Review

Targeting ATR Pathway in Solid Tumors: Evidence of Improving Therapeutic Outcomes

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Targeting ATR Pathway in Solid Tumors: Evidence of Improving Therapeutic Outcomes

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Abstract: The DNA damage response (DDR) system is a complicated network of signaling pathways that detects and repairs DNA damage or induces apoptosis. Critical regulators of the DDR network include the DNA damage kinases ataxia telangiectasia mutated Rad3-related kinase (ATR) and ataxia-telangiectasia mutated (ATM). The ATR pathway coordinates processes such as replication stress response, stabilization of replication forks, cell cycle arrest, and DNA repair. ATR inhibition disrupts these functions, causing reduction of DNA repair, accumulation of DNA damage, replication fork collapse, inappropriate mitotic entry and mitotic catastrophe. Recent data have shown that the inhibition of ATR can lead to synthetic lethality in ATM-deficient malignancies. In addition, ATR inhibition plays a significant role in the activation of the immune system by increasing the tumor mutational burden and neoantigen load as well as by triggering the accumulation of cytosolic DNA and subsequently inducing the cGAS-STING pathway and the type I IFN response. Taken together, we review stimulating data showing that ATR kinase inhibition can alter the DDR network, the immune system and their interplay and therefore potentially provide a novel strategy to improve the efficacy of antitumor therapy, using ATR inhibitors as monotherapy or in combination with genotoxic drugs and/or immunomodulators.

Keywords: ATR; ATR inhibitor;ceralasertib (AZD6738);DNA damage response; immune system; synthetic lethality; ATR-ATM interplay

1. Introduction

Cells face constant exposure to multiple DNA damage sources, both endogenous (e.g. oxidation, alkylation, hydrolysis, mismatch of DNA bases) and exogenous (genotoxic chemicals, UV light, ionizing radiation, etc.) [1–5]. To neutralize these threats and ensure genomic stability, cells have developed several mechanisms, collectively called the DNA damage response (DDR) network [6]. The DDR system includes damage sensors, transducer kinases, and effectors to maintain genomic integrity. Interestingly, recent data have shown that the deregulated DDR network is capable of activating the host immune system [7]. These results potentially provide a novel strategy for enhancing the efficacy of immunotherapy.

On the other hand, deregulated DDR pathways trigger mutagenesis and genomic instability, thus getting implicated in the onset and progression of cancer. Cancer cells divide rapidly and continuously due to a breakdown of the mechanisms regulating the cell cycle. The increased proliferation rate and the DNA repair defects in cancer cells make these cells more vulnerable to specific DDR inhibition [8]. Hence, DDR inhibitors, a class of drugs that can modify the DDR network, have recently gained great attention in the research of cancer treatment. The known DDR inhibitors include drugs that inhibit different DNA repair pathways or factors, such as the polyADP-ribose

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polymerase (PARP), the ataxia telangiectasia mutated kinase (ATM), the ataxia telangiectasia and Rad3 related kinase (ATR), the Checkpoint kinases 1 and 2 (CHK1/2), the Cyclin-dependent kinases 4 and 6, (DK4/6), the cell-cycle checkpoint kinase WEE1, and the DNA-dependent protein kinase (DNA-PK)[8].

Particularly, ATM and ATR kinases have a critical role in the activation of the DDR network. As for ATR, following the formation of the stable replication protein A (RPA)-single-stranded DNA (ssDNA) complex at sites of DNA damage, the ATR-interacting protein (ATRIP) will bind directly to RPA, resulting in the localization of the ATR kinase to these sites [9]. Next, to give more time for the DNA repair mechanism to proceed, the ATR-CHK1 signaling pathway causes cell-cycle arrest at G2-M phase. As for ATM, this kinase is activated via the MRN (meiotic recombination protein 11 - MRE11, Nijmegen breakage syndrome protein 1 - NBS1) complex, a DNA double-strand breaks (DSBs) sensor [10]. Then, ATM phosphorylates the H2A histone family member X (H2AX) at S139 (γH2AX), and induces the CHK2 kinase, resulting in the activation of the G1-S and intra-S-phase. Based on the above, ATR and ATM kinases may be promising molecular targets in the treatment of cancer. Currently, numerous small molecule ATM/ATR inhibitors have been discovered and are undergoing preclinical and clinical evaluation.

Herein, we present a review of the current literature summarizing the role of ATR inhibition in the modification of the DDR network, the immune system and their interplay. The latest advances of ATR inhibitors in preclinical and clinical states are also elucidated.

2. The ATR Pathway in the DNA Damage Response Network

The DNA damage response network is activated following the detection of DNA damage by specific sensors [6]. The next step is the activation of a signal transduction cascade which leads to the induction of genome protection mechanisms, such as DNA repair pathways, cell cycle checkpoints, or the initiation of apoptosis. Deregulated DDR may also result in mutagenesis and genomic instability. Since DDR is an important cellular network of molecular pathways that regulates the cell's decision to repair the DNA damage or to undergo apoptosis, it is implicated in both the onset and progression of a disease, as well as in the outcome of therapeutic treatment.

There are several DNA repair mechanisms active throughout the cell cycle, including the Fanconi Anemia (FA) pathway, which is implicated in the repair of interstrand crosslinks (ICLs), the Nucleotide Excision Repair (NER), which removes adducts that disrupt the DNA double-helix, the Base Excision Repair (BER), coping with alkylated, oxidized and deaminated bases, the Mismatch Repair (MMR) pathway that resolves mismatched bases that may occur during DNA replication, the homologous recombination (HR) repair and the non-homologous DNA end joining (NHEJ), two major subpathways for the repair of DSB, the most lethal type of DNA lesion [11].

It is generally accepted that in cells with dysfunctional DDR, such as cancer cells, DNA integrity is often compromised. During the S phase, the replication fork is usually stalled by DNA lesions and if these remain unresolved, the replication machinery eventually collapses [12]. This condition is referred to as “replication stress” and is a common characteristic of tumor cells due to chronic proliferation, being also the main cause of genomic instability in cancer. Nonetheless, it may also be noticed in normal cells, on account of oxidative stress or other endogenous damage [12]. DDR also needs to modulate cell cycle progression, as cell cycle arrest is required for the resolution of DNA lesions. Two major kinases appear to be the key players organizing the response right after DNA damage recognition, ATM and ATR (Figure 1)[13].

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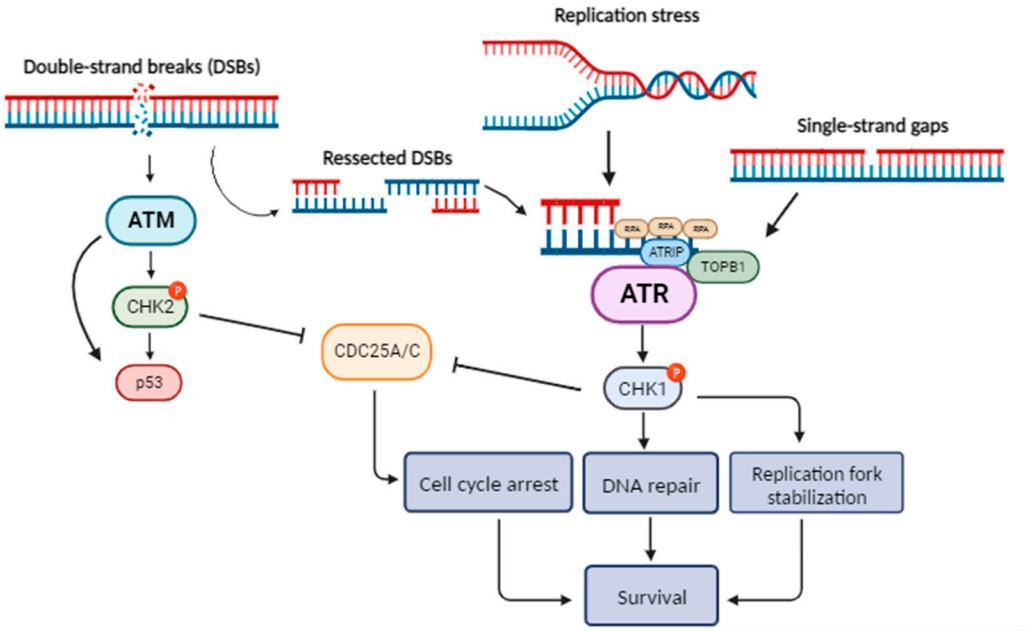

Figure 1. Schematic overview of the ATR/ATM pathways (Figure was created with BioRender.com).

ATM is mostly activated in response to DSBs during all phases of cell cycle [6,14], while ATR is involved in the recognition of single-strand breaks (SSBs), occurring as a response to numerous mechanisms (e.g. during replication fork stalling or as NER and DSBs repair intermediates) [13,15–17]. The broad involvement of ATR in various processes (replication stress response, SSBs and DSBs repair, interstrand crosslink repair, meiosis) is highlighted by that ATR, and not ATM, is indispensable for cell survival [18,19]. Particularly, ATR is an essential protein with scarce loss-of-function mutations in cancer [20], while it has been observed that impaired ATR function in mouse models leads to tumorigenesis resistance [21].

Of note, previous studies have shown crosstalk between the ATR and the ATM pathways [22,23]. As far as DSBs repair is concerned, ATM-dependent activation of ATR has been shown to occur [24,25]. DSBs are primarily detected by the MRN complex that is vital for the activation of ATM [26–30]. During the DSBs repair that is mediated by ATM signaling, ssDNA fragments are often accumulated as a result of the resection of DSBs by exo- and endo-nucleases [24]. These ssDNA fragments stimulate the ATR pathway, forming the ATR-ATM interplay during DSBs repair [23].

ATR pathway initiates with the RPA protein recognizing and coating ssDNA, followed by the binding of ATR-interacting protein (ATRIP) and the assembly of ATR-ATRIP complex at the DNA damage sites. However, this is not sufficient for the activation of ATR [31]. Several additional regulatory proteins, including the Rad17 complex, the Rad9–Rad1–Hus1 (9-1-1) complex, and the 9-1-1 interacting nuclear orphan (RHINO), need to be implicated [23,32,33] in order to recruit the DNA topoisomerase 2-binding protein 1 (TOPBP1) that finally stimulates the kinase activity of ATR [19,23,33]. Consequently, with the aid of mediators such as claspin[34], ATR phosphorylates the downstream Checkpoint Kinase 1 (CHK1). This pathway can result in cell cycle arrest either in the intra-S-phase or in the G2/M phase [12,13], as CHK1 is responsible for the phosphorylation of multiple substrates including phosphatases CDC25A, CDC25B and CDC25C [19]. This results in their inhibition, preventing them from keeping active the kinases CDK2 and CDK1, thus blocking cell cycle progression [35,36].

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3. The ATR Pathway and the Interplay between the DDR Network and the Immune System

The Immune System and the DDR network are important mechanisms that are implicated in the survival of the living organisms. Interestingly, a growing number of data have shown that these two systems play a crucial role in the onset and progression of cancer, as well as in the outcome of anticancer therapy [37]. Traditionally, conventional chemotherapy has been considered immunosuppressive and several chemotherapeutics are used to treat autoimmune conditions. On the other hand, accumulating data suggest that DNA damaging agents can promote immunogenicity in a variety of ways, some of which have the potential to be exploited in relation to immunotherapy. Several mechanisms are implicated in the DDR-mediated activation of the immune system, including the following:

a. The induction of immunogenic cell death, i.e. cell death which elicits an immune response [38]. Not all modes of cell death induce such a response which requires, in addition to neoantigen exposure, the presence of additional danger signals [39]. Such signals are provided by damage-associated molecular patterns (DAMPs), i.e. molecules released from dying tumor cells that stimulate the recruitment of antigen-presenting cells to the site, where they process and present tumor neoantigens, thereby priming an adaptive immune response. DAMPs released during chemotherapy-induced immunogenic cell death include, among others, DNA release in the cytoplasm where it leads to activation of stimulator-of-interferon genes (STING) and induction of type I interferon (IFN) and pro-inflammatory cytokines [40].
b. The increase in antigen presentation through the upregulation of MHC-1 (major histocompatibility complex type 1) expression on tumor cells and promotion of dendritic cell maturation, priming them for an adaptive immune response [41].
c. Changes in the cytokine milieu within the tumor microenvironment through the release of proinflammatory cytokines such as NF-κB and IFN-α [42], which has a bystander effect on neighboring cells that results in an immunogenic tumor microenvironment [43].
d. Downregulation of myeloid-derived suppressor cells (MDSC) and regulatory T-cells (Tregs), which play a role in dampening the host immune response [44,45].
e. Modification of the expression of the immune checkpoint factors PD-1/PD-L1. Some studies have reported a downregulation of PD-L1 expression following genotoxic chemotherapy [46] or a redistribution of PD-L1 from the tumor cell surface to nuclear membrane [47].
f. Increase of the tumor neoantigen burden. There are indications that genotoxic drugs may enhance tumor immunogenicity by causing, thanks to their mutagenicity, an increase of tumor neoantigens, which appear to play a critical role in the effectiveness of immune checkpoint blockade immunotherapy [48–50].

Interestingly, previous studies have demonstrated that a shift in the balance between DNA damage and repair causes the accumulation of cytosolic DNA that can act as potent immune-stimulator via the induction of the cGAS/STING pathway and the subsequent activation of the type-I interferon (IFN) signaling pathway [51–53]. Other studies have also shown that the progression of the cell cycle through mitosis in the presence of DNA DSBs results in the generation of micronuclei and the activation of the immune system [54,55]. Foreign DNA detection is a crucial step in the induction of immunity in many organisms. In mammalian cells, activation of the immune responses is contributed mainly by the cyclic GMP-AMP synthase (cGAS) –STING pathway, which plays an important role for coupling the detection of the DNA to the activation of the innate immune defence mechanisms [56]. In this pathway, the binding of cGAS to dsDNA induces its catalytic activity and results in the production of 2′,3′-cyclic GMP–AMP (cGAMP), a second messenger molecule, acting as a potent agonist of STING [57,58]. The synthesis of cGAMP is an important first step that results in the activation of the cGAS-mediated antiviral effects in several species [59]. Indeed, the cGAS molecule is activated by bacterial and viral DNA as well as by mitochondrial DNA and phagocytosed DNA that are abnormally localized in the cytosol. The induction of cGAS produces cGAMP that activates STING and leads to the induction of TANK-binding kinase 1 (TBK1), IkB kinase (IKK) and NF-kB inducing kinase (NIK) [60,61]. Together, induction of these kinases leads to the activation and nuclear translocation of IFN regulatory factor 3 (IRF3) and NF-kB, resulting in the expression of type

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I IFN, interferon-stimulated genes (ISGs) and inflammatory cytokines-further connecting the DDR network with the immune system [62,63]. On the other hand, extensive observations suggest that chronic activation of cGAS/STING can induce an immune suppressive tumor microenvironment (TME) that promotes the progression of the tumor [64–66]. In line with these data, activation of cGAS/STING pathway may have either a pro-tumor or an anti-tumor effect, depending on the stage of tumor progression and the tissue-specific context.

Since cytoplasmic dsDNA can activate STING, chemotherapies that result in the accumulation of cytoplasmic dsDNA may be an alternative strategy for STING activation. Indeed, genotoxic therapies including radiotherapy, cytotoxic chemotherapy, inhibitors of PARP and/or ATR augmented cytosolic DNA damage-induced dsDNA and activate the cGAS-STING-IFN response[67–70] with S-phase DNA damage being a particularly potent activator [71]. The activation of cGAS/STING inflammatory responses following DNA damage by PARP [72] or ATR [73] inhibition may also induce the formation of micronuclei with subsequent leaking of DNA from the micronuclei able to activate the innate immune response [52,74]. Micronuclei are small organelles that contain DNA and are produced as a result of genotoxic stress and chromosome missegregation in subsequent cell division [75]. Although these organelles are formed with a nuclear envelope (NE), after mitosis they lose compartmentalization as their NE ruptures [76]. A consequence of micronuclei rupture is that chromosomal DNA become accessible to cGAS and the subsequent induction of immune responses [52,54,55,77–79].

Concerning the ATR inhibition, an accumulating body of evidence suggests that the ATR pathway modulates the antitumor immunity (Figure 2). Indeed, ATR is induced in response to replication stress, single-stranded DNA and increased R-loops, and activates a kinase signaling cascade that involves CHK1 and WEE1 kinases that, in turn, leads to the activation of a checkpoint and the arrest of the cell cycle in order to give more time to the DNA repair mechanism to remove lesions [80]. In line with these data, the inhibition of ATR disrupts these functions of ATR, resulting in inappropriate mitotic entry and mitotic catastrophe. Moreover, the cytosolic DNA released may induce the cGAS-STING pathway and a type I IFN response. In addition, the inhibition of ATR plays an important role in augmenting tumor mutational burden (TMB) and neoantigen repertoire. Indeed, previous reports have studied the role of DDR inhibition as a means of increasing the TMB and the production of neoantigens[54] which may, in turn, increase the sensitivity to immune checkpoint blockade by elevated antigen presentation. Interestingly, samples harboring mutations in DNA damage signaling genes, such as ATR, showed increased neoantigen levels, thus enhancing the rationale for combination therapies using PD-1/PD-L1 blocking and ATR inhibitors. This is supported by preliminary data in a syngeneic mouse model of head and neck squamous cell carcinoma (HNSCC), where ATR inhibition by AZD6738 resulted in cGAS/STING pathway activation and induced tumor infiltration of cytotoxic T cells that eventually achieved tumor growth arrest and prolonged survival [81].

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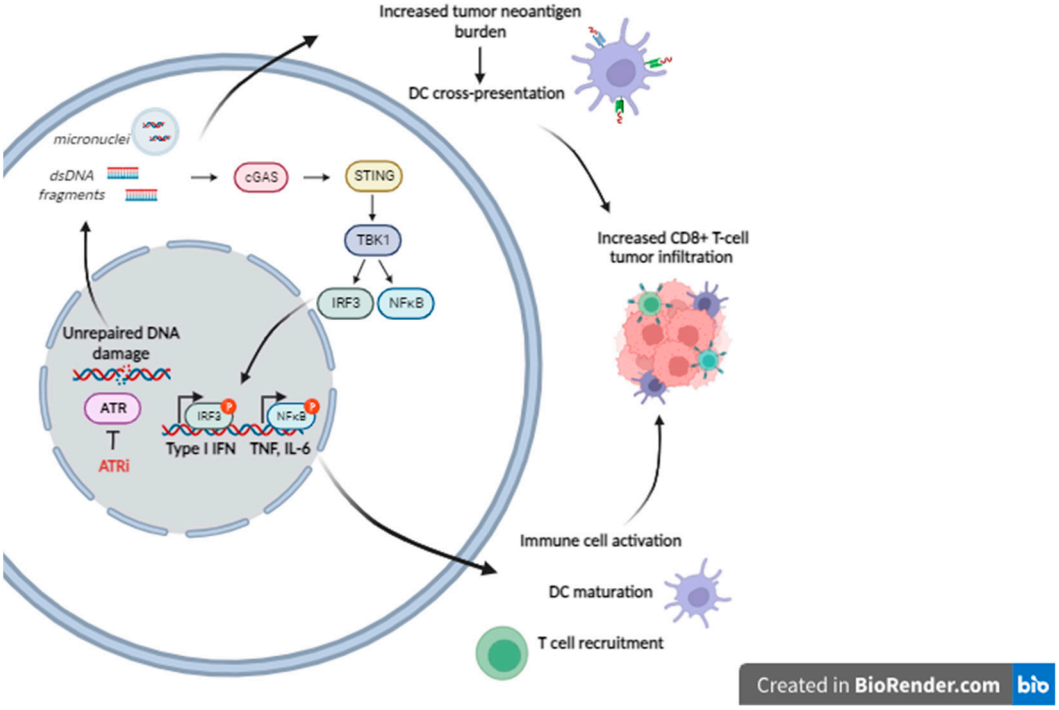

Figure 2. ATR pathway implication in the antitumor immunity (Figure was created with BioRender.com).

In line with these results, previous studies have shown that inhibitors of ATR potentiate immune stimulation following exposure to radiotherapy. Indeed, combined treatment with radiotherapy and ATR inhibitor induced type I/II IFN signaling and infiltration of CD8+ T-cells in a manner dependent on cGAS/STING [82–84]. While ATR inhibitors do not directly induce DNA damage, one may assume that the higher immunogenicity observed in irradiated tumors in the context of ATR inhibition is due to overriding of the G2/M cell cycle checkpoint. As a result, an increased proportion of cells with non-repaired DNA lesions enter mitosis, resulting in DNA fragmentation and micronuclei formation that trigger innate immune responses[54,85,86]. Furthermore, the inhibition of the ATR effector kinase CHK1 has been observed to abrogate the G2/M checkpoint after irradiation, resulting in the formation of micronuclei and type I IFN signaling in cancer cells [87]. Additionally, administering a combination of radiotherapy and the CHK1 inhibitor AZD7762 to mice led to increased CD8+ T-cell infiltration and a reduction in tumor volume compared to individual treatments with these agents [87]. Furthermore, ATR inhibition may enhance tumor immunogenicity by reducing the expression of programmed cell death 1 ligand 1 (PD-L1) in irradiated cancer cells [82,88,89]. Also, preclinical studies have indicated that cells that experience high replication stress may be selectively eliminated by ATR inhibition [90]. Indeed, the researchers observed that as the level of single-stranded DNA increased, a greater proportion of cells treated with ATR inhibitors underwent mitotic catastrophe. This finding suggests that the degree of cellular replication stress and the extent of ATR inhibitor-induced single-stranded DNA could potentially serve as predictive factors for the sensitivity to ATR inhibition.

Lately, it has been shown that except for the induction of immune response through canonical cGAS/STING signaling, the combination of irradiation therapy and ATR inhibition can also activate non-canonical STING pathway. As a result, a more robust immune activation was achieved leading to increased type I interferon-related gene expression and T cell infiltration turning the “cold” tumor microenvironment into “hot” and, thus, restoring sensitivity to PD-L1 immunotherapy [91].

Taken together, DDR-targeted therapies, including the inhibition of the ATR kinase, have the potential to enhance the antitumor immune response through various mechanisms, including the augmentation of antigenicity, the promotion of genomic instability in tumor cells, the activation of

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cytosolic immunity, as well as the modulation of different components that influence the interaction between tumor and immune cells [92].

4. The ATR Pathway as a Therapeutic Target

Chemotherapy resistance is a common challenge in cancer treatment, as the activation of a functional DDR can lead to cell cycle arrest and prolonged DNA repair [6]. Blocking the ATR pathway can reverse this state and enhance the cytotoxicity of genotoxic drugs by abrogating the cell cycle checkpoint [22,93].

4.1. ATM Functionality as a Predictive Biomarker of ATR-Blockade Response

A large-scale screening in *in vitro* and *in vivo* preclinical models of colorectal cancer has indicated that DDR inhibitors in general and ATR inhibitors specifically, are strong candidates for immunotherapy alternatives and has also suggested various response-predictive biomarkers for ATR inhibition, such as ATM protein loss [94]. Interestingly, recent data confirmed preclinical findings that the inhibition of ATR can lead to synthetic lethality in ATM-deficient malignancies. In fact, a study in ATM-deficient/p53-null cancer cells showed that ATR inhibition with VE-821 resulted in increased cytotoxicity after treatment with a variety of genotoxic agents, including platinum-based drugs, radiation, antimetabolites (gemcitabine), and topoisomerase inhibitors (camptothecin and etoposide). Importantly, VE-821 demonstrated a synergistic effect in tumor cells, but not in normal cells [95]. Another study has also presented synergy between cisplatin and the ATR inhibitor ceralasertib (AZD6738) in ATM-deficient NSCLC (Non-small cell lung cancer) cells [96]. Together, these data suggest that inhibition of ATR can lead to synthetic lethality in ATM-deficient/p53-null cancer cells that depends on alternative pathways to repair DSBs [97]. Strikingly, combining ceralasertib with cisplatin resulted in an enhanced cytotoxic effect even in ATM-proficient cell lines [98]. Of note, although previous studies have shown that ATM is implicated in preserving replication fork integrity and maintaining DNA replication [99], in ATM-proficient tumors, the ATR pathway also plays the most important role in replication stress management. For example, tumors expressing oncogenes (e.g. Ras, Myc) known to induce high replication stress [100], exhibited a strong response to ATR inhibition even without additional genotoxic treatment [101–104]. In fact, ATR appears to be crucial for the survival of those tumors, rendering ATR inhibition monotherapy a potential anticancer treatment [12,33].

4.2. ATR Inhibitors Synergy with Other Anti-Tumor Therapies

ATR inhibitor AZD6738 has been proved to synergize with chemotherapy agents like cisplatin in various solid tumor preclinical models, resulting in augmented antitumor activity [98,105]. Likewise, berzosertib (VE-822, VX-970, M6620) has been found to increase cell death both in cell lines and in patient-derived primary lung xenografts after cisplatin treatment, while also exhibiting a strong effect in tumor growth arrest in NSCLC models [106,107]. Other chemotherapy drugs may be also combined with ATR inhibition. Indeed, a recent study has shown synergism of the ATR inhibitor AZD6738 with the topoisomerase I inhibitor belotecan in ovarian cancer models [108], while combination with the antimetabolite gemcitabine in pancreatic models has been shown to instigate high replication stress leading to increased cell death and tumor shrinkage [109].

Recently, it has also been reported that AZD6738 can result in augmented cytotoxicity *in vitro* and tumor regression *in vivo*, when combined with Trastuzumabderuxtecan (T-DXd), an anti-HER2 antibody-topoisomerase I inhibitor hybrid [110], as well as improve the effectiveness of PI3K inhibitors, probably by DSBs-induced apoptosis as shown both *in vitro* and *in vivo* in preclinical models of breast cancer [111]

An intriguing idea has led to testing the combination of ATR inhibition with poly (ADP-ribose) polymerase (PARP) inhibitors. PARP is an essential protein for multiple DDR pathways and several inhibitors, such as olaparib, have been synthesized and are currently used in the clinical practice. Olaparib induces DNA damage and activates BRCA1/2-dependent homologous recombination.

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Thus, it is used to cause synthetic lethality in BRCA1/2-deficient cancers or with synchronous administration of HR-blocking agents [112]. Accumulating data show that the ATR inhibitor AZD6738 synergizes with olaparib to overcome resistance and achieve induced cytotoxicity in ATM-deficient tumors and/or tumors with impaired HR repair [113,114]. However, it has also been proved that AZD6738 combined with olaparib and radiotherapy can benefit therapeutically even HR-proficient tumors, through "PARP trapping" and the formation of PARP-DNA complexes that impede DNA replication [115].

A recent study underlined the significance of ATR inhibition scheduling during therapy [116]. The authors reported that to achieve increased cytotoxic T cells in the tumor-draining lymph node (DLN), radiation therapy or immune checkpoint inhibition must be followed by a short ATR inhibition, rather than a prolonged one.

4.3. ATR Inhibition in Clinical Studies

Several clinical trials of ATR inhibitors are ongoing and promise to radically alter the treatment landscape in a variety of solid tumors, either as monotherapies or in combinational treatments. As we anticipate the results of next phase trials in the upcoming years, we briefly present some of the most encouraging data from the clinic. One of the agents further along in clinical development is ceralasertib (AZD6738), with several phase 2 trials having completed recruitment and reporting clinical outcomes. Relevant clinical trials of ceralasertib in patients with solid tumors are listed in Table 1.

Table 1. Clinical trials of Ceralasertib (AZD6738) in patients with solid tumors.

NCT Number	Study Status	Conditions	Interventions	Primary Outcome	Phase	Enrollment	Completion Date
NCT03330847	Active, not recruiting	mTNBC	Ceralasertib + Olaparib	PFS	2	273	Sep-24
NCT03801369	Recruiting	mTNBC	Ceralasertib + Olaparib	ORR	2	132	Dec-27
NCT03740893	Recruiting	Operable TNBC	Ceralasertib	Biomarker	2	81	Dec-25
NCT03182634	Completed	mBC	Ceralasertib + Olaparib	ORR	2	70	Nov-23
NCT04090567	Recruiting	HER2-, BRCA+ mBC	Ceralasertib + Olaparib	ORR	2	60	Mar-25
NCT05582538	Recruiting	mTNBC	Ceralasertib followed by Durvalumab/nab-Paclitaxel	PFS	2	37	Nov-25
NCT05450692	Recruiting	mNSCLC	Ceralasertib + Durvalumab	OS	3	580	May-25
NCT03334617	Active, not recruiting	mNSCLC	Ceralasertib, Ceralasertib + Durvalumab	12-week ORR	2	531	Sep-24
NCT02664935	Active, not recruiting	mNSCLC	Ceralasertib + Durvalumab	ORR, PFS, 24-week DCR	2	423	Sep-23
NCT03833440	Recruiting	mNSCLC	Ceralasertib + Durvalumab	12-week DCR	2	120	Feb-24
NCT05941897	Recruiting	mNSCLC	Ceralasertib + Durvalumab	ORR	2	38	Jun-25

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NCT02937818	Active, not recruiting	ES-SCLC	Ceralasertib + Olaparib	ORR	2	72	Dec-23
NCT04361825	Active, not recruiting	ES-SCLC	Ceralasertib + Durvalumab	ORR	2	45	Dec-23
NCT04699838	Recruiting	ES-SCLC	Platinum-Etoposide-Durvalumab + maintenance Ceralasertib/Durvalumab	PFS	2	30	May-24
NCT03428607	Completed	ES-SCLC	Ceralasertib + Olaparib	ORR	2	26	Jan-21
NCT03579316	Recruiting	Ovarian Cancer	Ceralasertib + Olaparib	ORR	2	104	Dec-24
NCT04239014	Withdrawn	Ovarian Cancer	Ceralasertib + Olaparib	PFS	2	0	Jan-21
NCT04065269	Active, not recruiting	Gynaecological Cancers	Ceralasertib + Olaparib	ORR	2	168	Mar-23
NCT05061134	Active, not recruiting	Melanoma	Ceralasertib, Ceralasertib + Durvalumab	ORR	2	186	Apr-24
NCT03780608	Active, not recruiting	Melanoma, Gastric cancer	Ceralasertib + Durvalumab	ORR	2	61	Dec-23
NCT04298021	Active, not recruiting	Biliary Tract Cancer	Ceralasertib + Durvalumab, Ceralasertib + Olaparib	DCR	2	74	Dec-24
NCT04298008	Recruiting	Biliary Tract Cancer	Ceralasertib + Durvalumab	DCR	2	26	Dec-24
NCT04417062	Recruiting	Osteosarcoma	Ceralasertib + Olaparib	4-month EFS	2	63	Jun-25
NCT03787680	Active, not recruiting	Prostate Cancer	Ceralasertib + Olaparib	ORR	2	49	Jan-27
NCT03022409	Completed	HNSCC	Ceralasertib	Biomarker	1	21	Jan-21
NCT04704661	Recruiting	HER2+ GEJ/CRC	Ceralasertib + T-DXd	Toxicity	1	15	Mar-26
NCT02264678	Recruiting	Advanced Solid Tumors	Ceralasertib + Olaparib	Toxicity	1/2	466	Jul-26
NCT03682289	Recruiting	Advanced Solid Tumors	Ceralasertib, Ceralasertib + Olaparib	ORR	2	89	Jul-25

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NCT02223923	Active, not recruiting	Advanced Solid Tumors	Ceralasertib	MTD	1	87	Dec-23
NCT02576444	Terminated	Advanced Solid Tumors	Ceralasertib + Olaparib	ORR	2	67	Nov-19
NCT02630199	Completed	Advanced Solid Tumors	Ceralasertib + Paclitaxel	Toxicity, MTD	1	65	Apr-21
NCT03669601	Recruiting	Advanced Solid Tumors	Ceralasertib + Gemcitabine	DLT	1	55	Sep-24
NCT04564027	Active, not recruiting	Advanced Solid Tumors	Ceralasertib	ORR	2	54	Feb-24
NCT05514132	Active, not recruiting	Advanced Solid Tumors	Ceralasertib + Olaparib	DLT	1	14	Apr-25
NCT05469919	Active, not recruiting	Advanced Solid Tumors	Ceralasertib	DLT	1	12	Dec-24
NCT03878095	Suspended	IDH1/2mut Advanced Solid Tumors	Ceralasertib + Olaparib	ORR	2	50	Mar-24
NCT03330847	Active, not recruiting	mTNBC	Ceralasertib + Olaparib	PFS	2	273	Sep-24
NCT03801369	Recruiting	mTNBC	Ceralasertib + Olaparib	ORR	2	132	Dec-27
NCT03740893	Recruiting	Operable TNBC	Ceralasertib	Biomarker	2	81	Dec-25
NCT03182634	Completed	mBC	Ceralasertib + Olaparib	ORR	2	70	Nov-23
NCT04090567	Recruiting	HER2-, BRCA+ mBC	Ceralasertib + Olaparib	ORR	2	60	Mar-25
NCT05582538	Recruiting	mTNBC	Ceralasertib followed by Durvalumab/nab-Paclitaxel	PFS	2	37	Nov-25
NCT05450692	Recruiting	mNSCLC	Ceralasertib + Durvalumab	OS	3	580	May-25
NCT03334617	Active, not recruiting	mNSCLC	Ceralasertib, Ceralasertib + Durvalumab	12-week ORR	2	531	Sep-24

* Abbreviations: CRC, colorectal carcinoma; DCR, disease control rate; DLT, dose-limiting toxicity; EFS, event-free survival; ES-SCLC, extensive-stage small cell lung cancer; GEJ, gastroesophageal junction; HNSCC, head and neck squamous cell carcinoma; IDH1/2mut, isocitrate dehydrogenase 1/2-mutated; mBC, metastatic breast cancer; mNSCLC, metastatic non-small cell lung cancer; MTD, maximum tolerated dose; mTNBC, metastatic triple negative breast cancer; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; T-DXd, trastuzumabderuxtecan; TNBC, triple negative breast cancer; trAE, treatment-related adverse events.

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4.3.1. Breast Cancer

A phase I trial of ceralasertib and olaparib included patients with pretreated, HRR-wild type metastatic triple negative breast cancer (mTNBC) (n=25) or BRCA-mutated HER2-negative metastatic breast cancer (n=37). In the first cohort, no responses were observed, with a median progression-free survival (PFS) of 3.1 months (80% confidence interval [CI]: 2.0-3.9 months). In the BRCA-mutated cohort, the overall response rate (ORR) was 35%, with a median PFS of 7.7 months (80% CI: 5.8-11.4 months) [117]. The results of this study were further evaluated in VIOLETTE (NCT03330847), a randomized phase 2 trial evaluating the combination of ceralasertib and olaparib in comparison to olaparib monotherapy or the combination of olaparib and adavosertib, a WEE1 inhibitor, in patients with pretreated mTNBC. In this study (n=273 patients), the combination of ceralasertib and olaparib did not improve PFS over olaparib monotherapy (7.3 vs. 7.4 months, hazard ratio [HR]: 1.02, 90% CI: 0.63-1.66, p = 0.94). Interestingly, while response rates were similar between the combination therapy and olaparib monotherapy (50% vs. 44%), the ORR with the combination therapy was higher in patients without HRR gene mutations (15% vs. 4%, odds ratio: 4.45; 90% CI: 1.30-21.20, p = 0.04) [118]. In the plasmaMATCH trial, which included patients with pretreated mTNBC (n=70), the ORR was 17.1% (95% CI: 10.4%-25.5%), with a median PFS of 4.3 months. Responses were observed in patients without BRCA1/2 mutations that had functional HR-deficiency (HRD) by RAD51 foci [119]. This may account for the responses observed in patients without HRR gene mutations in the VIOLETTE study, and this subset of patients requires further evaluation in future clinical trials.

The combination of ceralasertib and olaparib, among other combinations, is being evaluated in mTNBC in another phase 2 trial that is actively recruiting patients (NCT03801369). Other than mTNBC, this combination is also being evaluated in HER2-negative, germline BRCA mutated advanced or metastatic breast cancer patients pre-treated with PARP inhibitors (NCT04090567). Trials evaluating other drug combinations include ATRiBRAVE (NCT05582538), an open-label phase 2 trial of mTNBC patients that have experienced disease progression after locoregional therapy that included chemotherapy and immunotherapy. In this trial, patients will receive a priming therapy by ceralasertib followed by the combination of paclitaxel and durvalumab, aiming to restore sensitivity to immunotherapy.

4.3.2. Lung Cancer

The HUDSON trial (NCT03334617) is an open-label, biomarker-directed trial for patients with metastatic NSCLC (mNSCLC) after progression on chemotherapy and immunotherapy. In this trial, the combination of durvalumab with ceralasertib demonstrated early signals of efficacy in patients with ATM alterations (n=18 patients, 6-month overall survival [OS]: 100%, 6-month PFS: 61.2%, ORR: 13.3%) and in unselected patients (n=20 patients, 6-month OS: 74.8%, 6-month PFS: 53.8%, ORR: 11.1%) [120]. As a result, a randomized phase 2 trial (NCT03833440) and a randomized phase 3 trial (NCT05450692) are ongoing, which compare the combination of durvalumab and ceralasertib in mNSCLC patients who have progressed on chemotherapy and immunotherapy with the standard of care in this indication (docetaxel) and may be practice-changing in this setting. In another open-label, biomarker-directed clinical trial, the National Lung Matrix Trial (NLMT), which included pretreated, KRAS-mutated or KRAS-wild type mNSCLC patients that had received prior immunotherapy, outcomes were numerically higher in patients with KRAS mutations (ORR: 13.8% vs. 4.8%, mPFS 5.95 vs. 3.9 months, mOS 30.9 vs 13.2 months).

In extensive-stage small cell lung cancer (ES-SCLC), a small phase 2 trial is ongoing in the first-line setting that evaluates the efficacy of maintenance therapy with ceralasertib plus durvalumab after 4 cycles of induction therapy with platinum-etoposide-based chemotherapy and durvalumab (NCT04699838). Results have been reported from an open-label phase 2 trial that included 21 patients with platinum-refractory ES-SCLC that received ceralacertib plus olaparib (NCT02937818), the ORR was 4.8%, with a 12-week disease control rate of 38.1%. Interestingly, despite the disappointing response rate, the median OS for patients receiving the combination therapy was 7.56 months, which is in line with approved therapies for this indication. Similar results were seen in SUKSES, a phase 2 umbrella trial that included patients with refractory ES-SCLC that received the combination therapy.

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In this study, the ORR was 3.8%, median PFS was 2.75 months (95% CI: 1.77–5.44 months), and median OS was 7.18 months (95% CI: 5.97-10.79 months) [121].

4.3.3. Gynaecological cancers

The combination of ceralasertib and olaparib has shown early clinical activity in patients with advanced, high-grade serous ovarian cancer (HGSOC) that have progressed after treatment with a PARP inhibitor. In OLAPCO, a basket trial of olaparib combinations in heavily pre-treated patients, responses or prolonged disease stabilization with ceralasertib plus olaparib were observed in patients with ATM mutations and patients who had received prior treatment with a PARP inhibitor [122]. In CAPRI, an open-label phase 2 trial of the same combination, promising clinical activity was seen in patients with HGSOC enrolled immediately after progression on a PARP inhibitor (n=13), with an ORR of 46% [123]. No responses were observed in patients with platinum-resistant disease (mPFS was 4.2 months overall (90% CI: 3.5–8.2 months) [124].

The combination of ceralasertib and olaparib has also been evaluated in rare gynaecological cancers in the open label phase 2 ATARI trial (n=78). In this study, outcomes were similar in patients with clear cell histology with or without AT-rich interactive domain-containing protein 1A (ARID1A) loss (ORR: 14% vs. 14%, median PFS: 3.6 vs. 3.5 months), while the clinical activity of the combination therapy may be higher in patients with non-clear cell histologies (ORR: 24%, median PFS: 5.6 months) [125].

4.3.4. Other Solid Tumors

In PATRIOT, the first-in-human trial of ceralasertib in patients with advanced solid tumors, a subset of patients with ARID1A-deficiency derived greater benefit from treatment [126]. Furthermore, an open-label phase 2 trial in patients with advanced solid tumors also included a cohort of ARID1A-deficient tumors. In this cohort, durable complete responses were achieved in 2/10 patients, for an ORR of 20% [127]. ARID1A-deficiency represents a promising target for ATR inhibition that warrants future evaluation.

The combination of ceralasertib plus durvalumab has been evaluated in various solid tumors. In an open label phase 2 trial of the combination therapy in patients with pretreated advanced gastric cancer (n=31), the ORR was 22.6% (95% CI: 9.6%-41.1%), the median PFS 3.0 months (95% CI: 2.1-3.9 months), and the median OS 6.7 months (95% CI: 3.8-9.6 months). The benefit was limited to patients with ATM deficiency or a mutational signature attributable to HRD (median PFS: 5.60 vs 1.65 months, HR: 0.13, 95% CI: 0.045-0.39, $p < 0.001$) [128]. This study also included a cohort of patients with melanoma who had progressed on treatment with a PD-1 inhibitor (n=30). In this cohort, the ORR was 31.0%, the median PFS was 7.1 months (95% CI: 3.6-10.6 months), and the median OS was 14.2 months (95% CI: 9.3-19.1 months) [129].

In addition to PARP inhibitors and immune checkpoint inhibitors, ceralasertib is also being evaluated in combination with chemotherapy. In a phase I trial of ceralasertib plus weekly paclitaxel in patients with advanced solid tumors, the combination was safe and showed preliminary signs of efficacy, with a ORR of 25.4%, including one complete response in a patient with melanoma [130]. A phase I trial of ceralasertib plus gemcitabine in patients with advanced solid tumors is ongoing (NCT03669601).

5. Conclusions

Taken together, data present in this report demonstrate that the inhibition of the ATR kinase can modify the DNA damage response network and the immune system. These results potentially offer a new approach to improving the effectiveness of anticancer therapy using combinations of an ATR inhibitor with genotoxic drugs and/or immunomodulators.

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