REVIEW

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When DNA damage responses meet tumor immunity: From mechanism to therapeutic opportunity

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Abstract

DNA damage is a prevalent phenomenon in the context of cancer progression. Evidence suggests that DNA damage responses (DDR) are pivotal in overcoming tumor immune evasion. Alternatively, traditional radiotherapy and chemotherapy operate by inducing DNA damage, consequently stimulating the immune system to target tumors. The intricate interplay between signaling pathways involved in DDR and immune activation underscores the significance of considering both factors in developing improved immunotherapies. By delving deeper into the mechanisms underlying immune activation brought on by DNA damage, it becomes possible to identify novel treatment approaches that boost the anticancer immune response while minimizing undesirable side effects. This review explores the mechanisms behind DNA damage-induced antitumor immune responses, the importance of DNA damage in antitumor immunity, and potential therapeutic approaches for cancer immunotherapy targeting DDR. Additionally, we discuss the challenges of combination therapy and strategies for integrating DNA damage-targeting therapies with current cancer immunotherapy. In summary, this review highlights the critical role of DNA damage in tumor immunology, underscoring the potential of DDR inhibitors as promising therapeutic modalities for cancer treatment.

KEYWORDS
DNA damage responses, immunotherapy, microenvironment, radiotherapy, tumor immunity

1 | INTRODUCTION

DNA damage can result from external factors such as ionizing radiation (IR), chemical exposure, viral infection, and internal factors such as cellular metabolism and DNA replication stress.[1] In pursuing DNA damage rectification and promoting cellular survival, the DNA damage response (DDR) pathway within tumor cells is elicited.[2] Within this context, these cells assume the multifaceted capacities of DNA lesion detection, signal transduction and the mending of compromised genetic material. Nonetheless, imbalances between DNA damage and repair, combined with the emergence of DNA lesions resulting from the maladjustment and mutations of unplanned or unregulated DDR constituents and their modulatory factors, propel the accumulation of mutations and genomic instability.[3]

Dong Pan and Qi Wang contributed equally to this work.

This sequence of events subsequently accentuates the trajectory of cancer progression.[4]

Genotoxic therapies, encompassing interventions such as radio-therapy (RT) and chemotherapy, frequently hinge on initiating DNA damage to debilitating tumor entities. The inherent activation of the DDR pathway within tumor cells emerges prominently as a primary instigator of therapeutic resistance. DNA damage and immunity are inherently interconnected and dynamically regulated biological processes that are increasingly acknowledged for their reciprocal interaction. DNA damage-induced strategies have therapeutic potential by enhancing innate and adaptive immune responses, increasing tumor immunogenicity, and minimizing toxicity.[5] These findings reveal a novel direction for tumor immunotherapy based on DDR damage. This, in turn, establishes a critical theoretical foundation for developing improved treatment methods tailored to the genetic characteristics of cancer patients.

This review summarizes and discusses the relationships and mechanisms between tumor DDR and the tumor immune response. Furthermore, we endeavor to provide insights into developing innovative treatment approaches for tumor therapy in the future. Specifically, we focus on the strategies that combine DNA damage-targeted therapies with tumor immunotherapy, presenting a panoramic view of ongoing advancements in pre-clinical and clinical research within this domain.

2 | MECHANISMS OF IMMUNE SURVEILLANCE ACTIVATION AND IMMUNE RESPONSE BY DNA DAMAGE

2.1 | Tumor DNA damage response and activated immune cells

The tumor immune microenvironment (TIME), consisting of antitumor immune effector cells, immune suppressive cells, and immune signaling molecules, is pivotal in tumor development and clinical management. The intricate interplay between DDR pathway activation and immune infiltration within the TIME is multifaceted (Figure 1). Elevating tumor mutation burden (TMB) enhances the infiltration of $CD8^+$ T cells.[6,7] Additionally, patients with gastrointestinal tumors harboring more than two DDR mutations exhibit heightened $CD4^+$/ $CD8^+$ T cell infiltration.[8] Various malignancies reveal substantial negative associations between mRNA levels of several DDR factors, including RPA1, Ku70, Ku80, MRE11A, RAD50, NBS1, PRKDC, RAD51, PARG, and XRCC4, and the extent of infiltration by cytotoxic $CD8^+$ T cells.[9] A distinct subset of T cells, known as γδT cells, exhibits a unique capacity for cancer cell recognition, distinct from conventional T cells. Through CRISPR screening, researchers have successfully identified molecular complexes involving butyrophilins, the activation of which becomes more facile with escalated stress pathways in cancer.[10] The Vγ9Vδ2 T cell subset, in particular, has garnered attention for its recognition of phosphoantigens, such as HMB-PP, which are upregulated in metabolically stressed cancer cells. This process is facilitated by the interaction of BTN3A1 and BTN2A1, with BTN2A1 playing a critical role in sensing these antigens and initiating an immune response.[11] The prospect of future immunotherapies involving the Vγ9Vδ2 T receptor engaged in DNA damage response pathways is poised to emerge as an enticing avenue of research.

The immunomodulatory effects of DNA damage induction or DDR inhibition include suppressing regulatory T cells (Tregs) and their potential contribution to antitumor immunity. In an additional study, treatment with small extracellular vesicles (sEVs) activated ATM, resulting in Treg expansion and immune evasion in pancreatic ductal adenocarcinoma (PDAC).[12] Moreover, inhibition of ATM effectively prevents Treg-induced $CD8^+$ T cell senescence.[13] Another investigation revealed a slight increase in Treg levels and an elevated $CD4^+$/ $CD8^+$ ratio under olaparib treatment.[14] However, combining olaparib with programmed cell death protein 1 (PD-1) antibodies yielded no notable shifts in Treg expression.[15] These findings underscore the intricate nature of tumor microenvironment (TME) alterations induced by distinct therapeutic interventions.

B cells augment the release of diverse cytokines, such as IL-12, IFNγ, granzyme B, and TRAIL57, to activate antitumor immune responses.[16] The development of a B cell-based vaccine (BVax) has yielded promising results, demonstrating superior antitumor effects compared to B cell depletion when combined with radiation, BVax administration, and programmed death-ligand 1 (PD-L1) blockade in a glioblastoma model. This approach elicits immune memory and activates $CD8^+$ T cells.[17] Furthermore, the indispensability of B cell responses for the efficacy of immune checkpoint inhibitors (ICIs) has been established in a murine breast cancer model, offering a robust avenue to enhance B cell responses through immune checkpoint blockade (ICB).[18] The concomitant application of RT and PD-L1 blockade augments the development of memory B cells, plasma cells, and antigen-specific B cells while also inducing significant B cell somatic hypermutation.[19] STING agonists facilitate the emergence of immature TLSs.[20] The STING-IL-35 axis within B cells has been identified as a potential regulator of NK-mediated antitumor responses.[21] Low-dose chemotherapy and oncolytic virotherapy effectively bolster tumor-infiltrating lymphocytes B cells (TIL-Bs), resulting in substantial tumor regression during ICB, and the depletion of B cells in murine models leads to complete therapeutic loss.[22] The plausible connections between DNA damage induction from therapeutic interventions such as RT and chemotherapy and subsequent TIL-B responses offer promising avenues for innovative combination therapies. Further inquiry is justified to delineate optimal strategies for augmenting B cell responses via DNA damage induction and ICIs and to formulate diagnostic and therapeutic paradigms directed toward B cells.

Dendritic cells (DCs) present antigens to $CD8^+$ T cells via MHC-I, generating CXCL9 and CXCL10, which recruit T cells to the tumor and produce IL-12.[23,24] DCs carrying tumor-associated antigens migrate to the tumor-draining lymph nodes (TdLNs), where they initiate the primary immune response of T cells.[25] The migration of DCs from the tumor to the TdLN is essential for the immunotherapeutic efficacy of the tumor in the MC38 model with RT.[26] In vitro, irradiation with 0.2 Gy x-rays significantly enhances DC migration and IL-12
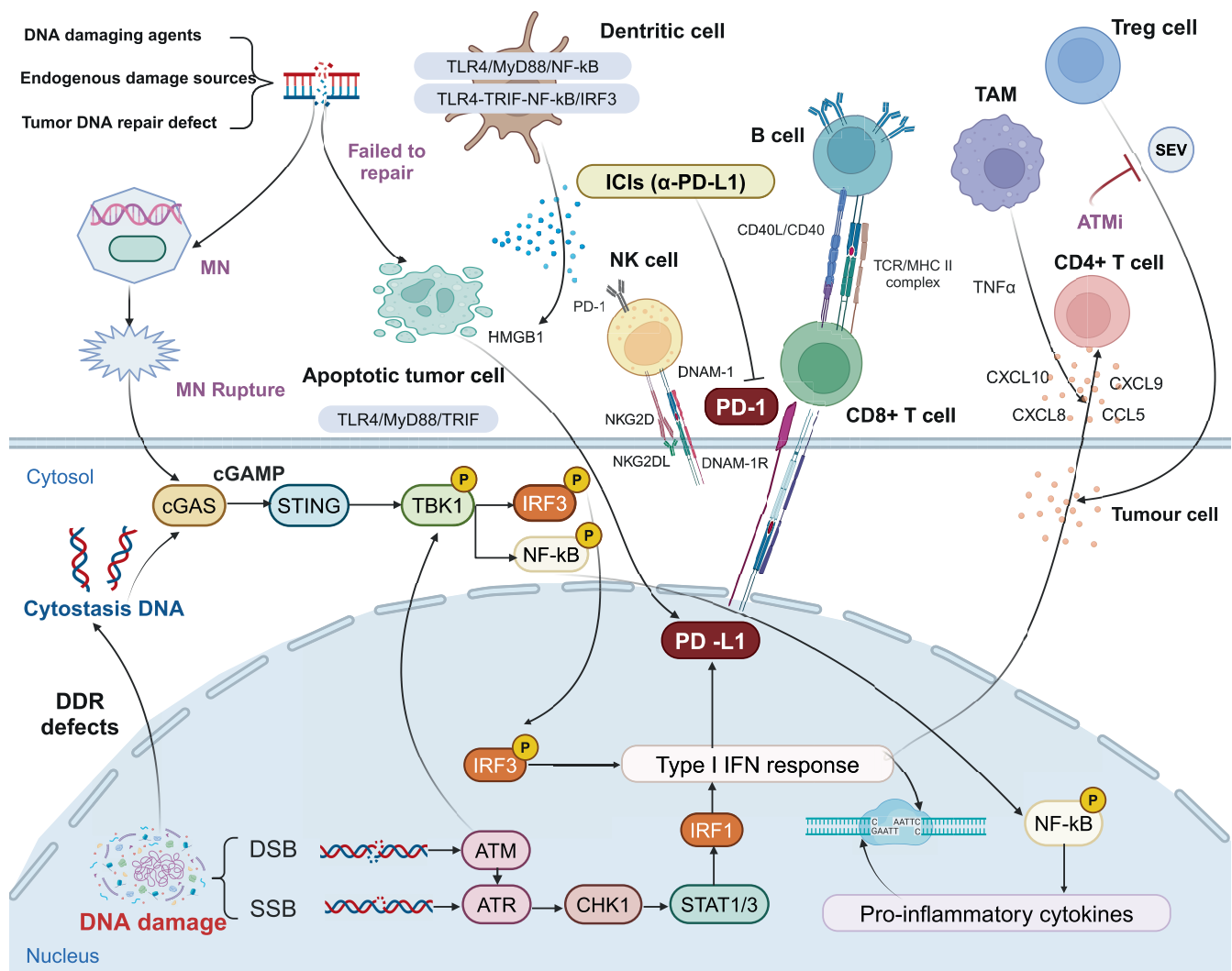
FIGURE 1 DNA Damage-Induced Remodeling of the TME. Accumulation of cytoplasmic DNA fragments due to DNA damaging agents, endogenous damage sources, and tumor DNA repair defect, or the entrapment of large chromosome fragments in micronuclei (MN) during persistent/unrepaired DNA damage in mitosis, leads to the accumulation of dsDNA within MN that is released upon MN rupture. The DNA damage response and neoantigen pathways activate cGAS-STING and IFN-I pathways, inducing PD-L1 expression, ultimately recruiting the release of chemokines (such as CXCL10 and CCL5) and CTL infiltration, as well as the activation of immune cells (e.g., T cells, NK cells, and DCs), while reducing Tregs and exhausted T cells that bind to ICB, collectively enhancing immune responses within the TME. In turn, these cytokines drive the migration of circulating lymphocytes, including B cells, CD4^{+} Th1 cells, and CD8^{+} effector T cells, as well as tumor-associated macrophages (TAMs). The ATM/ATR/CHK1 pathway also induces PD-L1 expression. ATM inhibitors can suppress tumor cell-derived small extracellular vesicle (sEV)-mediated Treg induction. Importantly, the HMGB1 protein released by tumor cells can, on one hand, engage TLR-4 on DC and macrophage surfaces, further activating the IRF3 and NF-κB pathways, and on the other hand, enhance PD-L1 expression in other tumor cells via the TLR4/MyD88/TRIF signaling. Figure created with Biorender.com. [Color figure can be viewed at wileyonlinelibrary.com]

production.[27] Depletion of the cytoplasmic DNA exonuclease three prime repair exonuclease 1 (TREX1) increases DC activation following treatment with DNA damage-inducing drugs, suggesting that increased cytoplasmic DNA originating from tumor cells might further enhance DC activation.[28] Combination therapy with RT and simulated viral mimetics within the tumor, involving DC vaccination and stereotactic ablative radiotherapy (SABR), has shown initial promising clinical outcomes.[29]

NK cells, a subset of innate lymphoid cells, exhibit direct cytotoxicity against cancer cells and participate in antitumor immune

responses by secreting cytokines.[30,31] Given RT's capacity as a potent DNA-damaging agent, the depletion of NK cells undermines the efficacy of previous successful treatments involving RT, anti-CD25, and anti-CD137 combination therapy.[32] Previous studies have emphasized the modulation of interactions between DCs and Tregs through anti-CD137 antibodies.[33] Importantly, NK cells play a crucial role in mediating this crosstalk.[32] Activation of NK cells through interleukin-2 (IL-2) leads to increased production of FLT3L, further activating DCs and CD8^{+} T cells. Additionally, RT can upregulate the expression of NKG2D ligands in tumors and modulate immune cells

by inducing the secretion of IFN-γ, TNF-α, perforin, and granzyme B through the p38-MAPK,[34] ATM,[35] and NF-kB pathways.[36,37] The radiosensitizing effect of combined RT and histone deacetylase inhibitor (HDACi) enhances the sensitivity of hepatocellular carcinoma (HCC) cells to NK cell-mediated cytotoxicity by elevating the expression of NKG2D ligands.[38] Notably, NK cells potentiate the therapeutic effect of DDR inhibition (DDRi), such as ATR inhibitors (ATRi), substantially intensifying the inflammatory response triggered by radiation.[39] The combination of RT and systemic NK cell therapy has shown promising success, significantly improving the migration and infiltration of NK cells into primary tumor sites and effectively inhibiting distant metastasis in mice.[40] This may serve as an attractive alternative to traditional T cell-based approaches.

2.2 | DNA damage induced innate immunity

2.2.1 | Cytoplasmic DNA sensing pathways

In the context of tumors, whether arising from exogenous genotoxic therapies such as RT or chemotherapy, or endogenous DNA damage due to DDR deficiencies or inhibition, the accumulation of cytoplasmic DNA has been observed.[41,42] Upon binding to double-stranded DNA (dsDNA), cGAS becomes activated, catalyzing the conversion of ATP and GTP into the secondary messenger 2′,3′-cyclic GMP-AMP (cGAMP). The effects of cGAMP can be mediated through either secretion or gap junctions formed between host cells and neighboring cells.[43] Subsequently, cGAMP triggers the activation of STING, a protein residing in the endoplasmic reticulum that translocates to the Golgi apparatus. Phosphorylated STING recruits interferon regulatory factor 3 (IRF3), acting as an adaptor molecule to facilitate IRF3 phosphorylation by TBK1.[44] The phosphorylated form of IRF3 forms dimers and translocates into the nucleus. Moreover, STING can also activate NF-κB, another pivotal transcription factor involved in proinflammatory signaling.[45] The concerted action of IRF3 and NF-κB leads to the transcriptional activation of cytokines, chemokines, and Type I interferons (IFN-I)[44] (Figure 2B). These immune factors are secreted by cells, promoting the infiltration of antitumor CD4$^+$ and CD8$^+$ T cells into the TME, thus boosting antitumor immunity. The viability and ability of CD8α$^+$ DCs to present antigens critically rely on IFN-I signaling, which enhances the process of DC cross-priming.[46]

DDRi, including ATM inhibitors (ATMi), ATRi, CHK1 inhibitors (Chk1i), PARP inhibitors (PARPi), and WEE1 inhibitors (WEE1i), have been demonstrated to activate the cGAS-STING pathway, facilitate T-cell recruitment, and augment the secretion of IFN-γ and TNFα within the TME.[15,47–52] Furthermore, endogenous DDR deficiencies, encompassing HR, non-homologous end joining (NHEJ), Fanconi anemia (FA), nucleotide excision repair and interstrand crosslink repair, single-strand break (SSB) repair, and double-strand break (DSB) repair, have also been found to upregulate the cGAS-STING pathway.[52–61] DDR factors play a pivotal role in suppressing immune signaling cascades mediated by DNA sensing pathways and preventing spurious activation of immune signals. Notably, certain DDR factors have been reported to indirectly modulate the activity of the cGAS-STING pathway by regulating the generation of cytoplasmic DNA. For instance, ATM,[62] PARP1,[15] RPA/RAD51,[63] SAMHD1,[64] TREX1,[65,66] MUS81,[67] and other DDR factors can curb excessive cytoplasmic DNA. In summary, targeted inhibition of DDR or therapies that induce DNA damage can exploit this innate immune activation pathway by fostering the accumulation of cytoplasmic dsDNA, thereby enhancing therapeutic effectiveness against cancer.

DNA sensors are vital for recognizing both endogenous and exogenous DNA in the cytoplasm or nucleus, contributing significantly to the DNA sensing pathways in innate immune signaling. In addition to cGAS, many other sensors, including Toll-like receptor 9 (TLR9), DHX9, DHX36, DDX41, IFI16, and POL III, initiate IFN-I responses. Remarkably, the DNA sensors AIM2 and IFI16 play crucial roles in activating the inflammasome complex (Figure 2D). Among these, IFI16, an IFN-γ-induced protein, has emerged as a cytoplasmic DNA sensor of notable importance. IFI16, a prominent nuclear protein, is recognized for its ability to detect and respond to the synthesis of both dsDNA and single-stranded DNA (ssDNA), as well as DNA damage induced by ultraviolet radiation.[68] Furthermore, it has been implicated in triggering innate immune responses following viral infections[69] (Figure 2D). Moreover, TLR9 stands out as the sole known DNA sensor within the TLR family, capable of perceiving CpG dsDNA leaked from mitochondria and triggering the production of IFN-I[70] (Figure 2C). However, a consensus regarding the precise mechanisms employed by DNA sensors to distinguish damaged DNA from diverse sources has yet to be reached within the current studies.

2.2.2 | Cytoplasmic RNA sensing pathways

The RNA sensing pathway mediated by the mitochondrial anti-viral signaling protein (MAVS) also generates IFN-I. Upstream of the adapter protein MAVS, cytoplasmic RNA sensors, including retinoic acid-inducible gene I (RIG-I, also referred to as DDX58) and melanoma differentiation-associated protein 5 (MDA5, also known as IFIH1), are involved. Following DNA damage induction or inhibition of the DNA damage response, cytoplasmic RIG-I or MDA5 binds to dsRNA, triggering IFN-I signaling mediated by the adapter protein MAVS.[71,72] Notably, MDA5 and RIG-I within the RNA sensing pathway can be activated by diverse molecules, including endogenous small non-coding RNAs (sncRNAs), dsRNA derived from downstream endogenous retroviral elements (ERVs), and RNA possessing a 5′-triphosphate moiety synthesized by DNA-dependent RNA polymerase III from AT-rich double-stranded DNA[71,73,74] (Figure 2A). Recent investigations have uncovered a novel role of RIG-I in suppressing DNA repair and viral integration into the host genome. Elevated RIG-I expression hampers DNA repair and heightens the susceptibility of cancer cells to radiation therapy.[75]

Incorporating epigenetic modulators and targeted delivery systems may enhance the effectiveness of STING agonists in cancer therapy. Reversing methylation-induced silencing of STING has been shown to enhance STING activation and T cell-mediated tumor
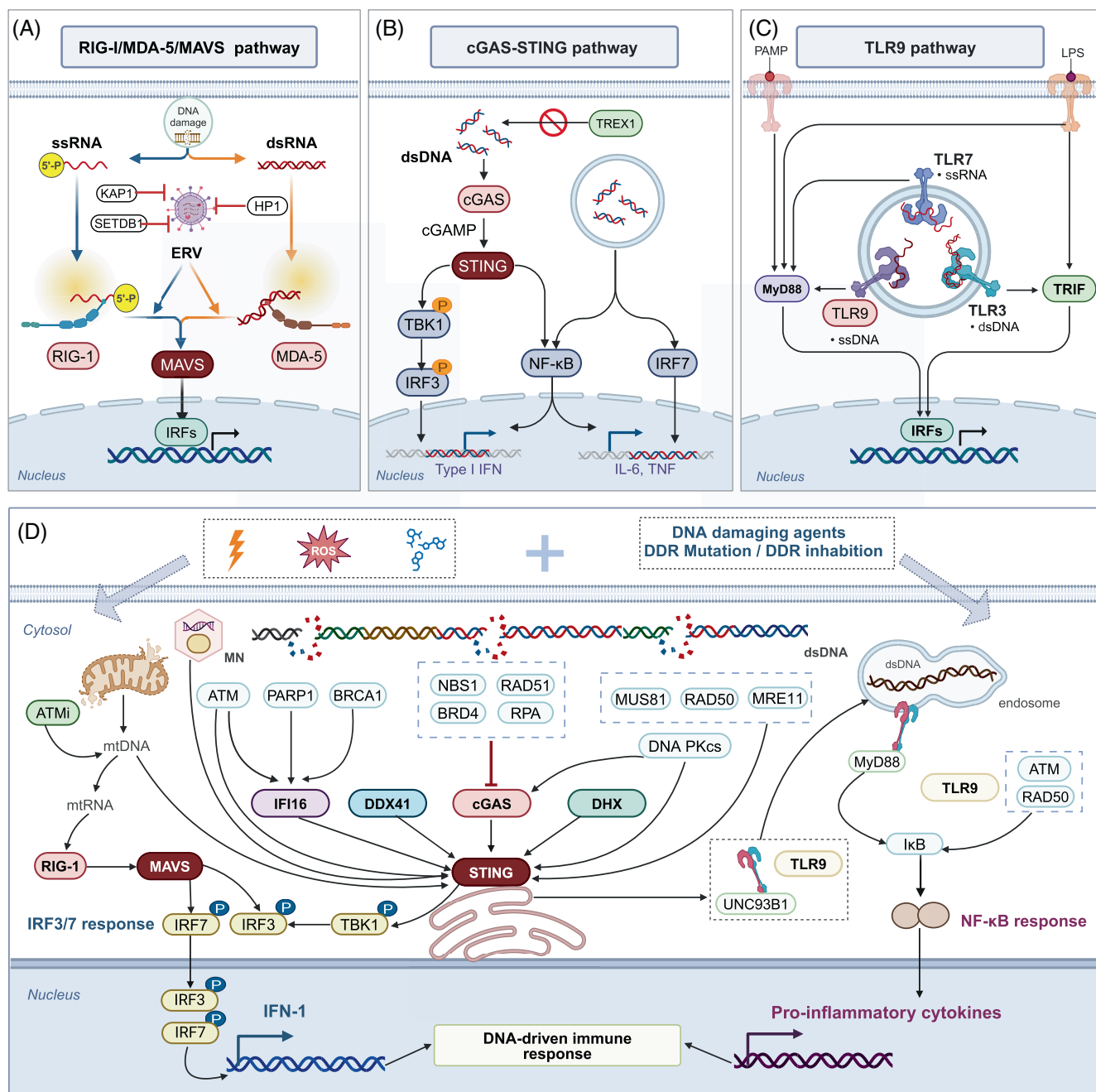
FIGURE 2 DNA damage involvement in cytosolic nucleic acid sensing pathways. (A) RIG-I/MDA-5/MAVS serves as the primary cytosolic RNA sensing pathway. RIG-I or MDA-5 detects cytosolic DNA damage or dsRNA from ERV sources, initiating the MAVS signaling to trigger type I interferon-mediated immune responses. Among them, KAP1, SETDB1, and HP1 have been reported as effective suppressors of ERV activation. (B) cGAS-STING acts as the main cytosolic DNA sensing pathway. cGAS detects cytosolic dsDNA and generates cGAMP, which binds to STING, inducing conformational changes that recruit TBK1, leading to STING phosphorylation. Phosphorylated STING recruits IRF3, which is subsequently phosphorylated by TBK1. Phosphorylated IRF3 dimerizes and translocates to the nucleus, where it acts as a transcription factor for type I IFN expression. Trex1, a DNA exonuclease, can degrade cytosolic DNA, thereby attenuating this pathway. Moreover, activation of the cGAS-STING pathway can also trigger the NF-κB pathway. (C) TLR9 pathway is involved in cytosolic DNA sensing. (D) DNA damage can lead to micronucleus formation and the release of dsDNA into the cytoplasm. Several cytosolic DNA sensors such as cGAS, IFI16, DDX41, DAI, and TLR9 can recognize cytosolic DNA. Some DDR components can directly or indirectly recognize cytosolic DNA, subsequently activating type I interferon-mediated immune responses. Mitochondria can also serve as sources of cytosolic DNA and RNA under genomic stress and participate in immune responses through the cGAS-STING pathway and the RIG-1-MAVS pathway, respectively. Figures (A), (B), and (C) are respectively adapted from "cGAS-STING DNA Detection", "RIG-I and MDA-5 Detect Cytosolic Viral RNAs" and "TLR Signaling Pathway", by BioRender.com (2020). Retrieved from https://app.biorender.com/biorender-templates. Figure (D) created with BioRender.com. [Color figure can be viewed at wileyonlinelibrary.com]

regression in murine models.[76] Strategies targeting the STING pathway through antibody-drug conjugates (ADCs) and nanoparticle delivery systems enable selective activation of STING in TME, enhancing immune responses while minimizing systemic toxicity.[77,78] Emerging evidence suggests that DNA methyltransferase inhibitors (DNMTi), HDACi, and RNA-induced silencing complex inhibitors, among other anticancer drugs, can induce the accumulation of cytoplasmic dsRNA by activating ERV transcription.[79–82] This activation subsequently triggers the MDA5/MAVS/TBK1 pathway and leads to the induction of an IFN-I response.[83] Interestingly, these anticancer drugs synergize with ICB and RT.[82,83] Supporting this, we previously investigated the potential of IR to activate ERVs and demonstrated that radiation alone stimulates ERV transcription and subsequent interferon production through the MDA5/MAVS/TBK1 dsRNA sensing pathway, with enhanced activation observed when KAP1 is absent.[84] Meanwhile, our work implies that histone methyltransferase SETDB1 (SET Domain Bifurcated 1) deficiency enhances ERV expression induced by radiation, resulting in increased tumor sensitivity and consequently improving the efficacy of RT.[85] Subsequent analyses revealed that this antitumor effect relied on MDA5/MAVS signaling and the augmentation of IFN-I. An earlier study established that the inhibition of SETDB1 synergized with ICB.[86] The successful development of histone methyltransferase inhibitors has provided a promising avenue for employing these targets in tumor immunotherapy, which are currently being evaluated in clinical trials[87] (Figure 2A). Although MAVS and STING have been reported as essential factors in IFN-I production, the precise contribution of each pathway may vary depending on the specific cell line under investigation.[88] Further research is required to delineate the relative involvement of dsDNA, dsRNA, and other potential sensing pathways in IFN-I production.

2.3 | DNA damage induced adaptive immune

Within the domain of adaptive immunity, DNA damage predominantly leads to a heightened TMB in tumor cells. Consequently, this instigates the production of tumor-specific neoantigens that differ from chromosomal structures and are readily recognized by the immune system.[89,90] The recognition and activation of these neoantigens on the tumor cell surface significantly contribute to a notable increase in the population of CD8+ T cells, thereby intensifying immune infiltration.[5] These findings underscore the alleged role of DDR deficiency as a wellspring for mutational burden and neoantigen generation within microsatellite instability (MSI) tumors. Critically, the inactivation of MMR amplifies the mutational burden and instigates a dynamic mutation landscape, thereby sustaining a continuous evolution of neoantigens both endogenously and exogenously.[91]

In contrast, proficient MMR cells manifest a stable mutation burden and a consistent repertoire of neoantigens over time. These findings underscore the potential of targeting DNA repair mechanisms to amplify the neoantigen load within tumor cells while highlighting the hypermutated state of MMR-deficient tumors as a catalyst for long-term immune surveillance, further augmented by immune modulators.[92]

Furthermore, an additional investigation has documented the involvement of epigenetic modulators in the induction of immunogenic neoantigens.[93] Moreover, within a cohort of non-small cell lung cancer patients undergoing combined anti-CTLA-4 therapy and RT, a rapid amplification of CD8+ antitumor T cells was observed, selectively recognizing a novel antigen generated by the radiation-induced upregulation of specific genes. This intriguing observation provides a plausible mechanism for radiation-induced abscopal responses, whereby RT prompts the exposure of immunogenic mutations to the immune system.[94] Additionally, analogous results have been reported in the context of chemotherapy.[95]

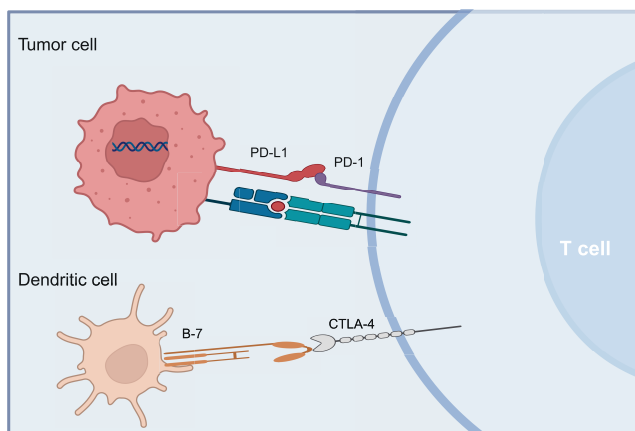# 3 | ADVANCEMENTS IN DNA DAMAGE TARGETING FOR COMBINED IMMUNOTHERAPY

3.1 | STING agonists

There is growing interest in exploring the potential of STING agonists as immunoadjuvants in combination with chemotherapy, RT, and ICB. Deng, L et al. were the first to discover the essential role of the cGAS-STING pathway in radiation-induced antitumor immune response and the induction of IFN-I. They found that combining exogenous cGAMP treatment with RT can enhance the efficacy of antitumor treatment by further promoting the activation of the immune system.[96] In models of B16-OVA and 4T1 lung metastasis, combined with IR, NP-cGAMP synergizes to induce systemic anticancer immunity and confer long-term survival in mice challenged with lung metastases and recurring tumors.[97] Additionally, using alginate-Mn materials with sustained release of Mn^{2+} accumulates DNA damage and synergistically amplifies the activation of the cGAS-STING pathway.[98] Significantly, administering Mn^{2+} 24 h after RT, rather than immediately, enhanced the therapeutic efficacy of RT. This observation suggests a difference in the metabolic timing of Mn^{2+} and RT-induced DNA damage in tumors. The combination of STING agonists with 5-fluorouracil (5-FU), a widely used anticancer drug, significantly mitigates the side effects of 5-FU, including nausea, mucosal necrosis, and bloody stools, while improving its anticancer efficacy.[99] These novel findings underscore the potential of targeting DNA damage to sensitize chemotherapy and warrant further exploration. Clinical trials investigating the combination of STING agonists and ICIs have yielded promising outcomes.[100,101]
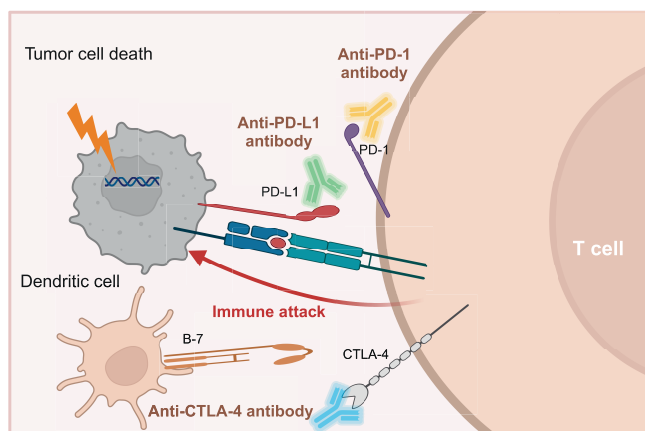
Nevertheless, potential hurdles may impede their future applications. For instance, most current STING agonists are synthetic analogs of 2′3′-cGAMP. However, their limited bioavailability, hydrophilicity, small molecular size, stability, deliverability necessitate predominantly intratumoral administration. Consequently, urgent efforts are required to develop prospective drugs capable of systemic delivery and activation of the cGAS/STING pathway while improving drug delivery systems. Intriguingly, Vanpouille-Box et al. discovered that TREX1 efficiently clears cytoplasmic dsDNA only when the radiation dose exceeds a critical threshold of 12 Gy, preventing the activation of the

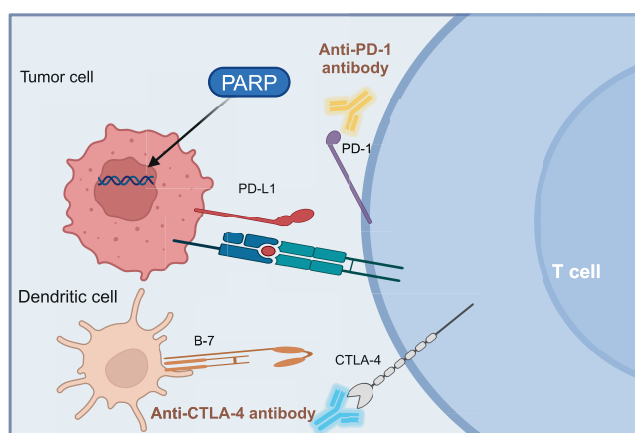## Molecular Mechanisms : Combination Therapy with PARP Inhibitors and ICIs

FIGURE 3 Molecular mechanisms: Combination therapy with PARP inhibitors and ICIs. (A) Immune checkpoint hinders T cell activation. Immune checkpoint 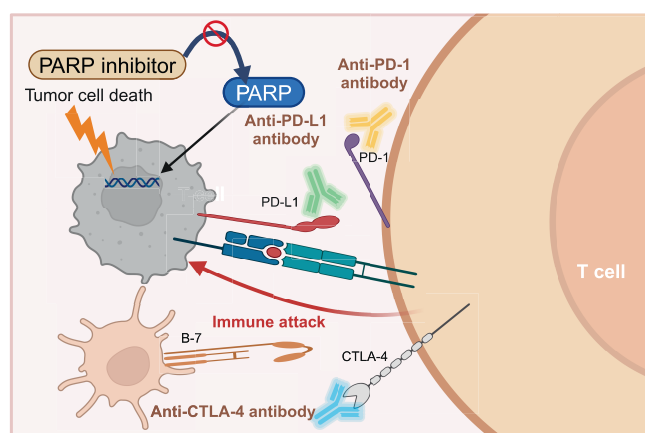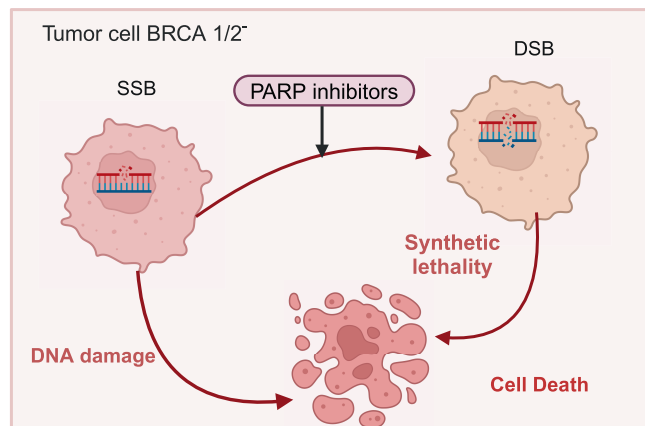molecules PD-1 and CTLA-4 on the surface of T cells bind to PD-L1 on tumor cells and B7 on antigen-presenting cells (APCs), facilitating tumor immune evasion. (B) Anti-PD-1/PD-L1/CTLA-4 antibodies permit T cell activation. Immune checkpoint inhibitors (ICIs) specifically bind to CTLA-4 and PD(L)1, effectively activating T cell-mediated anti-tumor immune responses. (C) PARP-mediated DNA damage repair. PARP repairs damaged DNA in tumor cells, aiding in tumor immune evasion. (D) PARP inhibitor facilitate DNA damage repair and assist ICIs. This exposes tumor cells to ICIs treatment, enhancing the anti-tumor immune response. (E) PARP inhibitors in normal cells. Failed DNA damage repair caused by PARP inhibitor is rescued by activated BRCA1/2, promoting cell survival. (F) Treatment for PARP inhibitors in BRCA mutant cancers. PARP inhibitors and defective BRCA1/2 form synthetic lethality, promoting cell death. Figure created with Biorender.com. [Color figure can be viewed at wileyonlinelibrary.com]
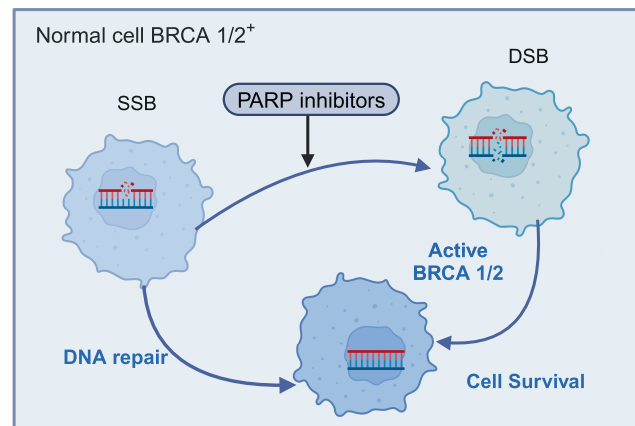
TABLE 1 Clinical trials of combination therapy with PARP Inhibitors and ICIs (ongoing and completed).

Trial ID	Phase	Tumor type	PARPi	ICI	Status
NCT04825990	Phase 2	Nasopharyngeal carcinoma	Olaparib	Pembrolizumab	Recruiting
NCT05033756	Phase 2	Breast cancer	Olaparib	Pembrolizumab	Recruiting
NCT04187833	Phase 2	Melanoma	Talazoparib	Nivolumab	Active not recruiting
NCT04306367	Phase 2	Cholangiocarcinoma	Olaparib	Pembrolizumab	Active not recruiting
NCT03559049	Phase 1/2	NSCLC Stage IV	Rucaparib	Pembrolizumab	Active not recruiting
NCT03297606	Phase 2	Lymphoma, multiple myeloma, advanced solid tumors	Olaparib	Nivolumab, Ipilimumab	Recruiting
NCT03995017	Phase 1/2	Esophagus cancer, stomach cancer	Rucaparib	Nivolumab	Active not recruiting
NCT05203445	Phase 2	Breast cancer	Olaparib	Pembrolizumab	Recruiting
NCT03958045	Phase 2	Small cell lung cancer	Rucaparib	Nivolumab	Active not recruiting
NCT04034927	Phase 2	Fallopian tube/ovarian carcinoma	Olaparib	Tremelimumab	Active not recruiting
NCT05201612	Phase 2	Metastatic colorectal cancer	Olaparib	Pembrolizumab	Recruiting
NCT04624204	Phase 3	Small cell lung cancer	Olaparib	Pembrolizumab	Recruiting
NCT03824704	Phase 2	Epithelial ovarian cancer, fallopian tube cancer, primary peritoneal carcinoma, high grade serous carcinoma, endometrioid adenocarcinoma	Rucaparib	Nivolumab	Terminated
NCT05623319	Phase 2	Extensive small cell lung cancer (ES-SCLC)	Olaparib	Pembrolizumab	Recruiting
NCT05485766	Phase 2	Triple negative breast neoplasms, triple negative breast cancer, breast neoplasms, breast cancer, BRCA1 mutation, BRCA2 mutation, BRCA mutation, BRCA-associated breast carcinoma	Olaparib	Pembrolizumab	Not yet recruiting
NCT04123366	Phase 2	Solid tumors	Olaparib	Pembrolizumab	Active not recruiting
NCT05366166	Phase 2	Squamous cell carcinoma of head and neck	Olaparib	Pembrolizumab	Recruiting
NCT05093231	Phase 2	Pancreatic cancer	Olaparib	Pembrolizumab	Not yet recruiting
NCT03308942	Phase 2	Neoplasms	Niraparib	Pembrolizumab, TSR-042	Completed
NCT02849496	Phase 2	Locally advanced breast carcinoma, metastatic breast carcinoma, Stage III breast cancer AJCC v7, Stage IV breast cancer AJCC v6 and v7, unresectable breast carcinoma	Olaparib	Atezolizumab	Active not recruiting
NCT03061188	Phase 1	Advanced solid neoplasm, aggressive non-Hodgkin lymphoma, recurrent solid neoplasm, refractory mantle cell lymphoma, T-cell non-Hodgkin lymphoma, unresectable solid neoplasm	Veliparib	Nivolumab	Unknown
NCT03834519	Phase 3	Prostatic neoplasms	Olaparib	Pembrolizumab	Active not recruiting
NCT05268510	Phase 2	Esophagogastric adenocarcinoma	Olaparib	Pembrolizumab	Active not recruiting
NCT02657889	Phase 1/2	Neoplasms, triple negative breast cancer, ovarian cancer, breast cancer, metastatic Breast cancer, advanced breast cancer, stage iv breast cancer, fallopian tube cancer, peritoneal cancer	Niraparib	Pembrolizumab	Completed
NCT05524935	Phase 2	Uveal melanoma, ocular melanoma	Olaparib	Pembrolizumab	Recruiting
NCT05174832	Phase 2	Triple negative breast cancer	Olaparib	Pembrolizumab	Recruiting
NCT03976323	Phase 3	Carcinoma, NSCLC	Olaparib	Pembrolizumab	Active not recruiting
NCT04334941	Phase 2	Extensive stage lung small cell carcinoma	Talazoparib	Atezolizumab	Active not recruiting
NCT04633902	Phase 2	Metastatic melanoma	Olaparib	Pembrolizumab	Recruiting
NCT03572478	Phase 1/2	Prostate cancer, endometrial cancer	Rucaparib	Nivolumab	Terminated
NCT03101280	Phase 1	Gynecologic neoplasms	Rucaparib	Atezolizumab	Completed
NCT03598270	Phase 3	Recurrent ovarian carcinoma	Niraparib	Atezolizumab	Active not recruiting
NCT03639935	Phase 2	Biliary tract cancer	Rucaparib	Nivolumab	Active not recruiting
NCT04483544	Phase 2	Cervical cancer	Olaparib	Pembrolizumab	Recruiting

Note: Full access to the research studies description is available on ClinicalTrials.gov.

cGAS pathway responsible for IFN-I induction and consequently diminishing the immunogenicity of RT.[65,66] Therefore, minimizing TREX1 expression and maintaining the "optimal point" of cGAS-STING signaling pathway activation is determined by radiation dose thresholds rather than cumulative doses. A fundamental question remains regarding selecting an optimal radiation scheme that can enhance dsDNA accumulation without surpassing the threshold for DNA enzyme activation.

3.2 | DDR inhibitions

DDR-targeted inhibition offers the optimal therapeutic combination for improving the treatment outcomes of cancers that are difficult to treat with existing RT and chemotherapy regimens.[102] In patients with advanced pancreatic cancer, the combination strategy of PARPi with platinum-based chemotherapy has shown significant superiority over monotherapy.[103] ATRi can attenuate radiation-induced CD8$^+$ T cell exhaustion.[104] Pre-clinical studies suggest that combining DNA-PK inhibition with RT may lead to persistent immune-mediated tumor control, as evidenced by experiments in mouse models, indicating a potential therapeutic strategy for future clinical evaluation.[105] Combining CHK1i with RT increases CD8$^+$ T cell infiltration and reduces mouse tumor volume.[106] However, the combination therapy of ATM with RT increases the induction of IFN-I signaling independently of cGAS/STING and relies on TBK1.[107] Additionally, inhibiting the function of POLQ can increase the sensitivity of tumor cells to RT.[108]

Considerable research findings have established that the coadministration of PARPi and PD-L1 antibodies promotes the infiltration of cytotoxic T cells into tumors by activating the cGAS-STING pathway. This therapeutic combination exhibits notable efficacy in tumors harboring BRCA1/2 or ERCC1 mutations.[52,109] Combining PARPi with other ICIs, such as CTLA-4 antibodies, yields similar effects.[110]

Notably, PARPi also induces an elevation in PD-L1 levels within tumors, providing a plausible rationale for the favorable outcomes observed when combining DDRi and ICIs[111] (Figure 3). The preclinical findings, such as those reported in Wang, Z. et al., have been instrumental in guiding the development of clinical trials examining the combined use of PARPi and ICIs[112] (Table 1). Additional investigations stemming from adjunctive studies evaluating ICIs have further revealed the potential of DDR-related biomarkers to predict ICI response.

Furthermore, several other DDRi are actively undergoing assessment in clinical trials (Table 2). Schoonen et al. reported that the use of PARPi alone results in dose-dependent G2 arrest in BRCA2-deficient cells, leading to a reduced percentage of cells in mitosis, indicating a dose-dependent delay in G2/M progression. Therefore, treatment with a mitotic entry-promoting drug (such as WEE1 or DDR kinase inhibitors) in combination with PARPi forces mitotic entry, enhancing the cytotoxic effect of olaparib in models of BRCA2 mutant and BRCA2 knockout cancer cell lines.[113] This provides a pre-clinical theoretical basis for the triple therapy of WEE1i, PARPi, and anti-PD-(L)1 blockade. Evidence for triple therapy involving two different DDRi remains an active area of research. Collectively, these findings substantiate the concept that combining ICIs with agents inducing DNA damage or targeting DDR can enhance the effectiveness of cancer immunotherapy, particularly in individuals nonresponsive to PD-L1/PD-1 pathway inhibitors.

3.3 | Combination therapy strategies

In addition to targeting DDR, classical DNA damage inducers such as RT and chemotherapy offer alternative strategies for ICI treatment and provide a pre-clinical rationale for combining radiation/chemotherapy with ICIs (Table 3). Several ongoing clinical trials

TABLE 2 Clinical trials of combination therapy with other DDR inhibitors (excluding PARP inhibitors) and ICIs (ongoing and completed).

Trial ID	Phase	Tumor tape		DDRi	ICI	Status
NCT04216316	Phase 1/2	Advanced squamous cell NSCLC	ATRi	Berzosertib	Pembrolizumab	Recruiting
NCT04266912	Phase 1/2	Solid tumors		Berzosertib	Avelumab	Recruiting
NCT05061134	Phase 2	Melanoma		Ceralasertib	Durvalumab	Recruiting
NCT03334617	Phase 2	NSCLC		Ceralasertib	Durvalumab	Recruiting
NCT04298008	Phase 2	Biliary tract cancer		Ceralasertib	Durvalumab	Recruiting
NCT03780608	Phase 2	Gastric adenocarcinoma, Malignant melanoma		Ceralasertib	Durvalumab	Active not recruiting
NCT03833440	Phase 2	NSCLC		Ceralasertib	Durvalumab	Recruiting
NCT02664935	Phase 2	NSCLC		Ceralasertib	Durvalumab	Active not recruiting
NCT02264678	Phase 1/2	Solid tumors		Ceralasertib	Durvalumab	Recruiting
NCT04095273	Phase 1	Solid tumors		Elimusertib	Pembrolizumab	Completed
NCT03495323	Phase 1	Advanced solid tumors	CHK1i	Prexasertib	LY3300054	Completed
NCT02546661	Phase 1	Bladder cancer	WEE1i	Adavosertib	Durvalumab	Active not recruiting
NCT02617277	Phase 1	Solid tumors		Adavosertib	Durvalumab	Active not recruiting

Note: Full access to the studies' description is available on ClinicalTrials.gov.

TABLE 3 Selected clinical trials of combination therapy with radiotherapy and ICIs.

Trial ID	Phase	Tumor type	RT type	ICI	Status
NCT05501665	Phase 1/2	NSCLC	RT	Pembrolizumab	Recruiting
NCT04754321	Phase 1	Head and neck squamous cell carcinoma	EBRT、IORT	Pembrolizumab	Recruiting
NCT04090710	Phase 2	Metastatic renal cell carcinoma	SBRT	Ipilimumab, Nivolumab	Recruiting
NCT04577638	Phase 2	NSCLC stage III	IMRT	Nivolumab	Active not recruiting
NCT03907488	Phase 3	Stage III-IV classic Hodgkin lymphoma	RT	Nivolumab	Active not recruiting
NCT03693014	Phase 2	Metastatic cancer, melanoma cancer, lung cancer, bladder cancer, renal cancer, head/neck cancers	SBRT	Ipilimumab, Nivolumab, Pembrolizumab, Atezolizumab	Recruiting
NCT03776487	Phase 2	Resectable gastric cancer	IMRT	Nivolumab, Ipilimumab	Recruiting
NCT05488366	Early phase 1	Sarcoma, soft tissue	RT	Pembrolizumab	Recruiting
NCT05327686	Phase 2	Metastatic renal cell carcinoma, unresectable renal cell carcinoma	SABR	Avelumab, Axitinib, Cabozantinib, Ipilimumab, Lenvatinib, Nivolumab, Pembrolizumab	Recruiting
NCT03486197	Phase 2	Urothelial carcinoma	RT	Pembrolizumab	Active not recruiting
NCT04214067	Phase3	Endometrial endometrioid adenocarcinoma	EBRT	Pembrolizumab	Active not recruiting
NCT04671667	Phase 2	Recurrent head and neck squamous cell carcinoma	IMRT、PBRT	Pembrolizumab	Recruiting
NCT05655715	Phase 2	Prostate cancer metastatic, castrate resistant prostate cancer	SBRT	Ipilimumab, Nivolumab	Recruiting
NCT04989283	Phase 2	NSCLC, superior sulcus lung carcinoma	EBRT	Atezolizumab	Active not recruiting
NCT03546582	Phase 2	Head and neck squamous cell carcinoma	SBRT	Pembrolizumab	Recruiting
NCT05204290	Early phase 1	Epidural spinal tumors	SBRT	Pembrolizumab	Recruiting
NCT05568550	Phase 2	Prostate cancer	RT	Pembrolizumab	Recruiting
NCT03425292	Phase 1	Newly diagnosed high grade glioma	CBRT	Nivolumab, Ipilimumab	Active not recruiting
NCT03223155	Phase 1	Stage IV small cell lung cancer	SBRT	Nivolumab, Ipilimumab	Active not recruiting
NCT04357587	Phase 1	Rectal neoplasms	EBRT	Pembrolizumab	Recruiting
NCT03952585	Phase 2/3	Basaloid squamous cell carcinoma, oropharyngeal squamous cell carcinoma, papillary squamous cell	IGRT、IMRT	Nivolumab	Recruiting
NCT04396860	Phase 2/3	Gliosarcoma, MGMT-unmethylated glioblastoma	RT	Ipilimumab, Nivolumab	Active not recruiting
NCT03686332	Phase 2	Penile cancer	RT	Atezolizumab	Active not recruiting
NCT03774732	Phase 3	Advanced NSCLC	SBRT	Pembrolizumab	Recruiting
NCT03110978	Phase 2	Stage I-IIA or recurrent NSCLC	SBRT	Nivolumab	Active not recruiting
NCT05431764	Phase 2	Nasopharyngeal carcinoma	SBRT、IMRT	Camrelizumab	Recruiting
NCT04581382	Early phase 1	Melanoma	RT	Nivolumab, Pembrolizumab	Recruiting
NCT03767582	Phase 1/2	Locally advanced pancreatic ductal adenocarcinoma	SBRT	Nivolumab	Recruiting
NCT03304639	Phase 2	Advanced MERKEL cell carcinoma	SBRT	Pembrolizumab	Active not recruiting

TABLE 3 (Continued)

Trial ID	Phase	Tumor type	RT type	ICI	Status
NCT04683679	Phase 2	Triple negative breast cancer, metastatic breast cancer	RT	Pembrolizumab, Olaparib	Recruiting
NCT05116917	Phase 2	Pancreatic cancer	SBRT	Nivolumab, Ipilimumab	Recruiting
NCT03614949	Phase 2	Recurrent, persistent, or metastatic cervical cancer	SBRT	Atezolizumab	Recruiting
NCT03317327	Phase 1/2	Head and neck squamous cell carcinoma	RT	Nivolumab	Recruiting
NCT04477759	Phase 1	Head and neck neoplasm	RT	Atezolizumab	Recruiting
NCT03867175	Phase 3	Metastatic lung cancer, stage iv lung cancer	SBRT	Pembrolizumab	Active not recruiting
NCT03811015	Phase 2/3	Clinical stage II/III HPV-mediated oropharyngeal carcinoma	IMRT	Nivolumab	Recruiting
NCT04454489	Phase 2	Advanced head and neck squamous cell carcinoma, locally advanced head and neck squamous cell carcinoma	RT	Pembrolizumab	Recruiting
NCT04402788	Phase 2/3	Extensive stage lung small cell carcinoma	RT	Atezolizumab	Recruiting
NCT03544736	Phase 1/2	Esophageal cancer	RT	Nivolumab	Active not recruiting
NCT04549428	Phase 2	NSCLC stage iv	RT	Atezolizumab	
NCT04659811	Phase 2	Grade I, II, III meningioma, recurrent meningioma	SRS	Pembrolizumab	Recruiting
NCT01810913	Phase 2/3	High-risk head and neck squamous cell carcinoma	RT	Atezolizumab	Recruiting
NCT05169957	Phase 2	Urothelial carcinoma bladder	SBRT	Sasanlimab	Recruiting
CT04902040	Phase 1/2	Various advanced cancers	RT	Atezolizumab, Avelumab, Durvalumab, Nivolumab, Pembrolizumab	Recruiting
NCT04430699	Phase 2	Vulvar cancer, vulvar squamous cell carcinoma	RT	Nivolumab, Ipilimumab	Recruiting
NCT04977453	Phase 1/2	Various advanced solid tumors	RT	Pembrolizumab	Recruiting
NCT04361162	Phase 2	Pancreatic cancer, metastatic pancreatic cancer	RT	Nivolumab, Ipilimumab	Active not recruiting
NCT03618134	Phase 1/2	HPV-mediated oropharyngeal squamous cell carcinoma	RT	Durvalumab, Tremelimumab	Active not recruiting
NCT04929041	Phase 2/3	NSCLC stage IV	SBRT	Ipilimumab, Nivolumab, Pembrolizumab	Recruiting
NCT02296684	Phase 2	Head and neck squamous cell carcinoma	IMRT	Pembrolizumab	Active not recruiting
NCT03445858	Early phase 1	Childhood solid tumor, lymphoma, relapsed/refractory cancer	HISR	Pembrolizumab	Active not recruiting
NCT03811002	Phase 2/3	Limited stage small cell lung cancer	RT	Atezolizumab, Pembrolizumab	Recruiting
NCT04271384	Phase 2	NSCLC stage I	SABR	Nivolumab	Recruiting
NCT03391869	Phase 3	NSCLC stage IV	RT	Ipilimumab, Nivolumab	Recruiting
NCT03658447	Phase 1	Metastatic castration resistant prostate cancer	177Lu-PSMA	Pembrolizumab	Completed

(Continues)

TABLE 3 (Continued)

Trial ID	Phase	Tumor type	RT type	ICI	Status
NCT04109729	Phase 1	Metastatic castration resistant prostate cancer	Radium-223	Nivolumab	Recruiting
NCT03805594	Phase 1	Metastatic castration resistant prostate cancer	177Lu-PSMA-617	Pembrolizumab	Active not recruiting
NCT03093428	Phase 1/2	Metastatic castration resistant prostate cancer	Radium-223	Pembrolizumab	Active not recruiting

Note: Full access to the research studies description is available on ClinicalTrials.gov.
Abbreviations: CBRT, conformal brain radiation therapy; EBRT, external beam radiation therapy; HISR, hypofractionated index site radiation; IGRT, image guided radiation therapy; IMRT, intensity-modulated radiation therapy; IORT, intraoperative radiation therapy; PBRT, proton beam radiation therapy; RT, radiation therapy; SABR, stereotactic ablative radiation therapy; SBRT stereotactic body radiation therapy; SRS, stereotactic radiosurgery.

represent an emerging field where targeted radionuclide therapies (α and β radiation therapies) are being combined with immunotherapy in the treatment of metastatic castration-resistant prostate cancer (mCRPC; NCT03658447, NCT03805594, NCT03093428 and NCT04109729). These indicate a strategic shift towards leveraging the inherent cytotoxicity of RT and the power of ICIs to activate the immune system, aiming to establish a more effective and potentially less toxic paradigm for mCRPC treatment.

Hence, we primarily elucidate a triple combination therapeutic strategy based on DNA damage induction, RT, and ICIs, offering a novel avenue to harness the immune-stimulating effects of cGAS-STING pathway activation. For example, in vitro experiments employing triple combination therapy involving ATM knockout, RT, and anti-PD-L1 demonstrated significant enhancements in CD8$^+$ T cell infiltration, stronger immune memory, and durable antitumor immunity.[51] In multiple models of head and neck cancer, lung cancer, and melanoma, RT and WEE1i amplified the cytotoxicity of T lymphocytes against tumors. Incorporating anti-PD-L1 on top of RT and WEE1i substantially improved survival outcomes in mouse models of head and neck squamous cell carcinoma, with the greatest tumor antigen-specific T-cell responses observed in this triple therapy.[114] Myeloid-derived suppressor cells (MDSCs) are recognized for their immunosuppressive properties and contribute to tumor immune evasion through various mechanisms. Targeting the downregulation of the CCR2 receptor can impede the mobilization of MDSC cells, ameliorate the immunosuppressive TME, and achieve radiation sensitization. In the MC38 tumor model, triple treatment with RT, a CCR2 antagonist, and the STING agonist cGAMP achieved 60% tumor inhibition, indicating that eliminating MDSCs from the TME can further potentiate radiation-induced antitumor immunity.[115] Thus, a promising and reliable strategy involves the utilization of STING agonists or DDRi to enhance immune stimulation via the cGAS-STING pathway and eliminate immune defects resulting from cGAS-STING pathway activation through ICB or CCR2 inhibition. Further pre-clinical investigations are warranted to explore the potential of this triple combination therapy.

However, the synergistic effects of combination therapy are highly complex, and a deeper understanding of the DDR-anticancer immune interaction is crucial. The heterogeneity of tumors needs to be considered to optimize personalized, biomarker-driven combination treatment approaches. In the process of individualized therapy, factors such as toxicity, immune-related adverse events, drug selection, dosage, timing of combination, and sequence of variety need to be carefully considered, in addition to factors such as resistance. Moreover, not all types of cancer are suitable for combination therapy, and more pre-clinical and clinical trials are needed to compare the choices between single ICIs and combination DDR-targeted therapy, with an expansion of study populations in clinical trials.

4 | CONCLUSION AND PERSPECTIVES

The role of DNA damage and DDR in activating the immune system is increasingly evident. In this context, DNA damage-induced immune activation and DDR-targeted inhibition have emerged as promising strategies for synergistic combination therapy with ICIs. As a potential therapeutic target that connects DNA damage to immune activation, the activation of the cGAS-STING pathway has spurred the development of STING agonists and analogs as immune adjuvants for combination therapies, including chemotherapy, RT, and ICB, intending to enhance treatment efficacy. However, activating the cGAS-STING pathway does not consistently benefit tumor immunotherapy. Chromosomal instability (CIN) tumors may inherently resist STING agonists, and a subset of patients could potentially benefit from the inhibition of cGAS-STING signaling to suppress chronic inflammation and its associated immune suppression within the TME.[116] Thus, the stratification of patients becomes pivotal in this context. Achieving the optimal balance in pathway activation to promote immune stimulation while suppressing negative immune responses remains challenging, leaving significant room for further advancements.

Clinical trials exploring the interplay between DDR and anticancer immunity have paved the way for the broader implementation of this therapeutic approach. Although combination therapy with PARPi and anti-PD-L1 monoclonal antibodies has shown promising results, additional investigations on DDRi combined with ICIs are needed in pre-clinical and clinical settings. It is important to note that the effects of DDR inhibition may vary across different tumor types, and not all types of cancer may benefit from combination therapy. Hence, identifying predictive biomarkers to guide patient selection is

crucial,[117] and further exploration of tools such as whole-exome sequencing, single-cell RNA-seq, and TME profiling is warranted in this regard. Furthermore, exploring the potential of combining DDR-targeted therapy with immunotherapies beyond ICIs requires further investigation. Emerging evidence has uncovered the utilization of retrotransposons to hijack host cells during the alternative end-joining (alt-EJ) DNA repair process, resulting in the generation of extrachromosomal circular DNA (eccDNA).[118] Despite being initially regarded as a backup pathway for canonical DNA repair, alt-EJ remains relatively understudied in DNA repair mechanisms.[119] An intriguing avenue for further investigation is whether the innate immune system can perceive the cyclic DNA arising from alt-EJ, potentially triggering an immune response.

In conclusion, the intrinsic events driven by DNA damage within tumor cells play a central role in immune regulation. Gaining a more comprehensive understanding of the immune pathways triggered by DNA damage can provide valuable insights for optimizing therapeutic combinations, harnessing the intrinsic effects of tumor cells, and overcoming resistance to ICB.

AUTHOR CONTRIBUTIONS

The work reported in the paper has been performed by the authors, unless clearly specified in the text. *Conceptualization and supervision*: B.H. and C.Z. *Writing original draft*: D.P. and Q.W. *Visualization*: A.S. and Z.Q. *Writing review & editing*: all authors.

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