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Review

Review

# Triple kill: DDR inhibitors, radiotherapy and immunotherapy leave cancer cells with no escape

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**Abstract**

Radiotherapy (RT) has been widely used in the clinical treatment of cancers, but radiotherapy resistance (RR) leads to RT failure, tumor recurrence and metastasis. Many studies have been performed on the potential mechanisms behind RR, and a strong link has been found between RR and DNA damage. RT-induced DNA damage triggers a protective mechanism called the DNA damage response (DDR). DDR consists of several aspects, including the detection of DNA damage and induction of cell cycle checkpoint, DNA repair, and eventual induction of cell death. A large number of studies have shown that DDR inhibition leads to significantly enhanced sensitivity of cancer cells to RT. DDR may be an effective target for radio- and chemo-sensitization during cancer treatment. Therefore, many inhibitors of important enzymes involved in the DDR have been developed, such as PARP inhibitors, DNA-PK inhibitors, and ATM/ATR inhibitors. In addition, DNA damage also triggers the cGAS-STING signaling pathway and the ATM/ATR (CHK)/STAT pathway to induce immune infiltration and T-cell activation. This review discusses the effects of DDR pathway dysregulation on the tumor response to RT and the strategies for targeting these pathways to increase tumor susceptibility to RT. Finally, the potential for the combination treatment of radiation, DDR inhibition, and immunotherapy is described.

**Key words** radiotherapy, radioresistance, DNA damage response, immunotherapy, DDR inhibitor

## Introduction

The rising incidence of cancer has a negative impact on life quality and life expectancy worldwide. The Global Burden of Diseases, Injuries, and Risk Factors Study 2019 (GBD 2019) reported 23.6 million cancer cases and 10 million deaths in 2019 across 204 countries and territories. Cancers are the second leading cause of death after cardiovascular diseases, with an increasing global burden [1]. In clinical practice, cancer treatments include surgery, chemotherapy, radiotherapy (RT), targeted therapy and immunotherapy. RT is widely used before surgery to reduce the size of tumors and is used after surgery to prevent recurrence, as it induces cancer cell senescence and apoptosis and regulates the tumor microenvironment (TME) [2]. RT can be used as a curative treatment in localized cancers and may be used as a palliative method to relieve pain in metastatic cancers. However, RT patients inevitably develop radiotherapy resistance (RR), resulting in cancer recurrence, poor prognosis, and additional treatment burden [3]. Over the years, numerous studies have been performed on the hidden mechanisms of RR. Some studies have suggested that cancer stem cells are radiation-resistant and can withstand radiation-induced damage [4]. Furthermore, the DNA damage signaling pathway is considered to be closely related to RR, as it is involved in cellular responses to radiation exposure. X-ray and γ-ray ionizing radiation (IR) are primarily used in RT to treat cancers. IR disassembles electrons from atoms. Consequently, the interactions between free radicals and molecular oxygen produce reactive oxygen species (ROS), which results in DNA damage and cell death [5]. Cells treated with radioactive rays undergo chemotaxis (attracted by calreticulin) and phagocytosis (enhanced by interferon from cGAS-STING signaling), followed by antigen presentation and later T-cell activation. Distorted DNA generated by irradiation causes replication stress, triggering the p53-mediated degradation process, or apoptosis, to prevent tumor expansion and then trigger the DNA damage response (DDR) [6]. DDR is a protective

mechanism that recognizes DNA lesions and activates DNA repair mechanisms. RT kills cancer cells by generating DNA damage. Studies have reported that cancer cells are more vulnerable than normal cells when exposed to the same dose of radiation due to impaired DDR [7]. Cancer cells with high proliferative rates and genome instability are more likely to directly or indirectly receive DNA damage, leading to cell death after absorbing radiation energy.

In the past, it was widely assumed that genetic defects in DNA damage signaling and repair pathways led to cancer-prone phenotypes. For example, mismatch repair (MMR) gene (*e.g.*, MSH2 and MSH6) mutations contribute to hereditary nonpolyposis colon cancer (Lynch syndrome). MMR defects (microsatellite instability) lead to the absence of TGF-β (epithelial cell tumor suppressor factor), promoting the survival of colon cancer cells [8]. Meanwhile, patients with xeroderma pigmentosa (XP) who are unable to repair UV-induced DNA damage by nucleotide excision repair (NER) due to mutations in the XP genes are highly susceptible to skin cancer [9]. Moreover, base excision repair (BER) defects have been associated with cancers. For example, mutation of *MUTYH*, which is involved in DNA glycosylation in BER, has been identified in multiple colorectal adenomas and carcinomas [10]. In addition, it has been revealed that patients with germline mutations in BRCA1/2 and ATM have an increased risk of aggressive prostate cancer [11]. However, these mutations also make cancer cells sensitive to therapeutic drugs due to accumulated DNA damage. For example, BRCA1-deficient tumors are sensitive to IR, especially when exposed to PARP inhibitors, and ATM-deficient tumors are hypersensitive to IR and radiomimetic drugs [12,13]. Therefore, it is speculated that DDR inhibition may enhance the sensitivity and efficiency of RT. Interestingly, DNA damage has been well documented in facilitating the protective immune infiltration and simultaneously upregulating immune suppressor PD-L1. We reasoned that DDR inhibition combined with RT and immune therapy may have the potential to achieve a favorable prognosis in cancer patients.

Here, we review the effects of DDR pathway dysregulation on the tumor response to RT and the strategies for targeting these pathways to increase tumor susceptibility to RT, and discuss the combination treatment of radiation, DDR inhibition and immunotherapy.

## DNA Damage Response Signaling Pathway and DNA Repair

IR-induced DNA damage includes base modifications such as 8-oxo-7,8-dihydroguanine (8-OxoG), single-strand breaks (SSBs) and double-strand breaks (DSBs). DSBs, accounting for a small proportion, are the most cytotoxic DNA damage that causes genetic mutations associated with tumorigenesis [14]. DNA DSBs are the primary damage induced by IR; complicated DNA DSBs (called clustered DNA damage) result in genomic instability. Once DNA damage occurs, it initiates DDR, which is a conservative mechanism in cells to resist DNA damage induced by external and internal factors [15]. DDR regulates many physiological processes, including apoptosis, terminal differentiation through senescence, activation of enhanced immune surveillance, and DNA repair itself [16]. DDR is mainly mediated by the phosphatidylinositol 3-kinase (PI3K)-like protein kinase (PIKK) family, including ataxia telangiectasia mutated kinase (ATM), ATM-Rad3 related kinase (ATR) and DNA-dependent protein kinase catalytic subunit (DNA-PKcs), and members of the poly(ADP-ribose) polymerase (PARP) family [17]. IR-induced DNA DSBs can activate the phosphorylation of all of the kinases mentioned above and trigger the DDR signaling pathway

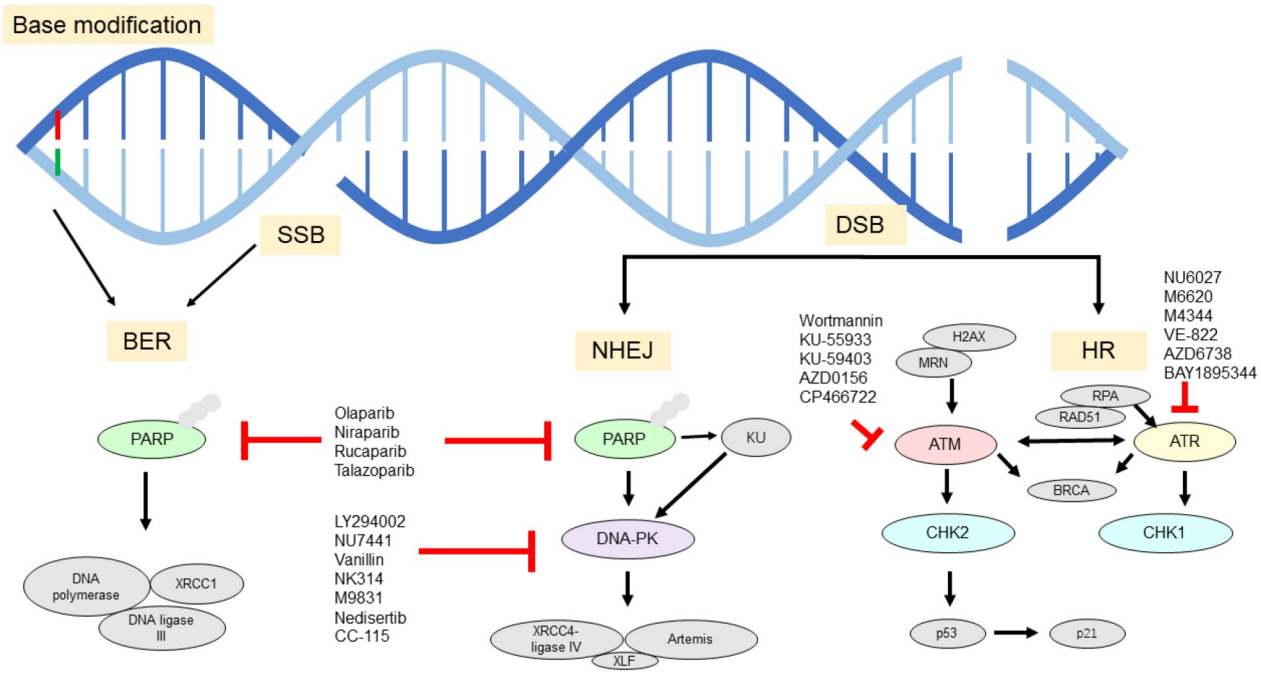


**Figure 1. Repair of DNA damage induced by irradiation** DNA damage induced by radiation can be repaired via several DNA repair pathways. Factors involved in these pathways can be targeted to improve the efficacy of RT. SSB, DNA single-strand break; DSB, DNA double-strand break; BER, base excision repair; NHEJ, non-homologous end joining; HR, homologous recombination; PARP, poly(ADP-ribose) polymerase; ATM, ataxia telangiectasia mutated kinase; ATR, ATM-Rad3 related kinase; DNA-PK, DNA-dependent protein kinase; CHK1, checkpoint kinase 1; and CHK2, checkpoint kinase 2.

[17] (Figure 1). DDR determines cell fate based on the extent of damage. If the damage is fetal, the cells will enter apoptosis and even necrosis; if the damage is repairable, the cells will initiate the DNA repair process and survive. However, DNA repair is not a perfect mechanism. Unsuccessful repair leads to cell death, and incorrect repair results in the accumulation of mutations that in turn make cells resistant to IR.

IR-induced base modifications and DNA SSBs both require BER which repairs damage based on the complementary strand. BER requires the involvement of X-ray cross complementing protein 1 (XRCC1) which helps to recruit other proteins that participate in BER, such as AP endonuclease 1 (APE1), DNA polymerase β (Pol β), and ligase III [18]. Meanwhile, PARP1 also participates in BER.

There are two main mechanisms to repair DSB: homologous recombination (HR) and non-homologous end-joining (NHEJ). In general, HR is a slow, template-dependent and accurate pathway that is restricted to the S and G2 phases of the cell cycle because it requires a homologous sequence located on the sister chromatid. In contrast, NHEJ is a fast, template-independent, error-prone and mutagenic pathway that occurs in all phases of the cell cycle [3]. NHEJ thus randomly erases segments close to the damaged site, causing a large loss of DNA fragments, point mutations, inversions, insertions, or translocations. The change in DNA sequence may induce cell apoptosis, which may be the mechanism for IR-induced cancer cell death.

Several key factors are involved in the DNA damage repair pathway described above, including PARP, DNA-PK, ATM and ATR. ATM initiates the DDR signaling pathway to repair DSBs induced by IR. However, ATR only responds to single-stranded DNA (ssDNA) regions that are RPA-coated and located at stalled replication forks and DSBs [16]. Meanwhile, DNA-PK is also activated by DSBs and participates in NHEJ to promote the ligation of DSBs [19]. The PARP family has 17 members, but only PARP1, PARP2 and PARP3 participate in DDR, where they recognize DNA damage, block local transcription, remodel chromatin, repair dmage or induce cell death [20].

In HR, a homologous sequence is needed on the sister chromatid as a template to replicate. In the ATM pathway, CtIP triggers the nucleolytic process of the DNA ends at the DSB. The MRN complex, which incorporates RAD50, NBS1 and MRE11, binds to DNA ends and recruits nucleases to remove damaged bases [21]. Meanwhile, the MRN complex activates ATM, which phosphorylates CHK2, BRCA1, and p53 that are involved in cell cycle checkpoints [16]. The stabilization of p53 can increase the expression of p21, which results in G1 arrest, while CHK2 and BRCA1 participate in S and G2/M checkpoints [22]. This prevents cells from entering mitosis until the damage has been repaired. In addition, BRCA1 can also interact with RAD51 indirectly and its paralogue XRCC3 whose function is to recognize a homologous sequence on the sister chromatid [21]. In the pathway that ATR participates in, the RPA complex keeps the undamaged ssDNA unwound, following the recruitment of RAD51. Afterwards, RAD51 nucleofilaments facilitate the invasion of the damaged DNA strand into the duplex of the sister chromatid to match the DNA strand [19]. In NHEJ, Ku proteins bind to DSBs, initiating the recruitment and activation of the catalytic enzyme DNA-PKcs, which phosphorylates many end-processing enzymes, polymerases and DNA ligase IV [7]. Finally, the XRCC4-ligase IV complex ligates the DNA ends after nucleolin changes the chromatin structure [23]. DNA damage and repair pathways are closely linked to each other, not only because a single DNA damage may require multiple repair mechanisms but also because a single repair mechanism plays a role in multiple types of DNA damage [24].

In summary, RT causes DNA damage, and cells initiate DDR as a protective mechanism to resist this damage. DDR determines whether DNA damage is repaired or apoptosis is induced to preserve the integrity of the genome. Thus, DDR greatly affects the efficacy of RT. In clinical practice, a large number of studies have found that the inhibition of DNA damage repair pathways such as HR and NHEJ can enhance RT sensitivity. For example, PI3K/Akt/mTOR inhibitors (BEZ235 and PI103) can enhance RT sensitivity in prostate cancer cells by repressing both HR and NHEJ pathways and increasing DSBs [25]. In addition, many studies have demonstrated that the inhibition or absence of PIKKs leads to enhanced sensitivity to RT. For example, a study proved that mice with diffuse intrinsic pontine glioma showed enhanced sensitivity to RT after the deletion of ATM by Cre/loxP [12]. To date, a number of DDR inhibitors have been tested in clinical trials or clinical treatment.

## DDR Inhibitors as Radiation Sensitizers

Although accumulated DSBs and SSBs induced by RT contribute to cell death, DNA repair is allowed to restore broken DNA and keep cells alive. Cancer cells with abundant endogenous DNA damage levels rely on DDR for tumor cell viability. Unrepaired DNA damage has been demonstrated to be associated with higher radiosensitivity. For example, combination treatment with cis-platinum and 5-FU reversed radiation resistance in patients with gastric and esophageal carcinoma. However, cancer cells have developed compensatory ways to invade cell death caused by catastrophic DNA breaks through reinforced DNA repair, which provides a target for cancer therapy [26]. For example, breast cancer with BRCA (DDR gene) mutation is considered to be a highly aggressive tumor with a high rate of relapse [27].

Many studies have demonstrated that inactivation of the *Ku70* or *Ku80* gene can enhance sensitivity to IR [28]. Furthermore, it is hypothesized that carriers of BRCA1/2 mutations might have increased sensitivity to IR. However, a retrospective cohort study indicated no evidence of increased radiosensitivity of mutant BRCA1/2 germline breast tissue in comparison with the control group [29]. It can be concluded that the relationship between DNA repair pathways and RT sensitivity is complicated. Based on these studies, many DDR inhibitors have been developed, and numerous clinical trials have demonstrated their efficacy (Table 1).

### PARP inhibitors

Among the DDR inhibitors that have been developed, PARP inhibitors are currently the most widely used ones. PARP plays a critical role in SSB repair. Inhibition of PARP results in unrepaired ssDNA breaks and finally contributes to DSB. PARP inhibitors allow synthetic lethality of BRCA mutant cancer cells (Figure 2). The BRCA2 protein and RAD51 initiate DSB repair through HR. Accumulated DSB caused by BRCA2 mutation in PARP inhibitor therapy increases radiosensitivity in tumor cells. Nonetheless, a few patients develop PARP inhibitor resistance, possibly mediated by silencing glycogen synthase kinase 3B (GSK3b) to increase PD-L1 level [39]. PARP inhibitors (olaparib, niraparib, rucaparib, and talazoparib) have been shown to prolong overall survival in BRCA mutant cancers in clinical practice [40].

The defect in HR caused by BRCA1 and BRCA2 mutations can

**Table 1. Different DDR inhibitors and their clinical trials**

| Target | Inhibitor | Mechanism | Clinical trials/clinical applications | Ref |
|---|---|---|---|---|
| PARP | Olaparib | Inhibition of BER results in selective cytotoxicity to cells carrying HR defects and enhanced sensitivity to radiation. | Olaparib is FDA approved to treat patients with advanced, deleterious germline BRCA-mutated ovarian cancer who have been treated with three or more prior lines of chemotherapy. | [30] |
| | | | PARP inhibitors were effective for treating breast cancer patients harboring germline BRCA1/2 mutations. Olaparib monotherapy showed a high response rate when treating naive, large triple-negative breast cancer with germline or somatic HR deficiency (NCT02624973). | [31,32] |
| | Niraparib | Targeting cells with impaired DNA repair due to HR deficiency, leading to cell death through synthetic lethality. | The first FDA-approved PARP inhibitor for the maintenance treatment of patients with recurrent ovarian and fallopian tube cancer who are in complete or partial response to platinum-based chemotherapy. | [33] |
| DNA-PKs | Nedisertib (M3814) | Inhibited DNA-PK kinase activity results in suppression of DNA DSB repair and enhanced sensitivity to radiation in solid cancer cells. | A phase a/b study tested the combination of nedisertib with cisplatin and radiation for the treatment of locally advanced squamous cell carcinoma of the head and neck (NCT02516813). | [34] |
| | | | A phase b/2 study tested the combination of nedisertib with capecitabine and radiation in locally advanced rectal cancer (NCT03770689). | [34] |
| | CC-115 | Inhibition of mTORC1, mTORC2 and DNA-PK kinase activity induces cell death and suppresses cell proliferation. | A phase a/b study tested the safety, pharmacokinetics, pharmacodynamic profile, and preliminary efficacy of CC-115 in advanced solid or hematologic malignancies and found that oral CC-115 10 mg BID was a promising novel anticancer treatment (NCT01353625). | [35] |
| ATM | KU59403 | Increased the cytotoxicity of topoisomerase I and II poisons (camptothecin, etoposide and doxorubicin) and enhanced the sensitivity to chemo- and radiotherapy by ATM inhibition. | None. | [36] |
| ATR | M6620 (VX-970) | A potent ATP-competitive ATR inhibitor with high selectivity which lethally sensitizes tumors to chemotherapy, especially with ATM pathway deficiency. | A phase dose-escalation trial tested the safety, tolerability, MTD, and antitumor activity of M6620 monotherapy combined with carboplatin in patients with advanced solid tumors. | [37] |
| | Ceralasertib (AZD6738) | A potent oral selective inhibitor of ATR which has anti-tumor effects in patients with solid and hematologic malignancies. | A phasetrial tested the combination use of oral ceralasertib with carboplatin chemotherapy in patients with advanced solid tumors and antitumor activity was observed. It suggested that the recommended phase II dose for ceralasertib plus carboplatin was ceralasertib 40 mg once daily on days 1–2 administered with carboplatin AUC5 every 3 weeks (NCT02264678). | [38] |

increase RT sensitivity in tumor cells and promote apoptosis, achieving synthetic lethality. Patients with pancreatic ductal adenocarcinoma (PDAC) with BRCA (HR gene) mutation have demonstrated clinical benefits through platinum therapy and RT. Considering the flawed HR pathway, DNA breaks caused by platinum promote NHEJ, leading to cancer cell death [41].

Furthermore, the PARP inhibitor olaparib has been described to exert an unfavorable effect on HR. PARP inhibitors (PARPis) interfere with SSB repair and cause DSB, resulting in cell death. Positive outcomes have been demonstrated in PDAC patients treated with PARPis and RT combination therapy [42]. However, cancer cells develop HR restoration, leading to PARPi resistance and recurrence [43]. Resensitization to PARPi has also been investigated clinically. A DNPH1 (target of overexpressed C-myC protein) inhibitor that prevents endogenous DNA damage from elimination has the potential to resensitize resistant cells and improve prognosis [44]. Furthermore, the therapeutic effect of PARPi has been demonstrated to be expanded 250-fold in cancer with both HR and ALC1 (PAR-dependent nucleosome sliding enzyme) deficiency [45]. The PARP inhibitors play a critical role in HR-deficient cancer treatment.

## DNA-PK inhibitors

Besides PARP inhibitors, other antitumour agents that target the main DDR components are still under investigation and are limited to early phase clinical trials. As mentioned above, the Ku70/Ku80 complex binds to DSB and activates the catalytic enzyme DNA-PKcs. DNA-PKcs forms a heterotrimeric complex with Ku, while its serine/threonine protein kinase activity is activated. The formed

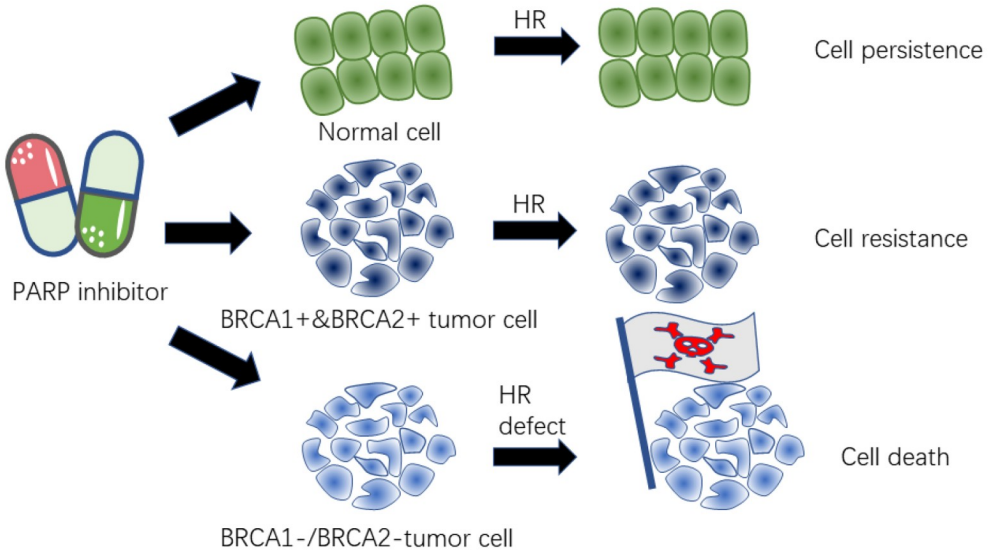


**Figure 2. Synthetic lethality of PARP inhibitors** PARP inhibitors have no adverse effect on normal cells, while BRCA mutant cancer cells are unable to repair SSB due to PARP inhibitors and defective HR mechanisms. Accumulated DNA breaks contribute to cancer cell death. Double failure of SSB and DSB caused by PARP inhibitors and HR gene mutation, respectively, results in catastrophic DNA breaks and finally eliminates cancer cells. PARP, poly(ADP-ribose) polymerase; HR, homologous recombination.

DNA-PK complex plays an important role in NHEJ by recognizing IR-induced DSBs, recruiting proteins to the DNA ends and ligating the broken ends. It utilizes proteins such as nucleases (Artemis) and polymerases (Pol X family) to process the DNA ends and recruits the ligase IV/XRCC4/XLF complex to ligate the DNA DSB [17].

DNA-PKcs is a member of the PI3K–mTOR enzyme family and is one of the best mediators of the IR-induced DDR and a good target in cancer treatment. As NHEJ is the primary mechanism of repair in traditional (nonheavy ion) RT, the specific targeting of NHEJ makes DNA-PK inhibitors particularly suitable for use in combination with RT [46]. The first identified DNA-PK inhibitor is wortmannin, but due to its lack of specificity and high cytotoxicity, it is rarely used in clinical practice. Since then, an increasing number of DNA-PK inhibitors have been developed, including LY294002, NU7026, NU7441, IC86621, IC87102, IC87361, OK-1035, SU11752, vanillin, NK314, IC486241, M9831, nedisertib, CC-115 and biotin-labelled peptide 3 [3,6,26,46]. Nedisertib in combination with RT has been tested in some advanced solid tumors and has shown promising efficacy [46]. These inhibitors can enhance radiosensitization and suppress the repair of IR-induced DSBs.

### ATM/ATR inhibitors

ATM and ATR have similar domain organizations, share certain substrates and have some overlapping functions. ATM is activated and recruited to the DSB site by MRN complexes that act as DNA damage sensors, while ATR is activated and recruited to the DSB site with its stable binding partner ATRIP (ATR-interacting protein). After irradiation, cells undergo DDR, while ATM phosphorylates hundreds of substrates. In this cascade, ATM activates CHK2 kinase, inducing apoptosis and cell cycle arrest.

Because ATM and ATR play predominant roles in activating the responses to both SSBs and DSBs, mediating cell cycle arrest and initiating DDR pathways, they are potential targets for cancer therapy. Since ATM-deficient cells are hypersensitive to radiation, ATM and its major downstream factor checkpoint kinase 2 (CHK2) are considered to be promising targets for radiosensitization. Currently, caffeine, wortmannin, KU-55933, KU-60019, KU-59403, AZD0156 and CP466722 have been shown to be effective ATM inhibitors. However, no clinical trials have been conducted thus far [6,17]. Schisandrin B, NU6027, NVP-BEZ235, M6620, M4344, VE-821, VE-822, AZ20, AZD6738 and BAY1895344 are ATR inhibitors that have been studied [3,26,46]. M6620 monotherapy is well tolerated with no dose-limiting toxicity (DLT) and could be used in combination with other chemotherapies, including topotecan, carboplatin, gemcitabine and cisplatin [37]. However, the combination use of M6620 and other chemotherapies resulted in increased bone marrow toxicity, and the maximum tolerated dose (MTD) was reduced. It has been shown that the combined use of DDR inhibitor chemotherapies causes bone marrow suppression and has no more benefit than monotherapy [46].

These ATM and ATR inhibitors were identified on the basis of extensive preclinical studies and literature. However, the possibility of increasing normal tissue toxicity remains an important issue, and their ability to selectively target tumor tissues requires further research [3].

## The Relationship between DDR and Immune Response in Clinical Practice

DDR gene distortion induces unrepaired DSBs, which interferes with immune responses from different pathways (Figure 3). DNA damage initiates immune signaling, and some classical factors involved in DSB repair have also been shown to modulate innate immunity [47]. The cGAS-STING signaling pathway is a good example. After DNA breaks, the interferon-stimulating gene (ISG) *STING* is stimulated by cGAMP produced from cyclic guanine adenosine monophosphate synthase (cGAS). Accumulated cGAS in the micronucleus mobilizes the downstream factor STING to activate STAT1 [2]. Therefore, STAT1 phosphoprotein triggered by DDR can be a marker of radiation-induced inflammation, since STING activation and interferon regulatory factor 3 (IRF3)

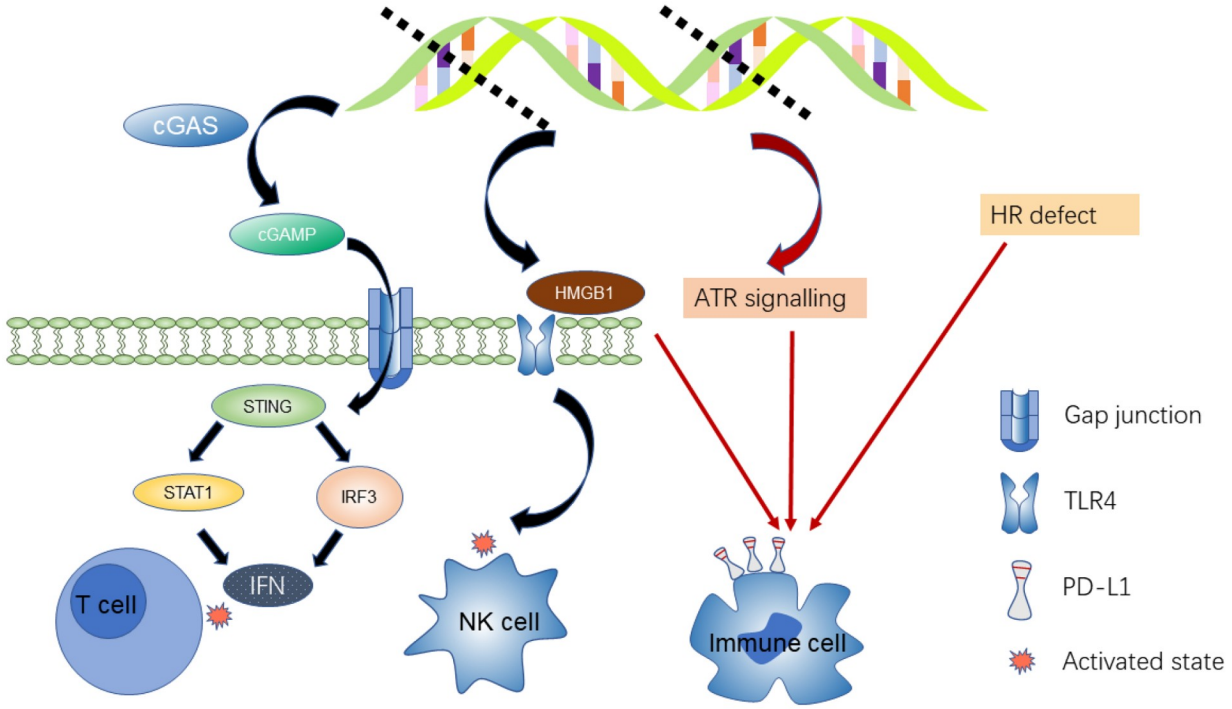


**Figure 3. Dynamic balance of immune response after DNA breaks** Broken DNA exerts both immunocompetent and immunosuppressive effects to manage TME. On the one hand, DNA breaks not only induce cGAS/STING/STAT1 (and IRF3)/IFN signaling to activate T cells but also release HMGB1 to trigger NK cells through TLR4, resulting in an immune-activated environment. On the other hand, the immunosuppressor PD-L1 is upregulated following DNA breaks, which is associated with HMGB1 and ATR signaling. cGAS, cyclic guanine adenosine monophosphate synthase; IRF3, interferon regulatory factor 3; IFN, interferon; HMGB1, high mobility group BOX-1; TLR4, toll-like receptor 4; ATR, ATM-Rad3 related kinase.

phosphorylation generate an immunocompetent TME that is infiltrated by interferon 1 (IFN1). Meanwhile, cGAMP released from tumor cells is transported to NK cells, tumor-associated dendritic cells and macrophages through gap junctions to develop protective antitumour surroundings with IFN1. Additionally, IFN1 has been demonstrated to be an indispensable factor for $CD8^+$ T cells to remove cancer cells. Mice with *STING* knockdown developed radiation resistance, implying that cGAS-STING may be a suitable marker for prognosis prediction [48]. As STING-mediated immune response is through IFN-dependent T cells, STING agonists are clinically used to prevent radiation resistance by intensifying the genome instability of tumor cells. For example, ADU-S100, DMXAA and di-ABZI have been described to facilitate the protective immune response to combat cancer [49]. Meanwhile, radiation sensitization activated by cGAS-STING and IFN-STAT1 has been demonstrated to be caused by mafosfamid (one of the metabolites of cyclophosphamide) [50]. Activation of STING has been reported not only in chemotherapy but also in RT. The abscopal effect is interpreted as the reversion of metastatic cancer outside the local radiation range and may be involved in immune activation by STING. Furthermore, delayed distal tumor degradation has been described in STING-deficient mice undergoing combination treatment with radiation and immunotherapy. The relationship between the abscopal effect and DDR is partly mediated by cGAS-STING sensing [2]. Although the activation of immune response by STING can prove the positive effects of radiotherapy and chemotherapy on tumors to a certain extent, the STING signaling pathway is not fully understood. The neoantigen hypothesis identifies that the neoantigens transcribed from an unstable genome may be used to identify tumor cells. By getting rid of central tolerance that is developed embryonically to prevent the immune system from attacking autoantigens, these neoantigens considerably enhance T-cell reactivity [51]. Activated T cells induced by neoantigens after irradiation may contribute to the abscopal effect of RT [52]. Cancer vaccines were designed to stimulate the immune system against cancer by using tumor-associated antigens with poor prognosis. Neoantigens are being tested in preclinical studies due to their high immunogenicity [53]. Furthermore, ATM/ATR(CHK)/STATs provide another signaling pathway for T-cell activation [54]. Active STATs increase IFNGR and IFNAR expressions, and produce IFN to facilitate resistance in tumors. In addition, high mobility group box-1 (HMGB1) released from irradiated tumor cells can facilitate immunostimulatory activity through binding to toll-like receptor 4 (TLR4) [39]. Another STING-regulated molecule, MRE11, is also involved in activating the immune response. MRE11 and RAD50 are constituents of the MRN complex, which recognizes DSBs and initiates HR repair through ATM phosphorylation. MRE11 can identify cytoplasmic dsDNA and activate downstream STING and IRF3 directly [55]. Meanwhile, Bhattacharya *et al.* [56] found that loss of RAD51 results in the accumulation of DNA fragments and triggers STING-mediated immune signaling.

Despite the immune augmentation caused by DNA damage, the immune suppressor PD-L1 is described to be induced by DSBs. For instance, HR defects (BRCA mutation and KU70/80 absence) are related to elevated PD-L1 through JAK/STATs/IRF signaling. Meanwhile, ATR signaling has been described to be associated with PD-L1 upregulation, resulting in a weakened immune response. Therefore, ATR blockade exerts an immunostimulatory

effect [57]. Moreover, HMGB1 increases the expression of PD-L1 in neighboring human melanoma cells [39,49]. Considering that PD-L1 level is increased in DDR defects, combination therapy with anti-PD1 shows promising preliminary outcomes. For example, PARPi combined with anti-PD-L1 therapy has been described to increase the radiosensitivity of cancer cells. In mouse skin cancer cells, Ahn *et al.* [58] found that sustaining STING activation favored chronic inflammatory infiltration and mediated immune resistance and cancer metastasis. Nevertheless, the protective immune response generated by STING outweighs its suppressive effect via an unknown mechanism [49]. While cumulative DNA breaks in the cytoplasm (mostly accumulated in micronuclei) induce immune infiltration, cells initiate an offset mechanism to avoid a long-term chronic inflammatory response. TREX1 is a DNA exonuclease involved in cytoplasmic DNA degradation and is partially responsible for RT resistance, since it decreases the downstream immunologic signaling of damaged DNA [59]. Furthermore, TREX1 also inhibits the antigen presentation process between tumor antigens and DCs. The survival of mice was improved when treated with TREX1 inhibition with radiation [6].

In general, DNA breaks dynamically regulate the tumor microenvironment and play a critical role in both immune promotion and immunosuppression. Taking advantage of the regulatory effects of DNA breaks caused by RT on immunity lends significant therapeutic promise to cancer treatment.

## Conclusion

In the past, it was widely accepted that defects in the DNA repair pathway could lead to tumorigenesis. Recently, many studies have revealed that by inhibiting the DNA repair pathway, cancer cells become more sensitive to irradiation. Currently, a large number of DDR inhibitors have been developed, and some are used in clinical practice. DDR disturbance combined with RT demonstrates favorable cancer clearance. However, further research is required to enable its personalized and targeted clinical application. In addition, DNA damage triggers various immune pathways, inducing immune infiltration and activation of immune factors. However, the activation of some immune factors may lead to immune resistance and immunosuppression. In this review, we suggest that the combination of RT, DDR inhibition, and immunotherapy in clinical practice can greatly improve the prognosis of cancer patients on account of their synergistic effects and their abilities to neutralize adverse responses, and provide a new perspective on the benefits of combining these three therapies to improve RT sensitivity and enhance immune response.

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## Conflict of Interest

The authors declare that they have no conflict of interest.

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