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Review

DNA Damage and Cancer Immunotherapy: A STING in the Tale

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SUMMARY

Cancer immunotherapies enhance anti-tumor immune responses using checkpoint inhibitors, such as PD-1 or PD-L1 inhibitors. Recent studies, however, have extended the scope of immunotherapeutics by unveiling DNA damage-induced innate immunity as a novel target for cancer treatment. Elucidating the interplay among the DNA damage response (DDR), cyclic GMP-AMP synthase-stimulator of interferon genes (cGAS-STING) pathway activation, and anti-tumoral immunity is critical for the development of effective cancer immunotherapies. Here, we discuss the current understanding of the mechanisms by which DNA damage activates immune responses that target and eradicate cancer cells. Yet, understanding how cancer cells can escape this immune surveillance and promote tumor progression represents an outstanding challenge. We highlight the most recent clinical advances, in particular how pharmacological fine-tuning of innate/adaptive immunity and its combination with DDR inhibitors, ionizing radiation (IR), and chemotherapy can be exploited to improve cancer treatment.

Cellular DNA is under constant threat of damage by exogenous and endogenous sources. Exposure to ionizing radiation (IR) and chemotherapeutic agents (e.g., platinum drugs) inflicts DNA double-strand breaks (DSBs), which are particularly lethal to cancer cells. Additionally, cell-intrinsic factors such as products of cellular metabolism (e.g., aldehydes and reactive oxygen species) generate DNA replication barriers that can lead to replication-associated DSBs or chromosome mis-segregation during mitosis, accompanied by DNA breakage. The DNA damage response (DDR) counteracts these threats and acts to maintain genome integrity through damage recognition and activation of a complex signaling network that promotes transient cell-cycle arrest and DNA repair (Blackford and Jackson, 2017). Ataxia telangiectasia mutated (ATM), ATM- and RAD3-related (ATR), and DNA-dependent protein (DNA-PK) are three related kinases that control the DDR and orchestrate DSB signaling. In cases where the DNA damage exceeds the repair capacity of the cell, DDR triggers senescence or apoptosis. By arresting proliferation and promoting clearance of damaged cells, the DDR acts as a barrier to tumorigenesis. Consequently, hereditary or sporadic mutations in DDR genes lead to mutations and chromosome rearrangements conducive for tumor initiation and progression.

Compelling evidence has accumulated linking DDR deficiency with activation of anti-tumor immunity. Innate immune responses, initially characterized as the first line of defense against pathogens, can also be activated by endogenous DNA. Genotoxic stress caused by DDR gene inactivation or DNA-damaging treatments generates chromosomal fragments that are recognized by the nucleic acid sensor cyclic GMP-AMP (cGAMP) synthase (cGAS; Figure 1). DNA binding triggers a conformational change in cGAS that enables cGAMP synthesis from ATP and GTP. cGAMP activates stimulator of interferon genes (STING) and promotes its re-localization to the Golgi apparatus. Through binding and activation of TANK-binding kinase 1 (TBK1) and the heterodimeric IKKα/β kinase, STING triggers transcriptional activation of interferon regulatory factor 3 (IRF3) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), respectively. These, in turn, mediate expression and secretion of proinflammatory cytokines, including the type I interferons (IFNs) IFN-α and IFN-β. Their binding to the heterodimeric IFN receptor IFNAR1/IFNAR2 activates Janus kinase 1 (JAK1), which phosphorylates members of the signal transducer and activator of transcription (STAT) family to induce expression of IFN-stimulated genes (ISGs; Table 1; Schoggins et al., 2011). Numerous ISGs have been identified with multiple antiviral functions, including inhibition of viral replication or translation. However, their functions in the specific context of the innate responses triggered by loss of genome integrity remain elusive (MacMicking, 2012; Zitvogel et al., 2015).

In addition, alternative, non-canonical pathways by which DNA damage can induce innate immune responses have been reported. For example, the ATM checkpoint kinase can activate STING in response to etoposide-induced DNA damage independently of cGAS, thereby triggering NF-κB-dependent transcription (Dunphy et al., 2018). Furthermore, DNA-PK activated by free DNA ends leads to STING-independent IRF3 phosphorylation and type I IFN gene expression (Burleigh et al., 2020; Karpova et al., 2002). Finally, it is important to mention that STING signaling also promotes cancer cell clearance through autophagy, necrosis, or apoptosis, independently of IRF3 and

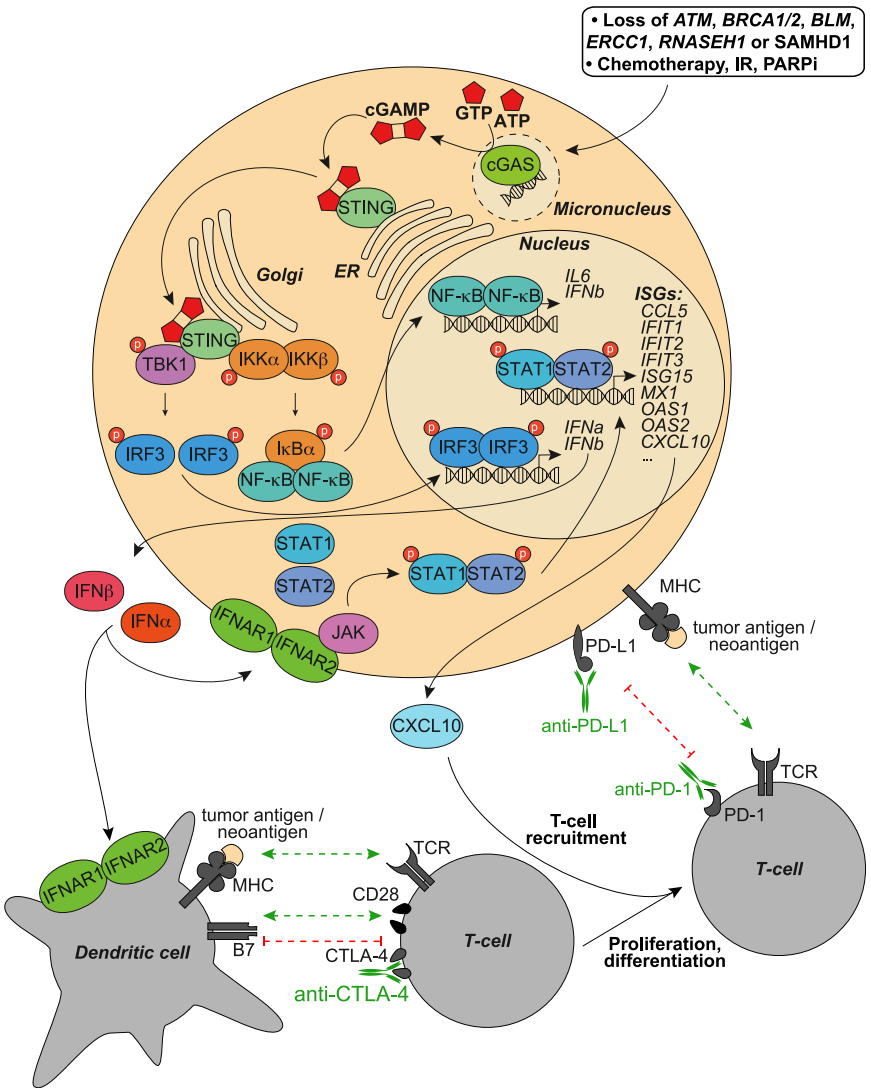

Figure 1. DNA Damage-Mediated cGAS-STING Activation Promotes Type I IFN Signaling and Anti-tumoral Immune Responses

Endogenous (e.g., DNA replication/repair gene inactivation) or exogenous (e.g., IR and PARPi) genotoxic stress triggers DNA damage accumulation, resulting in micronuclei formation. The DNA sensor cGAS binds the DNA encapsulated into micronuclei and catalyzes the synthesis of cGAMP using GTP and ATP. cGAMP activates endoplasmic reticulum (ER)-bound STING, which translocates to the Golgi apparatus, where it recruits IKKα/β and TBK1 kinases to phosphorylate IκBα and IRF3, respectively. NF-κB released from IκBα and IRF3 are activated and translocate into the nucleus, where they induce transcriptional targets, including type I interferons (IFNs). Secreted IFN-α and IFN-β bind to their heterodimeric receptor, IFNAR1/2, which activates JAK to phosphorylate STAT1 and STAT2. STAT1/2 heterodimer acts as a transcription factor and triggers expression of IFN-stimulated genes (ISGs). Type I IFN signaling activates immune effector cells by facilitating tumor antigen/neoantigen presentation on the major histocompatibility complex (MHC) of dendritic cells. This binds to the T cell receptor (TCR) and activates T cells in a process also dependent upon the balance between B7:CD28 stimulatory signals (green) and B7:CTLA-4 inhibitory signals (red). Activated T cells infiltrate tumors in response to chemokines (e.g., CXCL10) and recognize tumor antigens presented on the tumor cell surface. Interaction between PD-1 and PD-L1 limits the anti-tumor activity of the immune effectors. Inhibition of immunosuppressive signals (red) by anti-CTLA-4, anti-PD-1, or anti-PD-L1 antibodies (green) potentiates anti-tumorigenic immune responses.

NF-κB transcriptional activation (Gui et al., 2019; Gulen et al., 2017).

Due to their intrinsic genome instability, cancer cells can display constitutive activation of innate immunity and IFN signaling, which facilitates their targeting by cytotoxic lymphocytes. Thus, IFNs provide a link between anti-tumorigenic innate and adaptive immune responses (Chen et al., 2016; Parker et al., 2016; Zitvogel et al., 2015). IFN-α and IFN-β produced by cancer cells bind to IFN receptors on the same cell, neighboring cells, or immune cells, eliciting autocrine or paracrine signaling (Figure 1). The latter promotes mobilization of immune cells (e.g., dendritic cells and T cells) for tumor eradication (Diamond et al., 2011; Fuertes et al., 2011). Processing of cancer-associated antigens or neoantigens by dendritic cells is followed by their migration to the lymph node, where the antigens are presented on the major histocompatibility complex (MHC) to naive $CD8^+$ T cells. Binding to the MHC-antigen complex via their antigen-specific T cell receptor (TCR) primes $CD8^+$ T cells and enables them to detect MHC-antigen complexes on the surface of cancer cells upon tumor infiltration. T cell activation also depends on the balance between B7:CD28 stimulatory and B7:CTLA-4 inhibitory signals. Stimulatory signals promote survival and clonal proliferation of T cells recognizing a specific tumor antigen. In addition, ISG subsets encode proinflammatory chemokines (e.g., CXCL10; Figure 1), which play a major role in $CD8^+$ T cell recruitment from the lymph nodes to tumor and potentiate their cytotoxicity (Nagarsheth et al., 2017; Sen et al., 2019). $CD8^+$ T cells kill tumor cells primarily by secreting perforin, a protein that produces cytolytic pores in the membrane of cancer cells. Serine proteases such as granzyme B, also secreted by T cells, enter cancer cells via the perforin pores to induce caspase-mediated apoptosis (Lopez et al., 2013).

In order to evade type-I-IFN-mediated clearance, selection pressure leads to silencing of either cGAS or STING expression in tumors (Konno et al., 2018; Xia et al., 2016a, 2016b) or upregulation of cytoplasmic nucleases (e.g., TREX1; Vanpouille-Box et al., 2017). Although it is clear that cGAS-STING-activated type I IFN signaling has primarily anti-tumorigenic functions, paradoxically, it can also sustain tumorigenesis. For example, both type I and II IFN signaling activated JAK/STAT-dependent transcription and expression of programmed cell death ligand 1 (PD-L1) in human melanoma cells (Garcia-Diaz et al.,

Table 1. ISGs Upregulated in Response to Exogenous Genotoxic Stress or Loss of DDR Components

Gene Mutation/Treatment	ISGs	References
IR	*BST2*, *IFI6*, *IFI44*, *IFIT1*, *IFIT2*, *ISG15*, *MX1*, *OAS1*	Erdal et al., 2017
	CCL5, *ISG54*	Harding et al., 2017
	CCL5, *CXCL10*, *IFIT1*, *IFIT3*, *ISG15*, *OAS1*	Mackenzie et al., 2017
	IFI16, *IRF7*, *MX1*, *TMEM173*	Härtlova et al., 2015
MMC, cisplatin	*BST2*, *IFI6*, *IFI44*, *IFIT1*, *IFIT2*, *ISG15*, *OAS1*	Erdal et al., 2017
PARPi	*IFIT2*, *MX1*, *MX2*, *OAS2*	Wang et al., 2019
PARPi in BRCA1/2-deficient cells/tumors	*CCL5*	Chabanon et al., 2019
	CXCL10	Ding et al., 2018
	IFIT1, *IFIT2*, *IFI6*	Reisländer et al., 2019
ATM	*IFI16*, *IRF7*, *MX1*, *TMEM173*	Härtlova et al., 2015
BLM	*IFI27*, *IFI44L*, *IFIT1*, *IFIT2*, *ISG20*, *OAS1*, *MX1*	Gratia et al., 2019
BRCA1, *BRCA2*, or *FANCD2*	*CCL5*, *CXCL10*	Parkes et al., 2016; Pantelidou et al., 2019
	IFI6, *IFIT1*, *IFIT2*, *IFIT3*, *ISG15*, *OAS1*, *OAS2*	Reisländer et al., 2019
	IL-6, *IL-8*	Heijink et al., 2019
ERCC1	*CCL5*	Chabanon et al., 2019
RNASEH2A[a]	*CCL5*, *CXCL10*, *IFI44*, *IFIT1*, *IFIT3*, *OAS1*	Mackenzie et al., 2017
SAMHD1[a]	*ISG15*, *MX1*, *TNF*	Coquel et al., 2018
TREX1[a]	*BST2*, *IFI6*, *IFI44*, *IFIT1*, *IFIT2*, *ISG15*, *MX1*, *OAS1*	Erdal et al., 2017

[a]Mutations associated with Aicardi-Goutières syndrome.

2017). Furthermore, in a mouse model for carcinogen-induced skin tumors, chronic inflammation confers STING-dependent immune tolerance (Ahn et al., 2014). Chronic IFN signaling mediated by STAT1-dependent expression of two ISGs, *IFIT1* and *MX1*, was reported to have immunosuppressive and pro-tumorigenic functions in a melanoma mouse model (Benci et al., 2016). Chronic activation of the cGAS-STING pathway and downstream non-canonical NF-κB signaling promotes metastasis in mouse models of chromosome instability (Bakhoum et al., 2018).

It remains unclear how the tumor-promoting roles of cGAS-STING signaling become prevalent over its anti-tumorigenic activity. Fine-tuning the balance between inflammation-mediated tumor growth and tumor-suppressive cytotoxicity is required in order to exploit therapeutically cGAS-STING activation.

Activation of the Innate Immune Response by Loss of Genome Integrity Associated with DNA Replication/Repair Defects

It has been recognized early on that the efficacy of chemotherapeutic drugs (anthracyclines, oxaliplatin, and doxorubicin) relies in part on induction of anti-tumor immune responses mediated by type I IFN signaling and ISGs (Sistigu et al., 2014; Zitvogel et al., 2011). Likewise, IR-induced anti-tumor immunity was dependent on the cGAS-STING pathway (Deng et al., 2014). Recent studies (Erdal et al., 2017; Harding et al., 2017; Mackenzie et al., 2017) identified cytosolic DNA accumulation following chemotherapy or IR (Table 1) as the trigger to cGAS-STING activation, leading to IFN and ISG induction. Supporting this concept, innate immune responses were potentiated by loss of the 3′-5′cytoplasmic exonuclease TREX1, which can degrade either dsDNA or single-stranded DNA (ssDNA) cytosolic fragments (Lindahl et al., 2009; Wolf et al., 2016).

How cellular DNA, normally confined to membrane-enclosed nuclei or mitochondria, is evicted into the cytoplasm upon DNA damage remained an open question. Several studies have reported that DSB or stalled replication fork processing by nucleases and helicases (e.g., BLM, EXOI, DNA2, MUS81, and MRE11) can directly release fragments of genomic DNA into the cytoplasm (Coquel et al., 2018; Erdal et al., 2017; Ho et al., 2016). Two studies (Harding et al., 2017; Mackenzie et al., 2017) provided alternative insights into this conundrum by demonstrating that physiological breakdown of the nuclear envelope during mitosis or senescence provides the means for cytosolic DNA accumulation, a process potentiated by exposure to external sources of DNA damage. For example, IR generates DNA fragments, some of which are not incorporated into daughter nuclei during chromosome segregation and instead become enclosed in membrane-bound compartments termed micronuclei (Figure 1). cGAS associates with a subset of the IR-induced micronuclei, suggesting that recognition of cytosolic DNA encapsulated into micronuclei provides the signal for cGAS-STING pathway activation. Consistent with this, downstream events, including TBK1-dependent IRF3 phosphorylation, IFN-α and IFN-β expression, and STAT1 phosphorylation leading to ISG induction, were detected upon mitotic progression in irradiated human cells (Harding et al., 2017).

Innate immune responses following mitotic progression were also elicited by abrogation of *RNASEH2* (Mackenzie et al., 2017). RNase H2 is a nuclease whose mutations are associated with the autoinflammatory disorder Aicardi-Goutières syndrome, characterized by constitutive ISG expression (Crow and Manel,

2015). Loss of RNase H2 led to chromosome mis-segregation during mitosis and enhanced micronuclei formation. Importantly, cGAS was only associated with micronuclei with ruptured envelope, suggesting that its physical access to DNA is required for the activation of cGAS-STING signaling (Mackenzie et al., 2017). Similarly, chromosome mis-segregation caused by defects in chromosome attachment to microtubules during mitosis resulted not only in chromosomal instability but also in the formation of cGAS-infiltrated micronuclei (Bakhoum et al., 2018).

In addition to mitosis, cellular senescence provides a physiologically relevant setting for cytoplasmic DNA accumulation. A hallmark of senescence is loss of lamin B1, which leads to breakdown of the nuclear envelope and the release of cytoplasmic chromatin fragments (CCFs; Dou et al., 2017; Glück et al., 2017; Wang et al., 2017). Similarly to micronuclei, CCFs recruit cGAS and trigger proinflammatory cytokine production, a process known as senescence-associated secretory phenotype (SASP; Li and Chen, 2018; Tchkonia et al., 2013; Yang et al., 2017). cGAS-STING activation by CCFs not only regulates SASP but also contributes to preventing senescence evasion and tumorigenesis.

These studies reinforced the concept that DNA compartmentalization into the nucleus or mitochondria is essential to suppress illegitimate cGAS-STING activation during unperturbed cell proliferation. They did not explain, however, why during normal mitotic progression, when nuclear envelope breaks down, exposing chromosomal DNA to cytoplasmic activities, chromosomes remain refractory to cGAS recognition. The observation that cGAS binds nucleosomes with higher affinity than naked DNA led to the discovery that, although cGAS is recruited to chromatin during unperturbed mitosis, it only poorly stimulates phosphorylation of IRF3 (Zierhut et al., 2019). Moreover, when mitotic progression was arrested with Taxol, cGAS promoted IRF3-mediated apoptosis independently of its transcription activity, providing exciting novel insight into the mechanism of action of this drug routinely used in cancer therapeutics.

Interestingly, immunofluorescence and cell fractionation experiments revealed cGAS localization within the nucleus under physiological conditions (Gentili et al., 2019; Jiang et al., 2019; Liu et al., 2018; Volkman et al., 2019). Nuclear envelope breakdown during mitosis (Gentili et al., 2019; Yang et al., 2017; Zierhut et al., 2019) or active nuclear import (Liu et al., 2018) could explain cGAS localization in the nucleus. In contrast to cytosolic cGAS, chromosome-tethered cGAS lacks the ability to sense self-DNA and activate innate immune responses (Gentili et al., 2019; Jiang et al., 2019; Volkman et al., 2019). Instead, chromatin-bound cGAS binds poly(ADP-ribose) (PAR; Liu et al., 2018) and inhibits homologous recombination repair (Jiang et al., 2019; Liu et al., 2018). To date, the exact functions of cGAS nuclear tethering remain elusive, and further investigations are needed to unravel them.

Multiple studies reported that abrogation of DNA replication and/or repair factors is not only detrimental to genome integrity but also activates cGAS-STING signaling. This further substantiated the link between cancer-causing genomic instability and induction of innate immunity. For example, loss of ATM checkpoint kinase or ERCC1, a DNA repair factor implicated in excision repair, can trigger DNA damage and cytoplasmic DNA accumulation, which in turn activates cGAS-STING and type I IFN responses (Chabanon et al., 2019; Härtlova et al., 2015).

SAMHD1, another gene associated with Aicardi-Goutières syndrome, encodes a deoxyribonucleotide triphosphate (dNTP) triphosphohydrolase required for replication fork progression. Inhibiting *SAMHD1* expression causes replication failure, leading to genomic DNA accumulation in the cytosol and cGAS-STING activation (Coquel et al., 2018). BLM is a DNA helicase acting at the interface between replication and repair. BLM promotes not only the restart of stalled replication forks (Davies et al., 2007), but also DSB resection (Nimonkar et al., 2011) and Holliday junction dissolution (Bizard and Hickson, 2014), both key steps in homologous recombination repair. Consequently, *BLM* gene mutations cause Bloom syndrome, which is associated with genetic instability and cancer predisposition (Amor-Guéret, 2006). Recently, it was found that pathogenic *BLM* mutations also trigger activation of the cGAS-STING pathway, type I IFN responses, and induction of the ISG signature in the blood of Bloom syndrome patients (Gratia et al., 2019). This effect was potentiated by inactivation of the cytosolic nuclease TREX1 or treatment with replication inhibitors, aphidicolin or hydroxyurea.

A discovery of important clinical relevance was that abrogation of BRCA1 or BRCA2 tumor suppressors, known to cause replication failure and DSB accumulation (Tarsounas and Sung, 2020; Zhao et al., 2019), also elicited type I IFN signaling and anti-tumor immunity. The response was first identified as enhanced $CD4^+$ and $CD8^+$ T cell infiltration in BRCA1/2-deficient breast cancer patients (Parkes et al., 2016). Furthermore, overexpression of CCL5 and CXCL10 cytokines, dependent on STING, TBK1, and IRF3, was specifically detected in human cells lacking BRCA1 or BRCA2. Two subsequent studies (Heijink et al., 2019; Reisländer et al., 2019) identified an extensive transcriptional signature of innate immunity in human cells and tumors lacking BRCA1 or BRCA2, which was dependent on cGAS-STING pathway and accompanied by accumulation of cGAS-positive micronuclei.

These studies demonstrated that in the absence of BRCA1 or BRCA2, high endogenous levels of DNA damage caused by defective DNA repair and/or replication lead to cell-intrinsic type I IFN responses. These, however, were not sufficiently potent to prevent BRCA1/2-deficient tumor formation. Therefore, it was investigated whether exacerbating the intrinsic DNA damage with chemotherapeutic drugs can enhance innate immunity. BRCA1/2-deficient cells and tumors are hypersensitive to PAR polymerase (PARP) inhibitors (PARPi), which act by inhibiting PARP catalytic activity and the DNA repair mechanisms dependent on it. They also trap PARP on the DNA, thus obstructing replication fork progression. Both mechanisms inflict DNA damage lethal to BRCA1/2-deficient but not normal cells, which underpinned the approval of several PARPi for clinical use in *BRCA1/2*-mutated cancer patients (Lord and Ashworth, 2017). Studies carried out in BRCA1/2-deficient cells and tumors (Ding et al., 2018; Pantelidou et al., 2019; Reisländer et al., 2019; Wang et al., 2019) reported that PARPi treatment enhanced endogenous genomic instability and this, in turn, caused cGAS-STING activation and ISGs expression (Table 1). Moreover, PARPi stimulated T cell infiltration, leading to tumor eradication in BRCA1/2-deficient mouse models for ovarian and breast cancer. These results added a new dimension to the

Table 2. Clinical Trials of DDR and Immune Checkpoint Inhibitors, in Combination or as Single Therapy (Selected)

Clinical Trial ID	Phase	Cancer Type	Biomarker	Target	Drug	Target	Drug
NCT02563002 (KEYNOTE-177)	3	colorectal	MMR deficiency	–	–	PD-1	pembrolizumab
NCT01876511[a]	2	colorectal, solid tumors	MSI/MMR deficiency	–	–	PD-1	pembrolizumab
NCT02693535	2	various	DDR deficiency	–	–	PD-1	nivolumab
						CTLA-4	ipilimumab
NCT03025035	2	breast	*BRCA* mutations	–	–	PD-1	pembrolizumab
NCT03040791	2	prostate	DDR deficiency	–	–	PD-1	nivolumab
NCT03598270 (ANITA)	3	ovarian	–	PARP	niraparib	PD-L1	atezolizumab
NCT03602859 (FIRST)	3	ovarian	–	PARP	niraparib	PD-1	dostarlimab
NCT04380636 (KEYLYNK-012)	3	lung	–	PARP	olaparib	PD-1	pembrolizumab
NCT03834519 (KEYLYNK-010)	3	prostate	–	PARP	olaparib	PD-1	pembrolizumab
NCT04191135 (KEYLYNK-009)	2/3	breast	–	PARP	olaparib	PD-1	pembrolizumab
NCT03976362 (KEYLYNK-008)	3	lung	–	PARP	olaparib	PD-1	pembrolizumab
NCT03976323 (KEYLYNK-006)	3	lung	–	PARP	olaparib	PD-1	pembrolizumab
NCT03522246 (ATHENA)	3	ovarian	–	PARP	rucaparib	PD-1	nivolumab
NCT03824704	2	ovarian	*BRCA* mutations	PARP	rucaparib	PD-1	nivolumab
NCT02849496	2	breast	HR deficiency	PARP	olaparib	PD-L1	atezolizumab
NCT04276376	2	solid tumors	DDR deficiency	PARP	rucaparib	PD-L1	atezolizumab
NCT03565991	2	solid tumors	*BRCA* or *ATM* mutations	PARP	talazoparib	PD-L1	avelumab
NCT04034927	2	gynecologic	–	PARP	olaparib	CTLA-4	tremelimumab
NCT02657889[b] (KEYNOTE-162)	1/2	breast, ovarian	–	PARP	niraparib	PD-1	pembrolizumab
NCT04266912	1/2	solid tumors	DDR deficiency	ATR	M6620	PD-L1	avelumab
NCT02264678	1	head and neck, lung	–	ATR	ceralasertib	PD-L1	durvalumab
NCT04095273	1	solid tumors	DDR deficiency	ATR	BAY1895344	PD-1	pembrolizumab
NCT03495323	1	solid tumors	–	CHK1	prexasertib	PD-L1	LY3300054

DDR, DNA damage response; HR, homologous recombination; MMR, mismatch repair; MSI, microsatellite instability.
[a]Le et al., 2015
[b]Konstantinopoulos et al., 2019

highly specific toxicity characteristic of PARPi and set the stage for the development of novel combination therapies (see below; Table 2).

Expanding the Focus of Current Cancer Immunotherapy to Innate Immunity

The ability of cancer cells to escape immune detection and destruction relies in part on expression on their surface of proteins with immunosuppressive functions. One example is PD-L1, which engages the PD-1 receptor of T cells (Figure 1), thus arresting their proliferation and inhibiting their cytotoxic activity (Wei et al., 2018). Another negative regulator of immune effectors exploited by tumor cells is the cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4; Buchbinder and Desai, 2016; Zou et al., 2016). This receptor, present on the cell surface of T cells, interacts with B7 on the cell surface of antigen-presenting cells to prevent T cell activation (Buchbinder and Desai, 2016). Consequently, immune checkpoint blockade consisting of antibodies against PD-1, PD-L1, or CTLA-4 enhances adaptive immunity and inhibits tumor growth (Havel et al., 2019; Topalian et al., 2015; Zou et al., 2016). Several immunomodulatory therapies have been recently granted US Food and Drug Administration (FDA) breakthrough designation, including the anti-PD-L1 antibodies avelumab and durvalumab, the anti-PD-1 antibody pembrolizumab and the anti-CTLA-4 antibody ipilimumab (Table 2).

Although they show very promising results in the clinic, the benefits of immune checkpoint inhibitors are limited to a subset of cancer patients with a tumor microenvironment permissive to IFN secretion and T cell infiltration. One of the most pressing current clinical challenges is to convert nonresponsive, "cold" tumors to responsive, "hot" tumors (Sharma and Allison, 2015). Pharmacological activation of cGAS-STING signaling is under investigation for this purpose. DMXAA and ADU-S100 are STING agonists, which promote functional anti-tumoral responses, including type I IFN induction and $CD8^+$ T cell activation (Corrales et al., 2015; Sivick et al., 2018). Importantly, DMXAA can only activate mouse STING and has no effect on the human protein (Conlon et al., 2013). One major impediment to the clinical applications of these compounds is that they require intra-tumoral delivery, which limits their use to accessible solid tumors. A recently developed class of STING agonists, di-ABZI (Ramanjulu et al., 2018), can be administered intravenously and might overcome this limitation. However, these compounds

increase the blood levels of type I IFN and proinflammatory cytokines, which is reminiscent of systemic inflammation and may be detrimental to patients.

Tumor-targeted immunity remains the most desirable strategy for tumor eradication. Enhancing tumor-intrinsic genomic instability for the activation of the cGAS-STING axis represents a tumor-targeted approach with promising results in preclinical and clinical trials (Table 2). As a prominent example, PARPi treatment augmented micronuclei formation and induced cGAS-STING activation specifically in ERCC1-, BRCA1-, or BRCA2-deficient cells and tumors (Chabanon et al., 2019; Ding et al., 2018; Pantelidou et al., 2019; Reisländer et al., 2019). The functional relationship between PARPi-induced immune responses and anti-tumor immunity was highlighted in preclinical models of BRCA1-deficient ovarian (Ding et al., 2018; Huang et al., 2015) and triple-negative breast tumors (Pantelidou et al., 2019). Comparison of immuno-competent and immuno-deficient mice revealed that PARPi had greater anti-tumor effects in the mice with an intact immune system, where it increased the levels of infiltrating lymphocytes within the tumor (Ding et al., 2018; Pantelidou et al., 2019). Similarly, chemical inhibition of CHK1, a downstream target of ATR, in a mouse model for small cell lung cancer led to activation of the cGAS-STING axis and secretion of CXCL10 and CCL5 chemokines in the tumor microenvironment. Notably, both CHK1 inhibitors (CHK1i) and PARPi also enhanced PD-L1 expression (Sen et al., 2019), which provided a rationale for investigating the impact of PARPi or CHK1i in combination with PD-L1 inhibitors on tumor growth. Both combinations led to increased cGAS-STING-dependent innate immune responses and synergistic effects on tumor eradication, independently of BRCA status (Sen et al., 2019). A comparable synergistic and prolonged response was reported in BRCA1-deficient tumors treated with PARPi and anti-CTLA-4 antibody (Higuchi et al., 2015), as well as in BRCA1-proficient and BRCA1-deficient tumors treated with PARPi and anti-PD-1 antibody (Wang et al., 2019). The same combination demonstrated anti-tumor activity in a clinical study in patients with ovarian cancer (Konstantinopoulos et al., 2019; Table 2). Hence, concomitant PARP, ATR, or CHK1 inhibition with immune checkpoint blockade (e.g., PD-1, PD-L1, or CTLA-4) is extensively explored in the clinic. Phase 3 clinical studies together with selected phase 1/2 studies testing these drug combinations in the context of specific genetic or phenotypic backgrounds (e.g., *BRCA* or other DDR gene mutations) are summarized in Table 2.

Ongoing clinical trials also investigate the efficiency of checkpoint blockade as single treatment in tumors carrying inactivation of other DDR factors or pathways (Table 2). For example, the hyper-mutator phenotype characteristic of *BRCA1/2*-mutated or mismatch repair (MMR)-deficient cancers promotes adaptive immune responses independently of the cGAS-STING axis and IFN signaling (Germano et al., 2017; Strickland et al., 2016). These tumors accumulate mutations that alter the amino acid sequence of endogenous proteins, leading to their exposure on tumor cell surface as neoantigens, where they are recognized by tumor-infiltrated T cells (Figure 1). Hence, the immune checkpoint blockade may facilitate the detection and elimination of neoantigen-positive tumors by T cells in patients. Consistent with this, PD-1 checkpoint inhibition shows clinical activity against MMR-deficient, neoantigen-positive tumors (Le et al., 2015; Table 2), which encouraged further efforts toward exploiting neoantigen formation for cancer immunotherapy (Ali et al., 2019; Schumacher et al., 2019). It remains to be investigated whether PARPi and/or other DDR inhibitors can similarly increase mutation load and induce neoantigen expression in human tumors.

Conclusions/Perspectives

Tumor-intrinsic genomic instability can be further enhanced by chemotherapy, IR, or DDR inhibitors to instigate innate immune responses. These promote tumor eradication via IFN-dependent T cell activation. Consistently, DNA damage-inducing therapies act synergistically with the immune checkpoint blockade to inhibit tumor growth. This has invaluable therapeutic potential for the development of cancer treatments based on novel drug combinations. In the future, it will be essential to optimize predictive biomarkers that will enable design of the most effective combination regime for each patient. In addition to mutations in DNA repair/replication genes, such biomarkers include expression levels of immune checkpoint ligands or receptors (e.g., *CD274* encoding PD-L1 or *PDCD1* encoding PD-1) or cGAS-STING pathway components (e.g., *TMEM173* encoding STING). Furthermore, a comprehensive understanding of the DNA-damage-induced innate immune responses at the cellular and organismal levels is essential. In this respect, investigating potentially novel, virus-independent functions of ISGs in the context of genome instability might reveal novel targets for future cancer treatments.

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