AZD7762 | Cell lines | p53 deficiency | Cisplatin |
| | Barker et al., 2016 [126] | CCT244747 | Cell lines, cell-line xenografts, primary cells | — | Paclitaxel |
| Lung | Bartucci et al., 2012 [127] | SB218078, AZD7762 | Primary cells, PDX | — | Gemcitabine, cisplatin, paclitaxel |

**TABLE 10.2** Preclinical Studies Involving Chk1 Inhibitors (*cont.*)

| Malignancy | Study | Inhibitor | Experimental Model | Biomarker of Sensitivity | Treatments Potentiated by Chk1 Inhibitor |
|---|---|---|---|---|---|
| Lymphoma | Chila et al., 2015 [86] | PF477736 | Cell lines, cell-line xenografts | — | Wee1 inhibitor |
| | Zemanova et al., 2016 [128] | SCH900776 | Cell lines, primary cells, murine models | p53 deficiency | Fludarabine, cytarabine, gemcitabine |
| | Murga et al., 2011 [42] | UCN-01 | Murine models | Myc expression | — |
| Melanoma | Magnussen et al., 2015 [79] | AZD7762 | Cell lines, cell-line xenografts | — | Wee1 inhibitor |
| Myeloma | Pei et al., 2014 [129] | CEP3891 | Cell lines, primary cells | — | MEK1/2 inhibitor |
| | Dai et al., 2011 [130] | UCN-01 | Cell lines, primary cells, PDX | — | SRC inhibitor |
| Nasopharyngeal | Mak et al., 2015 [95] | AZD7762 | Cell lines, cell-line xenografts | — | Wee1 inhibitor |
| Neuroblastoma | Russell et al., 2013 [94] | MK-8776 | Cell lines, cell-line xenografts | — | Wee1 inhibitor |
| | Cole et al., 2011 [131] | SB218078, TCS2312 | Cell lines, primary cells | Myc expression | — |
| Oral SCC | Sankunny et al., 2014 [132] | PF477736 | Cell lines | ATM deficiency | Radiotherapy |
| Osteosarcoma | Duan et al., 2014 [84] | LY2603618 | Cell lines | — | Cisplatin |
| Ovarian | Kim et al., 2016 [81] | MK-8776 | Cell lines, PDX | — | PARP inhibitor |
| | Bryant et al., 2014 [87] | V158411, PF477736, AZD7762 | Cell lines | — | Gemcitabine, cisplatin |
| Pancreatic | Morgan et al., 2010 [92] | AZD7762 | Cell lines, cell-line xenografts, PDX | — | Radiotherapy |
| | Vance et al., 2011 [133] | | Cell lines | p53 deficiency | PARP inhibitor |
| | Engelke et al., 2013 [134] | MK-8776 | Cell lines, cell-line xenografts | HRR deficiency | Gemcitabine, radiotherapy |

(*Continued*)

**TABLE 10.2** Preclinical Studies Involving Chk1 Inhibitors (*cont.*)

| Malignancy | Study | Inhibitor | Experimental Model | Biomarker of Sensitivity | Treatments Potentiated by Chk1 Inhibitor |
|---|---|---|---|---|---|
| Various | McNeely et al., 2010 [135] | AZD7762 | Cell lines | BRCA2 deficiency, XRCC3 deficiency, DNA-PK deficiency | Gemcitabine |
| | Krajewska et al., 2015 [59] | | Cell lines | HRR deficient, Rad51 deficiency | — |
| | Sakurikar et al., 2016 [82] | MK-8776 | Cell lines | CDK2 activation in S phase | — |
| | Dietlein et al., 2015 [96] | PF477736 | Cell lines, cell-line xenografts, primary cells | KRAS mutation | MK2 inhibitor |

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; SCC, squamous cell carcinoma; PDX, patient-derived xenografts.

therapeutic combinations with ATR pathway inhibitors are not confined to agents targeting DDR. ATR inhibitors are capable of enhancing the effect of a BET bromodomain inhibitor in Myc-driven lymphoma and a B-cell receptor signaling inhibitor in CLL [31,73].

## 10.6 CLINICAL TRIALS OF SMALL-MOLECULE INHIBITORS TARGETING THE ATR PATHWAY

### 10.6.1 Clinical Studies Involving Chk1 Inhibitors

To explore the use of selective Chk1 inhibitors in oncology practice, several clinical studies have been carried out on AZD7762, LY2603618, LY2606368, and MK-8776 (Sch900776). These were phase I or II trials with the aim to assess safety and tolerability, to establish the maximum tolerable dose and, within certain studies, to determine response rate and durability. Unfortunately, the clinical development of AZD7762 was hampered by drug-related cardiac toxicity, which was dose limiting in some cases [97,98]. In contrast, LY2603618, LY2606368, and MK-8776 (Sch900776) had an acceptable toxicity profile with rare serious adverse events [99–102]. Moreover, early evidence of clinical activity was seen with these agents. In particular, eight (out of 24) refractory AML patients achieved complete remission and 15 (out of 30) individuals with advanced solid malignancy had partial response or stable disease following treatment with MK-8776 (Sch900776), either alone or in combination with chemotherapy [101,102]. Likewise, partial response was seen in two patients with advanced cancer following LY2606368 monotherapy [100].

Despite this, two phase II trials investigating LY2603618 in advanced lung and pancreatic cancer reported nonsuperiority of LY2603618, used in combination with premetrexed or gemcitabine, respectively, when compared to premetrexed or gemcitabine alone [103,104]. However, since the patients enrolled on these trials were unselected, a substantial proportion would unlikely have harbored high RS phenotypes or lesions that would yield synthetic lethality with ATR/Chk1 inhibition. Indeed, genomic analysis of an exceptional responder, who was cured of her invasive ureteric cancer with AZD7762 in combination with irinotecan, revealed mutation within *RAD50* which attenuated ATM signaling and enforced dependence on the ATR pathway [105]. This underscores the importance of the use of ATR pathway inhibitors for individuals who are likely to benefit.

Unfortunately, the clinical development of AZD7762, LY2603618, and MK-8776 has been suspended. Currently, the Chk1 inhibitors under active clinical investigation include LY2606368 and CCT245737, as detailed in Table 10.3.

### 10.6.2 Clinical Studies Involving ATR Inhibitors

With regard to ATR inhibitors, both AZD6738 and VX-970 have now entered phase I/II clinical testing. However, the majority of these studies have been initiated recently, and no results have yet been reported. As of March 2017, 11 clinical trials on ATR inhibitors are ongoing for a range of malignancies. These studies examine the use of the ATR inhibitor either alone or in combination with a range of conventional and novel therapeutic agents, including cisplatin, carboplatin, gemcitabine, etoposide, irinotecan, the PARP inhibitors olaparib and veliparib, and the anti-PD-L1 immune checkpoint inhibitor durvalumab.

A summary of the completed and ongoing clinical trials involving Chk1 and ATR inhibitors is presented in Table 10.3.

## 10.7 TARGETING THE ATM PATHWAY: IS THIS OF ANY VALUE IN OVERCOMING CHEMORESISTANCE IN CANCER?

There is considerable cross-talk between the ATR and ATM signaling pathways. Given the central importance of ATM in DDR, it is reasonable to also examine whether there is potential utility in inhibiting ATM to overcome chemoresistance, particularly since a number of selective ATM inhibitors are now available, including KU-55933, KU-60019, KU-59403, and AZD0156. In comparison with the plethora of literature on ATR pathway targeting, the number of studies on ATM targeting is relatively limited. It is generally recognized that ATM-deficient cells are hypersensitive to ionizing radiation (IR) on account of their inability to repair DSBs. Therefore, historical studies have focused on the use of ATM inhibitors to sensitize tumor cells to radiotherapy. Indeed, ATM inhibitors are potent radiosensitizers, both *in vitro* and *in vivo* [106–108].

More recently, Batey et al. [109] showed that ATM inhibitors can also be used to potentiate the cytotoxic effect of topoisomerase inhibitors such as camptothecin, etoposide, and doxorubicin, but lacked single-agent activity. However, the prolonged use of ATM inhibition remains questionable. In contrast to ATR inhibitors with target predilection for p53- and HRR-deficient cells, the lack of such predilection of ATM inhibitors raises the possibility of toxicity

TABLE 10.3 Clinical Studies Involving Chk1 and ATR Inhibitors

| Inhibitor | Phase | Study | Treatment | Malignancy | Pt no | Endpoint | Conclusions |
|---|---|---|---|---|---|---|---|
| AZD7762 | I | Seto et al., 2013 [98] | Monotherapy; combination with gemcitabine | Advanced solid tumor | 20 | Safety, pharmacokinetics, preliminary efficacy | Cardiac and hematologic toxicity was dose limiting at 30 mg. Maximum tolerable dose of AZD7762 in combination with gemcitabine was 21 mg |
| | | Sausville et al., 2014 [97] | Monotherapy; combination with gemcitabine | Advanced solid tumor | 42 | Safety, tolerability | Cardiac toxicity was dose limiting |
| LY2603618 | I | Doi et al., 2015 [99] | Monotherapy; combination with gemcitabine | Advanced solid tumor | 17 | Safety, tolerability | Acceptable safety and tolerability in combination with gemcitabine. Partial response in four patients. Most common high-grade toxicities were hematological |
| | | Calvo et al., 2016 [136] | Monotherapy; combination with gemcitabine | Advanced/metastatic solid tumor | 50 | Safety, tolerability | Adverse events include fatigue, thrombocytopenia, neutropenia, nausea, and anemia. Maximum tolerated dose was 200 mg/m$^2$ when combined with gemcitabine. Proceed to phase II trial with a fixed dose of 230 mg |
| | II | Scagliotti et al., 2016 [103] | Combination with pemetrexed | Metastatic non-small cell lung cancer | 53 | Response rate, pharmacokinetics, safety | Partial response in five patients. No association between p53 status and response. Efficacy and safety similar to pemetrexed monotherapy |
| | | Laguente et al., 2017 [104] | Combination with gemcitabine | Metastatic pancreatic cancer | 99 | Response rate, PFS, pharmacokinetics, safety | Maximum pharmacodynamic response was achieved after 230 mg of LY2603618. Combination of gemcitabine with LY2603618 was not superior to gemcitabine alone |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| LY2606368 | I | Hong et al., 2016 [100] | Monotherapy | Advanced cancer | 45 | Safety, pharmacokinetics, pharmacodynamics | Dose-limiting toxicities in seven patients were all hematological. Partial response was seen in two patients. Phase II study will utilize LY2606368 dose of 105 mg/m$^2$ |
| MK-8776 (Sch900776) | I | Karp et al., 2012 [101] | Combination with cytarabine | Refractory acute leukemia | 24 | Safety, tolerability, preliminary efficacy | Complete response seen in eight patients (33%). Dose-limiting toxicities were cardiac. Recommended dose of Sch900776 for phase II study is 100 mg |
| | | Daud et al., 2015 [102] | Monotherapy; combination with gemcitabine | Advanced solid tumor | 43 | Safety, pharmacokinetics, pharmacodynamics | Well tolerated as single agent and in combination with gemcitabine. Of 30 evaluable patients, 2 showed partial response and 13 had stable disease. Recommended phase II dose was 200 mg MK-8776 + 1000 mg/m$^2$ gemcitabine |

(*Continued*)

**TABLE 10.3** Clinical Studies Involving Chk1 and ATR Inhibitors (*cont.*)

| Inhibitor | | Phase | Start Date | Malignancy | Treatment | Pt no | ClinicalTrials.gov Identifier |
|---|---|---|---|---|---|---|---|
| ATR | AZD6738 | I | 2014 | Advanced solid tumor | Monotherapy; combination with palliative radiotherapy | 100 | NCT02223923 |
| | | | | Advanced solid tumor with ATM deficiency | Monotherapy; combination with carboplatin, olaparib, or durvalumab | 114 | NCT02264678 |
| | | | 2015 | Advanced solid tumor | Combination with paclitaxel | 21 | NCT02630199 |
| | VX-970 | I | 2012 | Advanced solid tumor | Combination with gemcitabine, cisplatin, etoposide, and/or carboplatin | 205 | NCT02157792 |
| | | | 2016 | Advanced head and neck squamous cell carcinoma | Combination with cisplatin/X-ray therapy | 45 | NCT02567422 |
| | | | | Non-small cell lung cancer with brain metastases | Combination with whole brain radiotherapy | 46 | NCT02589522 |
| | | | | Advanced solid tumor | Combination with irinotecan | 51 | NCT02595931 |
| | | | | Advanced solid tumor | Combination with veliparib and cisplatin | 60 | NCT02723864 |
| | | I/II | 2015 | Small cell lung cancer | Combination with topotecan | 55 | NCT02487095 |
| | | II | 2016 | Metastatic urothelial cancer | Combination with cisplatin/gemcitabine (vs cisplatin/gemcitabine alone) | 90 | NCT02567409 |
| | | | | Platinum-resistant recurrent ovarian or fallopian tube cancer | Combination with gemcitabine (vs gemcitabine alone) | 70 | NCT02595892 |
| Chk1 | CCT 245737 | I | 2016 | Advanced solid tumor | Monotherapy | 40 | NCT02797964 |
| | | | | Advanced solid tumor | Monotherapy | 70 | NCT02797977 |
| | LY 2606368 | I | 2017 | Advanced pediatric solid tumors | Monotherapy | 65 | NCT02808650 |
| | | II | 2014 | BRCA mutant or high-grade breast and ovarian cancer, metastatic prostate cancer | Monotherapy | 144 | NCT02203513 |
| | | | 2016 | Solid tumors with replication stress or HRR deficiency | Monotherapy | 38 | NCT02873975 |

Published studies are listed in the upper panel, and ongoing studies in the lower panel. Abbreviations: Pt no, patient number; PFS, progression-free survival.

to healthy cells [109]. ATM inhibitors may be more effective in certain tumor types, such as glioblastoma, where tumor-associated overexpression of platelet-derived growth factor alpha (PDGFRA) can be normalized with ATM inhibition [110]. While there is no published clinical data on ATM inhibition, a phase I study of AZD0156, alone and in combination with chemotherapy or olaparib, is currently underway (NCT02588105). Therefore, the clinical utility of ATM inhibition remains to be seen.

## 10.8 CONSIDERATIONS, CAVEATS, AND UNRESOLVED QUESTIONS IN ATR TARGETING

### 10.8.1 ATR vs Chk1 targeting

While ATR and Chk1 act in the same pathway, ATR is upstream of Chk1 and therefore controls additional downstream processes. Targeting ATR may therefore produce more wide-ranging effects than targeting Chk1, and this is reflected in the ability of ATR inhibitors to potentiate a broader range of chemotherapeutic agents. It remains to be determined whether ATR inhibitors are clinically more effective than Chk1 inhibitors. This might vary from one malignancy to another, or indeed from one tumor phenotype to another. For example, tumors with ATM deficiency might benefit more from ATR inhibition than from Chk1 inhibition, given the functional cross-talk between the ATM and ATR.

### 10.8.2 Potential Mechanisms of Resistance to ATR Pathway Inhibition

A fundamental assumption underpinning models of synthetic lethality is that there are only two major pathways regulating a process. Therefore, if one pathway is defective, cellular demise is assured when the other pathway is blocked. However, this notion is inevitably an oversimplification of the myriad of collateral pathways regulating a cellular process, many of which are hitherto underappreciated or unknown. When one collateral pathway is therapeutically inhibited, tumor cells may upregulate an alternative collateral pathway to mitigate the effects of the initial block, thereby resulting in therapeutic resistance.

Alongside ATM, DNA-dependent protein kinase (DNA-PK) plays a role in DSB repair through control of NHEJ. Recently, there have been reports pointing to a possible functional redundancy between ATR and DNA-PK in regulating downstream Chk1 activity [111]. Buisson et al. [112], in particular, demonstrated the existence of a DNA-PK-Chk1 backup pathway that can mediate resistance to ATR inhibitors. In this model, ATR inhibition is cytotoxic to a proportion of tumor cells with the highest levels of replication stress, but those with moderate levels are protected through the backup pathway [112]. Simultaneous targeting of ATR and Chk1 or DNA-PK could potentially overcome this. Indeed, a potentiating interaction between ATR and Chk1 inhibitors has been reported, suggesting that the combined use of these inhibitors could be more efficacious than either agent alone [113]. However, the potential toxicities and adverse effects of any combined use of ATR and Chk1 inhibitors will need to be properly assessed.

Finally, both ATR and Chk1 inhibitors are kinase inhibitors. As observed from the small-molecule kinase inhibitors currently in clinical use, point mutations in the target kinase can

develop. This constitutes another potential source of therapeutic resistance that physicians and investigators will need to anticipate and overcome.

### 10.8.3 Precision Medicine and the Selection of Patients for ATR/Chk1 Targeting

The long-term effects and toxicity of ATR and Chk1 inhibition are unknown and this is often a source of contention. Targeting a process of such importance to genome integrity can be potentially dangerous if it also affects healthy cells. Moreover, sublethal targeting of tumor cells with ATR pathway inhibitors can inadvertently promote tumorigenesis and clonal evolution, as these cells accumulate replication stress and DNA damage but escape death [114]. It is therefore of paramount importance that patients are selected for ATR pathway inhibitor treatment according to whether their tumor cells are differentially sensitive to these inhibitors. By selecting patients whose tumor is hypersensitive to these inhibitors, tumor killing can be maximized while minimizing treatment duration, thereby reducing both toxicity to healthy tissues and the risk of tumor evolution and escape. While a number of biomarkers of sensitivity to ATR pathway inhibition have been discussed earlier, it is likely that many others have yet to be discovered. The onus is on investigators to continue to identify predictive biomarkers of sensitivity to ATR and Chk1 inhibitors, specific for each tumor type, in order to enable accurate therapeutic stratification.

### 10.8.4 The Use of ATR/Chk1 Inhibitors with Other Therapeutic Agents

Finally, should ATR/Chk1 inhibitors be administered as a single agent or in combination with chemotherapy? If so, what is the ideal therapeutic regimen to be used? An argument in favor of combined use with chemotherapy is that the addition of the latter would increase the potency of ATR pathway inhibitors, but this needs to be balanced by a possible increase in toxicity. In addition, the use of therapeutic combinations may potentially reduce the likelihood of developing resistance to single agents. The ideal chemotherapeutic agent to be used in combination with different tumor types will need to be ascertained through mechanistic and clinical studies. In addition, ATR/Chk1 inhibitors can potentially be combined with other novel small-molecule inhibitors or monoclonal antibodies. This may be of particular relevance to a number of malignancies where a substantial proportion of tumor cells is quiescent. These cells are unlikely to be susceptible to ATR pathway inhibition and may give rise to a pool of residual cells from which therapeutic resistance can arise. Therefore, combination of ATR pathway inhibitors with agents targeting pathways unrelated to replication could allow the simultaneous eradication of both proliferating and quiescent tumor populations. The scheduling and sequencing of ATR pathway inhibitors within therapeutic regimens will require continuous optimization.

### 10.8.5 Concluding Remarks

The availability of ATR pathway inhibitors offer a beacon of hope that chemoresistance, which is a barrier to effective cancer treatment, could finally be overcome. Preclinical results

of ATR and Chk1 inhibitors have shown remarkable promise. However, our understanding of the mechanistic basis of ATR pathway inhibition is by no means complete, and the clinical development of ATR pathway inhibitors is still in its infancy. Identification of predictive biomarkers of response underpinned by sound mechanistic understanding remains a research priority, and the rational selection of patients into clinical studies based on these biomarkers could translate into better therapeutic outcomes. In conclusion, the development of ATR pathway inhibitors epitomizes the emerging role of precision medicine in cancer treatment, through which vulnerabilities in cancer are systematically identified and targeted.

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