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# Roles of the cGAS-STING Pathway in Cancer Immunosurveillance and Immunotherapy

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## Keywords

cGAS, cGAMP, STING, antitumor immunity, cancer immunotherapy

## Abstract

Cyclic GMP-AMP synthase (cGAS) is a cytosolic DNA sensor that initiates innate immune responses. DNA-bound cGAS produces cyclic GMP-AMP (cGAMP), which activates stimulator of interferon genes (STING) to induce inflammatory cytokines and other immune mediators. cGAS detects DNA without sequence specificity and responds to both cytosolic foreign DNA from pathogens and self-DNA leaked into the cytosol due to genome instability or cellular damage. Because of the diverse sources of cytosolic DNA, the cGAS-STING pathway plays a critical role during infection, autoimmune diseases, and senescence. Moreover, cGAS detects tumor-derived DNA and stimulates endogenous antitumor immunity. Thus, the cGAS-STING pathway is a promising target for cancer immunotherapy. Here, we review the role of the cGAS-STING pathway in various diseases and highlight various approaches targeting the cGAS-STING pathway for cancer therapy.

## The cGAS-STING PATHWAY

The innate immune system is composed of molecules and cells that respond to external and internal danger signals, such as pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). PAMPs and DAMPs bind to their respective pattern recognition receptors (PRRs) to initiate immune responses. Membrane-bound PRRs such as Toll-like receptors (TLRs) detect extracellular pathogens on the cell surface or pathogen-derived nucleic acids in the endosomes (Kawai & Akira 2009). On the other hand, cytosolic PRRs including nucleotide-binding oligomerization domain–like receptors, retinoic acid–inducible gene I–like receptors (RLRs), and cyclic GMP-AMP synthase (cGAS) detect intracellular pathogens (Wu & Chen 2014). Upon ligand binding, PRRs activate downstream signaling cascades to induce inflammatory responses, providing early protection against pathogen invasion or cellular damage. PRR-induced innate immune responses further activate the adaptive immune system that specifically eliminates pathogens or damaged or malignant cells. In this review, we focus on the cGAS-STING (stimulator of interferon genes) pathway and its role in infectious diseases, autoimmune diseases, senescence, tumor immunity, and cancer immunotherapy.

DNA is often called the blueprint of life, as it encodes the genetic information for all living organisms except for RNA viruses. Accordingly, hosts have evolved innate immune pathways to recognize the invasion of pathogen DNA into the cytosol; one of these is the cGAS-STING pathway (Chen et al. 2016, Ishikawa & Barber 2008, Sun et al. 2013, Wu et al. 2013, Zhong et al. 2008). Two molecules of cGAS bind to two molecules of double-stranded DNA (dsDNA) to form a $cGAS_2$-$DNA_2$ complex (X. Li et al. 2013, Zhang et al. 2014). Upon binding DNA, cGAS undergoes conformational changes at the active site that allow cGAS to convert ATP and GTP into 2′3′-cyclic GMP-AMP (cGAMP) (Ablasser et al. 2013; Civril et al. 2013; Diner et al. 2013; Gao et al. 2013a,b; Kranzusch et al. 2013; Wu et al. 2013; X. Li et al. 2013; Zhang et al. 2013, 2014).

cGAMP functions as a second messenger that binds to its endoplasmic reticulum (ER)-resident adaptor protein STING (**Figure 1**) (Wu et al. 2013). cGAMP binding induces a conformational change in STING that may expose the C-terminal tail for TBK1 binding and activation (Gao et al. 2013b, Tanaka & Chen 2012, Zhang et al. 2013). TBK1 phosphorylates IRF3, which induces type I interferons (IFNs) (Fitzgerald et al. 2003, S. Liu et al. 2015, Sharma et al. 2003, Tanaka & Chen 2012). Type I IFNs bind to the type I IFN receptor, which activates a signaling cascade leading to the expression of hundreds of IFN-stimulated genes (ISGs) (Schneider et al. 2014). Type I IFNs and ISGs elicit antiviral responses by promoting antiproliferative and immunomodulatory activities. STING also activates IKK and subsequently NFκB for proinflammatory cytokine induction. After cGAMP binding, STING traffics from the ER to the ER-Golgi intermediate compartment and then to the Golgi apparatus (Ishikawa et al. 2009, Saitoh et al. 2009). After signaling, STING is transferred to autophagosomes and lysosomes, where it is degraded. In addition to transmitting inflammatory signals by secreting cytokines, cGAMP can be transferred into nearby cells through gap junctions or packaged into viruses during viral replication (Ablasser et al. 2013, Bridgeman et al. 2015, Gentili et al. 2015). Thus, the cGAS-STING pathway initiates immune responses and plays an important role in infections and diseases involving cytosolic DNA.

## PHYSIOLOGICAL AND PATHOLOGICAL ROLES OF THE cGAS-STING PATHWAY

cGAS is a critical PRR that senses pathogen DNA in the cytosol. Interestingly, cGAS recognizes dsDNA irrespective of sequence or species; thus, activation of the cGAS-STING pathway by self-DNA is avoided using two main strategies: compartmentalization of self-DNA by nuclear or



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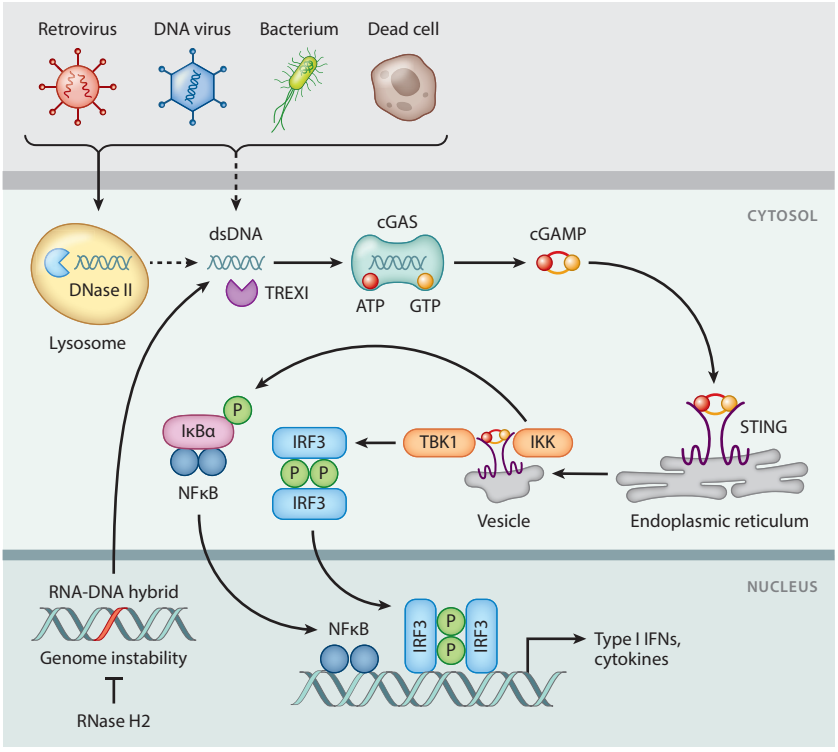


**Figure 1**

The cGAS-STING pathway. Pathogen-derived DNA (i.e., dsDNA from DNA viruses and bacteria or reverse-transcribed DNA from retroviruses) or self-DNA (from mitochondria or nuclei of dead or damaged cells) can be exposed to the cytosol by an unknown mechanism. To regulate the level of cytosolic DNA, RNase H2 maintains genome stability by degrading RNA-DNA hybrids or removing misincorporated ribose from DNA while DNase III (also known as TREX1) and DNase II degrade DNA in the cytosol and lysosomes, respectively. Cytosolic DNA binds to and activates cGAS, which catalyzes the production of cGAMP from GTP and ATP. cGAMP binds to its ER adaptor STING, which then translocates from the ER to the Golgi apparatus to activate IKK and TBK1. IKK phosphorylates and induces IκBα degradation, thereby activating NFκB. TBK1 phosphorylates IRF3, which causes IRF3 dimerization. Activated NFκB and the IRF3 dimer then migrate to the nucleus, where they induce type I IFNs and proinflammatory cytokines. Abbreviations: cGAMP, cyclic GMP-AMP; cGAS, cyclic GMP-AMP synthase; dsDNA, double-stranded DNA; ER, endoplasmic reticulum; IFN, interferon; STING, stimulator of interferon genes.

mitochondrial membranes and negative regulation of the pathway to prevent DNA accumulation in the cytosol. If the cGAS-STING pathway is aberrantly activated by lack of regulation, the immune system is activated without pathogen invasion, resulting in autoimmune diseases. The cGAS-STING pathway also senses the leakage of nuclear DNA into the cytoplasm and acts as a danger sensor by inducing senescence.

## Infectious Diseases

The cGAS-STING pathway detects the invasion of pathogens that release DNA into the cytosol of host cells. These pathogens include DNA viruses, retroviruses that reverse-transcribe their single-stranded RNA genome into DNA, and intracellular bacteria. Upon infection with these

pathogens, the cGAS-STING pathway is required for induction of ISGs and proinflammatory cytokines. Furthermore, mice deficient in cGAS or STING display greater pathogen burden and lethality. The essential role of the cGAS-STING pathway in infectious disease is summarized in other reviews and in the **Supplemental Appendix** (Cai et al. 2014, Chen et al. 2016, Ma & Damania 2016).

Supplemental Material >

## Sterile Inflammation, Autoimmune Diseases, and Ischemia

Preventing aberrant activation of the cGAS-STING pathway is important for maintaining immune homeostasis. Several enzymes regulate cGAS activation by controlling the basal level of cytosolic DNA: RNase H2, TREX1, and DNase II. Mice deficient in these functional enzymes develop autoimmune disease with inflammation and lethality in a cGAS- and STING-dependent manner, suggesting a critical role for the cGAS-STING pathway in the pathogenesis of autoimmune diseases (Ahn et al. 2012, Gall et al. 2012, Gao et al. 2015, Gray et al. 2015, Mackenzie et al. 2016, Pokatayev et al. 2016). In addition, the activation of the cGAS-STING pathway by self-DNA may be involved in sterile inflammation. Recent studies show that myocardial infarction damage is mediated in part by the cGAS-STING pathway and type I IFNs (Cao et al. 2018, King et al. 2017). Activation of cGAS and STING has also been linked to age-related macular degeneration (Kerur et al. 2018) and liver fibrosis (Iracheta-Vellve et al. 2016). The critical role of the cGAS-STING pathway in autoimmune diseases and inflammatory diseases suggests that cGAS is a promising therapeutic target. Details of the effect of the cGAS-STING pathway in autoimmune and inflammatory disease mouse models and patients are summarized in the **Supplemental Appendix** and in other reviews (Chen et al. 2016, Crowl et al. 2017).

## Senescence, Genomic Instability, and Oncogenesis

Stressed and damaged cells induce senescence, a state of irreversible cell cycle arrest, to provide a natural barrier to tumorigenesis. The known causes of senescence include laminopathies, oncogene activity, DNA damage, and telomere attrition (Campisi & d'Adda di Fagagna 2007). All of the above stimuli directly or indirectly induce DNA damage followed by the DNA damage response, formation of micronuclei and cytoplasmic chromatin fragments (CCFs), and the senescence-associated secretory phenotype (SASP). SASP includes the secretion of inflammatory cytokines, growth factors, and proteases that regulate immune cell recruitment and the tissue microenvironment. Recent studies revealed that the cGAS-STING pathway detects micronuclei and CCFs and is essential for SASP in senescent cells (**Figure 2**).

During senescence, the integrity of the nuclear envelope decreases via downregulation of Lamin B1. Damaged CCFs may bud off or leak into the cytosol through the fragile nuclear membrane (Gluck et al. 2017). DNA damage also promotes chromosome mis-segregation during cell division and increases CCF formation (Mackenzie et al. 2017). CCFs recruit nuclear envelope components and form micronuclei that have less membrane integrity and are prone to rupture. Micronuclei colocalize with cGAS, which induces a type I IFN response (Bartsch et al. 2017, Gluck et al. 2017, Harding et al. 2017, Mackenzie et al. 2017, Yang et al. 2017). Recognition of micronuclei by cGAS was essential for SASP induction in senescence induced by drug treatments, oxidative stress, oncogenic Ras, or ionizing radiation in vitro (Dou et al. 2017, Gluck et al. 2017, Yang et al. 2017). Furthermore, cGAS- and STING-deficient mice did not show senescence and SASP in response to whole-body ionizing radiation or expression of the oncogene *RasV12* (Dou et al. 2017, Gluck et al. 2017). Notably, *RasV12*-expressing senescent cells persisted in cGAS- and STING-deficient mice, while wild-type mice cleared these senescent cells. This observation

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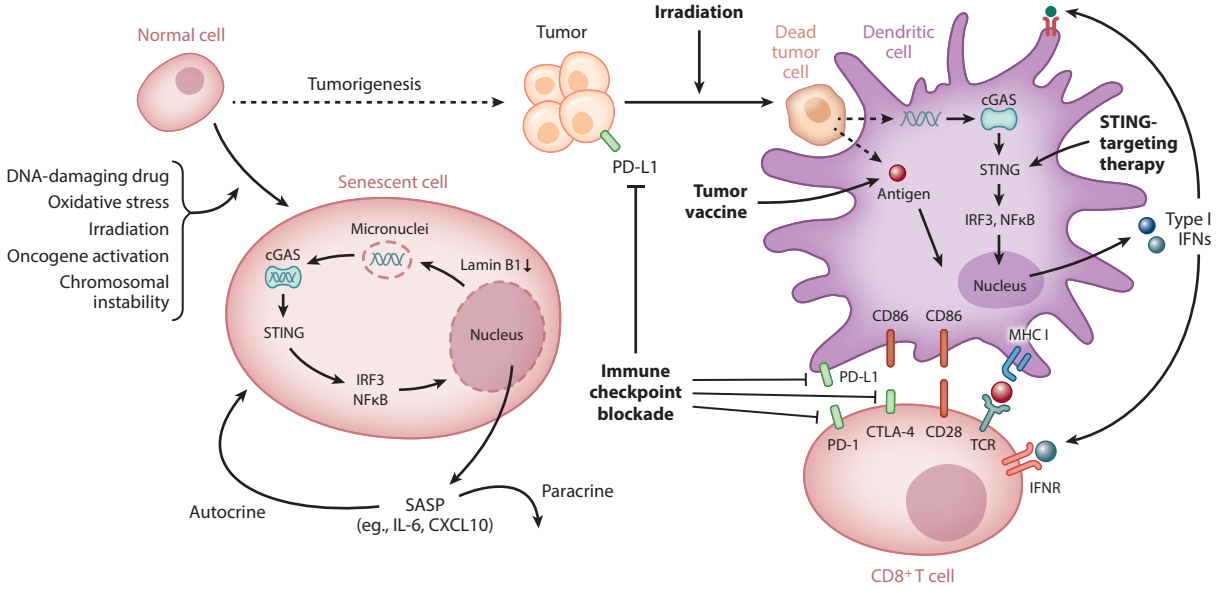


**Figure 2**

Roles of the cGAS-STING pathway in cellular senescence and tumor immunity. Normal cells undergo senescence after exhaustive proliferation or senescence-inducing treatments. Senescent cells show decreased nuclear membrane integrity due to downregulation of Lamin B1. DNA released from the nucleus or micronuclei activate the cGAS-STING pathway. Activation of the cGAS-STING pathway leads to SASP, which includes the production of proinflammatory cytokines. When normal cells overcome cell cycle checkpoints and senescence, they become tumor cells with the ability to undergo massive proliferation. Dying tumor cells can be taken up by dendritic cells, allowing tumor-derived antigens to be presented to $CD8^+$ T cells via MHC class I molecules. Tumor-derived DNA is exposed to the cytosol by an unknown pathway to activate the cGAS-STING pathway. This activation induces type I IFN production and promotes cross-priming of $CD8^+$ T cells. Multiple STING-targeting anticancer therapies and tumor radiation therapy promote STING activation. Tumor vaccines and immune checkpoint blockade therapies cooperate with STING-targeting agents to enhance antitumor effects. Abbreviations: cGAS, cyclic GMP-AMP synthase; IFN, interferon; IFNR, interferon receptor; SASP, senescence-associated secretory phenotype; STING, stimulator of interferon genes; TCR, T cell receptor.

suggests that cGAS-induced SASP may play a role in preventing tumorigenesis. Supporting this idea, low expression of cGAS and STING in tumors is linked to decreased survival of human lung adenocarcinoma patients and poor prognosis of gastric cancer and hepatocellular carcinoma patients (Bu et al. 2016, Song et al. 2017, Yang et al. 2017). In addition, many tumor cell lines have lost the expression of cGAS, STING, or both (Bhatelia et al. 2014; Chen et al. 2017; Sun et al. 2013; Wu et al. 2013; Xia et al. 2016a,b).

Despite the critical role of the cGAS-STING pathway in senescence, *cGAS*-knockout mice do not spontaneously develop tumors, indicating that other tumor-suppressing pathways are sufficient to prevent tumor development (Yang et al. 2017). Moreover, multiple human cancers preserved the cGAS-STING pathway during tumorigenesis and showed a correlation between STING expression and an increased proinflammatory gene profile (Dou et al. 2017, Yang et al. 2017). The presence of multiple tumor-suppressing pathways, the complex origin of tumors, and the role of the cGAS-STING pathway in both senescence and inflammation makes the role of cGAS in tumorigenesis very complex. Nevertheless, recent studies on senescence propose a new function for cGAS as a danger sensor that detects self-DNA in damaged, stressed, or transformed cells. Immune cells recruited by SASP will then remove dangerous cells harboring damaged or mutated DNA that could potentially develop into cancer.

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Although cGAS deficiency does not itself promote spontaneous tumorigenesis, it will be interesting to test if cGAS deficiency promotes tumorigenesis in oncogene- or carcinogen-driven tumor models. Downregulation of type I IFN signaling was reported to decrease oncogene-induced senescence and promote tumorigenesis in chemically induced tumors (Dunn et al. 2005, Katlinskaya et al. 2016, Katlinski et al. 2017). As cGAS is essential for type I IFN production during senescence, cGAS may play a similar role in suppressing tumorigenesis. Senescence is also involved in tissue regeneration and repair, thus playing an important role in aging. The role of the cGAS-STING pathway in age-related diseases such as atherosclerosis or neurodegenerative diseases remains to be further investigated. If cGAS plays a critical role in age-related diseases, inhibition of cGAS may provide benefits to a large population of patients with these diseases.

## Tumor Immunity

In 1863, Rudolf Virchow observed lymphoid cells in a tumor, providing a possible link between the immune system and tumors (Balkwill & Mantovani 2001). The presence of adaptive immunity against tumors was later evidenced by the spontaneous activation and infiltration of tumor antigen–specific $CD8^+$ T cells (Anichini et al. 1999, Lee et al. 1999, Valmori et al. 2002). The activation of the innate immune system precedes the priming of tumor antigen–specific $CD8^+$ T cells, and the level of ISGs is correlated with infiltrating T cell markers in metastatic melanoma, suggesting activation of IFN-inducing pathways in response to tumors (Harlin et al. 2009). In murine tumor models, type I IFN signaling in dendritic cells (DCs), specifically $CD8\alpha^+$ DCs, was shown to be essential for antitumor T cell priming (Diamond et al. 2011, Fuertes et al. 2011). Thus, type I IFN production in response to tumors is a critical factor in the formation of antitumor immune responses; however, how tumors induce type I IFNs was not understood until recently (**Figure 2**).

Multiple innate immune signaling pathways such as TLRs, RLRs, and cGAS-STING can produce type I IFNs. Using genetically modified mice that are deficient in PRRs or signaling adaptors, it was shown that the cGAS-STING pathway is essential for type I IFN production in tumors and the generation of antitumor immune responses. Mice lacking components of the TLR or RLR pathway, but not STING-deficient mice, can still spontaneously prime tumor antigen–specific $CD8^+$ T cells (Woo et al. 2014); moreover, tumor-infiltrating antigen-presenting cells (APCs) produced type I IFNs in a STING-dependent manner. The activation of STING indicates the presence of cytosolic DNA that activates cGAS; indeed, tumor-infiltrating DCs contain tumor-derived DNA that is not restricted to lysosomes (Woo et al. 2014). Another study suggested that endothelial cells are also a source of type I IFN in the tumor microenvironment (Demaria et al. 2015). These studies suggest that tumor-derived DNA is transferred into host cells and is then released into the cytosol of those cells to activate cGAS. Dying tumor cells can be phagocytosed by immune cells or can directly transfer DNA into endothelial cells (Arandjelovic & Ravichandran 2015, Ehnfors et al. 2009). How tumor-derived DNA reaches the cytosol of immune cells is yet to be understood. In addition, tumor cells that express cGAS may generate cGAMP after cellular stress or DNA damage. It will be interesting to dissect the role of cGAS in tumor cells versus host cells in antitumor immunity in vivo.

Due to the existence of endogenous antitumor immunity, immunotherapy that stimulates the host immune system has opened a new era in cancer therapy. In fact, immune checkpoint blockade therapy has shown remarkable effects in clinics, improving long-term survival in approximately 20% of patients on average, although the response rates vary greatly among different tumor types (Postow et al. 2015). Immune checkpoint inhibitors include neutralizing antibodies blocking T cell inhibitory molecules such as CTLA-4, PD-1, and PD-L1. CTLA-4 and PD-1 are expressed on the T cell membrane and inhibit T cell activation when they are bound to their cognate ligands,



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namely costimulatory molecules on APCs for CTLA-4 or PD-L1 expressed on APCs or tumor cells for PD-1. The physiological role of CTLA-4 and PD-1 is to maintain immune homeostasis (Nishimura et al. 1999, Tivol et al. 1995, Waterhouse et al. 1995). Despite great success in treating cancer patients, immune checkpoint blockade therapy is not effective when the tumor is cold, that is, lacking infiltrating T cells or possessing a microenvironment that suppresses T cell function. As the cGAS-STING pathway is shown to be essential in priming $CD8^+$ T cells and promoting leukocyte infiltration, the therapeutic efficacy of the anti-PD-L1 antibody depends on cGAS and STING activation in the mouse melanoma model (Wang et al. 2017).

## APPLICATION OF THE cGAS-STING PATHWAY IN CANCER IMMUNOTHERAPY

Following the development of immune checkpoint blockade therapy, a major focus of current immunotherapy is to augment innate immunity and foster a $CD8^+$ T cell–rich tumor environment. The essential role of the cGAS-STING pathway in spontaneous type I IFN induction and $CD8^+$ T cell priming reveals that this pathway is a potential immunotherapy target. Using rodent tumor models, many preclinical studies assessed the therapeutic index of STING agonists and other anticancer modalities that trigger the cGAS-STING-IFN axis. Moreover, the combination of STING agonists and immune checkpoint inhibitors showed a synergistic effect in treating tumors. In this section, therapeutic approaches utilizing the cGAS-STING pathway are categorized into STING agonists, radiation therapy, chemotherapy, antibody therapy, viral therapy, and therapeutic vaccines, as summarized in **Table 1**. In addition, combinations of STING-targeting therapies and other therapies are listed.

### STING Agonists

Detection of tumor-derived DNA by cGAS produces cGAMP, which activates STING and subsequent antitumor immune responses. To enhance antitumor immune responses, researchers have used various tumor models to test several natural and synthetic STING agonists.

**Mouse STING agonist.** 5,6-Dimethylxanthenone-4-acetic acid (DMXAA) was developed as a cell-permeable drug to disrupt tumor vasculatures and cause hemorrhagic necrosis of tumor tissue (Daei Farshchi Adli et al. 2017, Rewcastle et al. 1991, Zwi et al. 1994). Unlike other vascular disrupting agents, DMXAA was found to activate mouse STING and innate immune responses. DMXAA dramatically decreased the size of implanted tumors, such as non-small-cell lung cancer (NSCLC), melanoma, colon cancer, breast cancer, and glioma (Bahr et al. 2017, Corrales et al. 2015, Downey et al. 2014, Wang et al. 2009, Weiss et al. 2017); additionally, DMXAA extended the survival of mice with myeloid leukemia (Curran et al. 2016). STING activation by DMXAA induced type I IFNs and proinflammatory cytokines and led to the recruitment of more immune cells such as tumor antigen–specific T cells, macrophages, or neutrophils into the tumor. Despite this potent immunostimulatory effect in various tumor types, DMXAA failed to protect mice from metastatic NSCLC or human breast cancer xenografts (Downey et al. 2014). Furthermore, DMXAA failed to show an effect in a clinical trial due to its inability to bind human STING (Conlon et al. 2013, Lara et al. 2011).

Another mouse-specific cell-permeable STING agonist, 10-carboxymethyl-9-acridanone (CMA), induces malignant T cell apoptosis and inhibits tumor growth (Gulen et al. 2017). Intratumoral injection of CMA prevented growth of T cell acute lymphoblastic leukemia (T-ALL) in both wild-type and STING-deficient mice, suggesting that CMA directly activated STING

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**Table 1 Preclinical studies for cGAS-STING pathway–targeting immunotherapies[a]**

| Treatment | Administration | Tumor model | Reference |
|---|---|---|---|
| **STING agonists** | | | |
| DMXAA | IP | MC38 colon cancer (SQ) | Wang et al. 2009 |
| | IP | 344SQ-ELuc lung cancer (SQ) | Downey et al. 2014 |
| | IT | B16 melanoma, TRAMP-C2 prostate cancer, 4T1 breast cancer, Ag104L fibrosarcoma (SQ) | Corrales et al. 2015 |
| | IP | MMTV-PyMT breast cancer (mammary fat pad) | Weiss et al. 2017 |
| | IP | LN-229 glioma (SQ) | Bahr et al. 2017 |
| | IV | C1498 leukemia (IV), *CMM*$^{+}$ mouse leukemia model | Curran et al. 2016 |
| CMA | IT | Cpc46 T-ALL, EL4 lymphoma (SQ)[a] | Gulen et al. 2017 |
| c-di-GMP | IP | 4T1 breast cancer (mammary fat pad)[a] | Chandra et al. 2014 |
| | IT | GL261 glioma (intracranial) | Ohkuri et al. 2014 |
| | IT | Spontaneous papilloma (Pdx-Cre$^{+/-}$, Kras$^{G12D+/-}$, and Trp53$^{R172H+/-}$) | Baird et al. 2017 |
| 3′3′-cGAMP | IP | 5TGM1 multiple myeloma (SQ, IV), Eμ-TCL1 mouse CLL model[a] | Tang et al. 2016 |
| cGAMP | IT with lipofectamine | B16F10 melanoma (SQ, IV), MC38 colon cancer (SQ) | Demaria et al. 2005 |
| | IV | MC26 colon cancer (SQ) | Li et al. 2016 |
| | IM | B16F10 melanoma (SQ) | Wang et al. 2017 |
| | IT | 4T1 breast cancer, CT26 colon cancer, mSCC1 squamous cell carcinoma, B16F10 melanoma (SQ) | Ohkuri et al. 2017 |
| ADU-S100 | IT | B16F10 melanoma (SQ, IV), 4T1 breast cancer, CT26 mammary carcinoma (SQ) | Corrales et al. 2015 |
| | IT | NT2.5 breast cancer (SQ) in tolerant FVB/N mice | Foote et al. 2017 |
| | IV | C1498.SIY (IV) | Curran et al. 2016 |
| Dithio c-di-GMP | IT | MOC1 head and neck cancer (SQ) | Moore et al. 2016 |
| c-di-GMP/YSK05 liposome | IV | B16F10 melanoma (IV) | Nakamura et al. 2015 |
| ADU-S100/PBAE + PD-1 Ab | IT | B16F1melanoma (SQ) | Wilson et al. 2017 |
| **Anticancer therapies targeting the STING pathway** | | | |
| IR | Local IR | MC38 colon cancer (SQ) | Deng et al. 2014b |
| Topotecan | IP, IT | E0771 breast cancer (SQ) | Kitai et al. 2017 |
| CD47 Ab | IP, IT | A20 B cell lymphoma, MC38 colon cancer (SQ) | X. Liu et al. 2015 |
| | IT | MC38 colon cancer, A20 B cell lymphoma, B16.SIY melanoma (SQ) | Xu et al. 2017 |
| Heat-iMVA | IT | B16F10 melanoma, MC38 colon cancer (intradermal) | Dai et al. 2017 |
| LM vaccine | IV | CT26 colon cancer (IV, SQ) | Brockstedt et al. 2004 |
| | IV | B16-MO5 melanoma (IV) | Starks et al. 2004 |

(*Continued*)

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**Table 1** (*Continued*)

| Treatment | Administration | Tumor model | Reference |
|---|---|---|---|
| PC7A nanoparticle vaccine | SQ | B16F10 melanoma, MC38 colon cancer, TC-1 myeloma (SQ) | Luo et al. 2017 |
| T-MP-loaded DCs | SQ | B16 melanoma (SQ, IV) H22 liver cancer, CT26 colon cancer (SQ) | Zhang et al. 2015 |
| **Combination immunotherapies with STING activation** | | | |
| **Combination with tumor vaccines** | | | |
| STINGVAX | SQ | CT26 colon cancer, SCCFVII head and neck cancer, TRAMP prostate cancer, B16 melanoma (footpad), PANC02 pancreatic cancer (hemispleen injection) | Fu et al. 2015 |
| c-di-GMP, TriVax | IV | B16F10 melanoma (SQ) | Wang & Celis 2015 |
| c-di-GMP, ovalbumin peptide | CDN (IT), peptide (SQ) | Quad-GL261 glioma (intracranial) | Ohkuri et al. 2014 |
| c-di-GMP, LM vaccine | CDN (IP), vaccine (IP) | 4T1 breast cancer (mammary fat pad) | Chandra et al. 2014 |
| **Combination with immune checkpoint blockade** | | | |
| cGAMP, PD-1 Ab + CTLA-4 Ab | CDN (IT), Ab (IP) | B16F10 melanoma (SQ) | Demaria et al. 2005 |
| cGAMP, PD-L1 Ab | cGAMP (IM), Ab (IP) | B16F10 melanoma (SQ) | Wang et al. 2017 |
| c-di-GMP, CTLA-4 Ab + PD-1 Ab + 4-1BB Ab | CDN (IT), Ab (IP) | TRAMP-C2 prostate cancer (SQ) | Ager et al. 2017 |
| dithio c-di-GMP, PD-L1 Ab | CDN (IT), Ab (IP) | MOC1 head and neck cancer (SQ) | Moore et al. 2016 |
| ADU-S100, PD-L1 Ab, OX40R Ab | CDN (IT), Ab (IP) | NT2.5 breast cancer (SQ) in nontolerant neu/N mice | Foote et al. 2017 |
| IR, CTLA-4 Ab, PD-L1 Ab | Local IR, Ab (IP) | B16F10 melanoma, PDA.4662 pancreatic cancer (SQ) | Twyman-Saint Victor et al. 2015 |
| IR, CTLA-4 Ab | Local IR, Ab (IP) | 4T1 breast cancer (SQ) | Demaria et al. 2005 |
| IR, PD-L1 Ab | Local IR, Ab (IP) | TUBO breast cancer, MC38 colon cancer (SQ) | Deng et al. 2014a |
| PC7A vaccine, PD-1 Ab | Vaccine (SQ) Ab (IP) | TC-1 myeloma (SQ) | Luo et al. 2017 |
| STINGVAX, PD-1 Ab | Vaccine (SQ), Ab (IP) | B16 melanoma, CT26 colon cancer (footpad) | Fu et al. 2015 |
| Heat-iMVA, CTLA-4 Ab, PD-1 Ab, or PD-L1 Ab | Heat-iMVA (IT), Ab (IP) | B16F10 melanoma (intradermal) | Dai et al. 2017 |
| **Combination with chemotherapy** | | | |
| cGAMP, 5FU | IV | MC26 adenocarcinoma (SQ) | Li et al. 2016 |
| DMXAA, cisplatin, or CP | IP | KHT sarcoma (IM), SKBR3 breast cancer, OW-1 ovarian cancer (SQ) | Siemann et al. 2002 |

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**Table 1** (*Continued*)

| Treatment | Administration | Tumor model | Reference |
|---|---|---|---|
| **Combination with other cancer therapies** | | | |
| cGAMP, CpG ODN | IT | B16F10 melanoma, EG-7 lymphoma (SQ) | Temizoz et al. 2015 |
| IR, cGAMP | Local IR, CDN (IT) | MC38 colon cancer (SQ) | Deng et al. 2014b |
| IR, dithio c-di-GMP | Local IR, CDN (IT) | Panc02 pancreatic cancer, 3LL lung cancer (SQ) | Baird et al. 2016 |
| c-di-GMP, CAR T cells | IT biopolymer scaffold | KPC-luc pancreatic cancer (pancreas, IV), B16F10 melanoma (SQ) | Smith et al. 2017 |
| DMXAA, CD20 Ab | DMXAA (IP), Ab (IV) | $BCL_1$ lymphoma (IV) | Dahal et al. 2017 |

[a]Outcomes of cGAS-STING pathway–targeting immunotherapies include inhibition of tumor growth, extended mouse survival, reduced metastases, systemic and long-lasting antitumor responses, and tumor cell apoptosis.

Abbreviations: +, co-treatment; Ab, antibody; CAR, chimeric antigen receptor; c-di-GMP, 3′,5′-cyclic diguanylic acid; CDN, cyclic dinucleotide; cGAMP, cyclic GMP-AMP; CLL, chronic lymphocytic leukemia; CMA, 10-carboxymethyl-9-acridanone; *CMM*$^+$, genetically modified *Cbfb-MYH11/Mpl*-induced; CP, cyclophosphamide; DC, dendritic cell; DMXAA, 5,6-dimethylxanthenone-4-acetic acid; heat-iMVA, heat-inactivated modified vaccinia virus Ankara; IM, intramuscular injection; IP, intraperitoneal injection; IR, irradiation; IT, intratumoral injection; IV, intravenous injection; LM, *Listeria monocytogenes*; ODN, oligodeoxynucleotide; PBAE, poly(beta-amino ester); SQ, subcutaneous injection; STING, stimulator of interferon genes; T-ALL, T cell acute lymphoblastic leukemia.

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in tumor cells and caused apoptosis. STING activation–induced cell death is not common, but specific cell lines are vulnerable, as a recent study showed that STING activation can induce lysosomal cell death in human myeloid cells (Gaidt et al. 2017).

**Cyclic dinucleotides.** 3′,5′-Cyclic diguanylic acid (c-di-GMP) is a mouse and human STING agonist found in bacteria. c-di-GMP induced profound reduction of papillomas, orthotopic gliomas, breast cancer growth, and metastases in mouse models (Baird et al. 2017, Chandra et al. 2014, Ohkuri et al. 2014). 3′3′-cGAMP, another bacterial STING agonist, caused tumor regression and improved survival of mice bearing chronic lymphocytic leukemia and myeloma. 3′3′-cGAMP directly induces apoptosis in malignant B cells and activates the immune system (Tang et al. 2016).

**2′3′-cGAMP.** 2′3′-cGAMP is the natural product of cGAS and the endogenous STING agonist that is produced in response to DNA. Intratumoral injection of cGAMP alone or in liposomes inhibited the growth of implanted breast cancer, squamous cell carcinoma, colon cancer, and melanoma (Demaria et al. 2015, Ohkuri et al. 2017). cGAMP treatment induced a systemic antitumor response, controlling both local and distant tumor growth (Demaria et al. 2015). Similarly, cGAMP administration at a site distant from the tumor also controlled the growth of melanoma and colon cancer (Li et al. 2016, Wang et al. 2017). Type I IFNs induced by cGAMP promoted cross-presentation of tumor-associated antigens and infiltration of tumor antigen–specific $CD8^+$ T cells (Demaria et al. 2015, Wang et al. 2017). Macrophages with proinflammatory cytokine expression were recruited into the tumor in response to cGAMP and played an essential role in the antitumor response (Li et al. 2016). cGAMP did not have a direct cytotoxic effect in the studied tumor cell lines in vitro, but cGAMP treatment in vivo induced apoptosis of tumor cells by possibly activating antitumor $CD8^+$ T cells (Li et al. 2016). Host STING and $CD8^+$ T cells were essential for the antitumor effect of cGAMP (Demaria et al. 2015, Li et al. 2016).

**Cyclic dinucleotides engineered for efficient delivery.** The administration of cyclic dinucleotides (CDNs) such as c-di-GMP, c-di-AMP, 3′3′-cGAMP, and 2′3′-cGAMP activates human STING and induces profound antitumor immune responses. However, CDNs are

susceptible to hydrolysis by phosphodiesterases. Specifically, ecto-nucleotide pyrophosphatase/phosphodiesterase (ENPP1) was reported to be the dominant cGAMP phosphodiesterase (Li et al. 2014). ENPP1 is rich in the ER lumen, extracellular space, and serum, where it can degrade the majority of administered cGAMP before entering cells. Moreover, CDNs contain two negatively charged phosphate groups that impair passive diffusion through the plasma membrane. To improve CDN delivery, researchers synthesized CDNs that are resistant to degradation and utilized various drug delivery methods.

A modified CDN, dithio-(Rp,Rp)-[cyclic[A(2′,5′)pA(3′,5′)p]], also known as ML RR-S2 CDA or ADU-S100, is resistant to phosphodiesterases and has high potency in activating STING (Corrales et al. 2015). ADU-S100 reduced tumor growth in mice bearing melanoma, colon cancer, breast cancer, or myeloid leukemia (Corrales et al. 2015, Curran et al. 2016, Foote et al. 2017). The synthetic CDN dithio c-di-GMP also does not contain a phosphodiesterase target site; treatment using dithio c-di-GMP led to efficient rejection of implanted head and neck tumors (Moore et al. 2016).

YSK05 liposomes loaded with c-di-GMP disassemble upon endosomal acidification to activate STING, achieving reduction of mouse melanoma lung metastases (Nakamura et al. 2015). Cationic poly(beta-amino ester) (PBAE) polymers loaded with ADU-S100 also promoted ADU-S100 delivery and showed better tumor growth control than ADU-S100 alone in the presence of anti-PD-1 (Wilson et al. 2017). Virus-like particles (VLPs) filled with cGAMP were shown to have cytotoxicity to T lymphoma cells in vitro (Gulen et al. 2017). Whether VLP-cGAMP improves drug delivery and has an antitumor effect in vivo remains to be studied.

As mentioned above, a variety of small-molecule STING agonists promoted antitumor immunity and were well-tolerated in mouse models. The translation of these results to the clinic needs to proceed carefully, since immune and inflammatory side effects may be different in humans than in rodents. It has been reported that STING activation induces the immune suppressor IDO (indoleamine 2,3-dioxygenase) and promotes tumor growth in the case of lung carcinoma characterized by low antigenicity, suggesting that the outcome of STING activation may differ based on the tumor type (Lemos et al. 2016). Three ongoing clinical studies are addressing this question. In one trial, the STING agonist MK-1454 is given alone intratumorally or with the anti-PD-1 pembrolizumab for solid tumors (**https://www.clinicaltrials.gov/** identifier NCT02675439). In the other two trials, ADU-S100 is administered intratumorally to metastatic cancers or lymphoma alone or with a CTLA4 antibody (NCT02675439) or with a PD-1 antibody (NCT03172936).

## Anticancer Therapies Targeting the cGAS-STING Pathway

Several anticancer therapies that were designed to directly target cancer cells are now shown to indirectly activate the cGAS-STING pathway, which induces antitumor immunity. These anticancer therapies and their therapeutic effects on tumors are discussed below.

**Radiation-induced STING activation.** Approximately 50% of all cancer patients are treated with radiotherapy, a method using ionizing radiation to disrupt cancer cells locally by inducing DNA damage and apoptosis (Begg et al. 2011). Interestingly, local radiation treatment not only treated the targeted tumor but also reduced the size of distant tumors (Mole 1953); this phenomenon is termed the abscopal effect of radiation therapy. Abscopal effects in metastatic melanoma and NSCLC are associated with increased tumor infiltration of PD-1$^+$ cytotoxic T lymphocytes (CTLs) and elevated PD-L1 expression in tumor cells or stromal cells, suggesting activation of antitumor immune responses (Golden et al. 2013, Postow et al. 2012, Twyman-Saint Victor et al. 2015). Preclinical studies with mouse tumor models showed that the antitumor effect

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of radiation therapy required STING and type I IFNs signaling (Burnette et al. 2011, Deng et al. 2014b). Moreover, cGAS was required for DC sensing of irradiated tumors, suggesting that the therapeutic activity of radiation depends on the cGAS-STING-IFN axis (Deng et al. 2014b). Interestingly, dose fractions above 12–18 Gy in mouse models induced TREX1 expression, which attenuated the immunogenicity and the abscopal effects by degrading cytosolic DNA (Vanpouille-Box et al. 2017). This study emphasizes the importance of selecting an appropriate dose of radiation to prevent a negative feedback loop. Multiple clinical studies of combination radiation therapy and immune checkpoint blockade are ongoing.

**Chemotherapy-induced STING activation.** Several cytotoxic drugs increase genomic DNA damage and other cellular stresses that may induce cytosolic DNA. Topotecan, a topoisomerase I inhibitor, triggers cGAS-STING activation, leading to DC and $CD8^+$ T cell recruitment to murine breast tumors and tumor regression in vivo (Kitai et al. 2017). The S phase–dependent alkylating agents hydroxyurea and cisplatin induced DNA damage in BRCA1-deficient breast tumors (Parkes et al. 2017). In vitro, these chemotherapy drugs induced ISGs in a STING-dependent manner. Cytosine arabinoside, an antimetabolite, drove IFN expression in B cell lymphoma in vitro (Shen et al. 2015). More DNA-damaging chemotherapy drugs remain to be tested for their role in activating the cGAS-STING pathway and the antitumor immune responses in vivo.

**Antibody-mediated STING activation.** CD47 is a transmembrane protein upregulated in malignant cells that binds to its receptor SIRPα on phagocytes to inhibit phagocytosis. CD47 blockade with anti-CD47 antibodies promotes phagocytosis of tumor cells. CD47 blockade also induced type I IFNs and required host type I IFN signaling for its therapeutic effect (X. Liu et al. 2015). Moreover, the anticancer effect of anti-CD47 antibodies required $CD8^+$ T cells and STING in DCs, suggesting activation of the cGAS-STING pathway (X. Liu et al. 2015, Xu et al. 2017). At the molecular level, CD47 blockade prevented phagosome acidification and degradation of tumor mitochondrial DNA (mtDNA), which activates cGAS in DCs (Xu et al. 2017).

**Virus-induced STING activation.** Heat-inactivated modified vaccinia virus Ankara (heat-iMVA) injected intratumorally into melanoma or colon cancer–bearing mice induced tumor regression in a manner that depended on cGAS, STING, IFN signaling, and Batf3 DCs (Dai et al. 2017). Heat-iMVA may have been taken up by DCs, delivering viral DNA into the cytosol. Another modified DNA virus, talimogene laherparepvec (T-vec), is a genetically engineered HSV-1 strain that lacks certain viral immunoinhibitory genes while expressing human GM-CSF for an immunostimulatory effect (Andtbacka et al. 2015). T-vec is currently the only oncolytic virus approved by the US Food and Drug Administration (FDA), showing a 26% response rate and increased $CD8^+$ T cell infiltration in melanoma patients (Ribas et al. 2017). Although the principal mechanism of action of T-vec is to specifically replicate in tumor cells and induce lysis, increased tumor cell death may also activate the cGAS-STING pathway. Moreover, cGAS is the natural PRR for HSV-1, raising the possibility that T-vec may directly activate the cGAS-STING pathway. Similar to STING-targeting therapies, T-vec displayed synergistic effects with immune checkpoint therapy in clinical studies (Chesney et al. 2017, Ribas et al. 2017). Other oncolytic viruses are undergoing clinical trials; however, no evidence for innate immune activation by these viruses has yet been reported (Babiker et al. 2017).

**Therapeutic cancer vaccine–induced STING activation.** *Listeria monocytogenes* therapeutic vaccines were engineered to express tumor antigens while lacking cell-to-cell bacterial



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dissemination and infectivity to nonphagocytic cells (Brockstedt et al. 2004); nevertheless, these vaccines retain the ability to stimulate STING (Hansen et al. 2014). Recombinant *Listeria* vaccines inhibited metastases of tumors and prolonged survival of tumor-bearing mice (Brockstedt et al. 2004, Starks et al. 2004). *Listeria* vaccines, the first STING-related vaccines, have shown an effect in patients with pancreatic carcinoma and advanced cervical cancer (Cory & Chu 2014; Le et al. 2012, 2015). Although *Listeria* vaccines potently trigger adaptive immunity, they also cause cytokine release syndrome, an excessive inflammatory side effect that needs to be overcome (Miles et al. 2017). There are currently eight active *Listeria* vaccine trials for glioma, colorectal cancer, cervical cancer, head and neck cancer, ovarian cancer, mesothelioma, and prostate cancer.

PC7A nanoparticles are pH-sensitive vehicles that allow efficient delivery of a cargo into the cytosol. A PC7A nanovaccine conjugated with tumor-associated antigens showed potent growth inhibition of cervical cancer, myeloma, and melanoma (Luo et al. 2017). Interestingly, PC7A nanoparticles themselves induced ISGs and promoted cross-priming of $CD8^+$ T cells in a STING- and IFN$\alpha/\beta$ receptor–dependent manner. PC7A-induced ISGs were partially dependent on cGAS, suggesting an increase of cytosolic DNA by PC7A treatment; alternatively, PC7A may directly function on STING due to its weak affinity for STING.

Tumor cell–derived microparticles (T-MPs) are generated from ultraviolet-irradiated tumor cell supernatants and contain tumor-specific antigens and DNA fragments. T-MPs effectively deliver DNA fragments into DCs and activate the cGAS-STING pathway (Zhang et al. 2015). T-MP-loaded DCs showed a therapeutic effect in implanted hepatocellular carcinoma, colorectal cancer, and melanoma models.

Chitosan nanoparticles loaded with tumor-derived antigens displayed a growth inhibition of mouse thymoma, cervical cancer, and melanoma (Han et al. 2016, Shi et al. 2017). Moreover, chitosan nanoparticles alone were reported to have an adjuvant effect (Lin et al. 2014). After being phagocytosed by macrophages or DCs, chitosan induces mtDNA-driven cGAS-STING activation (Carroll et al. 2016). Mechanistically, chitosan damages mitochondria, releasing mtDNA into the cytosol to activate cGAS. Whether chitosan cancer vaccines activate the cGAS-STING pathway in vivo to trigger antitumor immune responses remains to be studied.

## Combination Immunotherapy with STING Activation

Tumor cells evade immune responses by various strategies, forming a complex microenvironment composed of multiple immune-suppressing cells. Targeting multiple pathways and enhancing the antitumor response are key therapeutic approaches for cancer therapy. In 2015, the FDA approved nivolumab (anti-PD-1) and ipilimumab (anti-CTLA-4) combination therapy, which significantly delayed progression of metastatic melanoma compared to either therapy alone (Larkin et al. 2015). Thus, combination immunotherapy has the potential to induce synergistic antitumor responses. The following studies report the effect of combination immunotherapy with STING activation.

**Combination with tumor vaccines.** Combining STING agonists with therapeutic vaccines delivering antigens enhances activation of adaptive immunity (X.-D. Li et al. 2013). STINGVAX, a vaccine containing both GM-CSF-producing tumor cells and ADU-S100, increased antitumor responses in melanoma, pancreatic cancer, colon cancer, and head and neck cancer models when compared to GM-CSF-producing tumor cell vaccines alone (Fu et al. 2015). The efficacy of STINGVAX depended on STING and was further enhanced by anti-PD-1 treatment. The TriVax vaccine mixture containing anti-CD40, poly-IC, and a modified $CD8^+$ T cell epitope is used as a vaccine to induce systemic immune responses against tumors. Combination of TriVax and c-di-GMP showed a synergistic antitumor response against melanoma (Wang & Celis 2015).

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c-di-GMP combined with an ovalbumin peptide or *Listeria*-based vaccine enhanced the antitumor effects of the peripheral vaccine in glioma and breast cancer (Chandra et al. 2014, Ohkuri et al. 2014).

**Combination with immune checkpoint blockade.** Various immune checkpoint inhibitors combined with STING activation improved antitumor response and survival in rodents. cGAMP combined with anti-PD-L1 or a mixture of anti-PD-1 and anti-CTLA-4 showed a synergistic effect in controlling murine melanoma growth (Demaria et al. 2015, Wang et al. 2017). Similarly, c-di-GMP combined with triple checkpoint inhibitors (anti-CTLA-4/PD-1/4-1BB), dithio-c-di-GMP combined with anti-PD-L1, and ADU-S100 combined with anti-PD-L1 or anti-OX40R all enhanced the antitumor effect of immune checkpoint blockade therapy (Ager et al. 2017, Foote et al. 2017, Moore et al. 2016).

Irradiation induced potent antitumor immune responses by activating the cGAS-STING pathway. Radiation therapy enhanced the effect of anti-CTLA-4 and anti-PD-L1 antibodies in melanoma and breast cancers (Demaria et al. 2005, Deng et al. 2014a, Twyman-Saint Victor et al. 2015). More than 300 clinical trials combining radiotherapy and immune checkpoint inhibitors are ongoing.

Tumor vaccines promote T cell priming and enhance the effect of immune checkpoint blockade therapy. PC7A nanoparticle vaccines and STINGVAX further improved tumor growth control and mouse survival when treated together with anti-PD-1 (Fu et al. 2015, Luo et al. 2017).

Virotherapy such as heat-iMVA activates the cGAS-STING pathway and induces antitumor immune responses. Heat-iMVA treatment combined with systemic immune checkpoint inhibitors including anti-CTLA4, anti-PD1, or anti-PD-L1 provided a synergistic antitumor effect in eradicating tumors and extending mouse survival (Dai et al. 2017).

**Combination with chemotherapy.** The chemotherapy drug fluorouracil (5FU) inhibits cancer cell proliferation by blocking thymidine synthesis and DNA replication. The combination of 5FU and cGAMP exhibited a synergistic antitumor activity against colon cancer (Li et al. 2016). Furthermore, cGAMP administration alleviated intestinal atrophy, a side effect of 5FU. Similarly, the mouse-specific STING agonist DMXAA synergistically increased cell death of sarcomas when treated together with cisplatin or cyclophosphamide without aggravating the side effects of cyclophosphamide (Siemann et al. 2002). The antitumor effects and the toxicity of combination therapy involving STING agonists and other chemotherapy drugs require further study.

**Combination with other cancer therapies.** Activating several innate immune signaling pathways may amplify antitumor responses. Co-administration of TLR9-agonist CpG oligodeoxynucleotides and cGAMP displayed synergistic tumor growth inhibition in melanoma and thymoma (Temizoz et al. 2015). Irradiation combined with STING agonist treatment enhanced immune responses against colon cancer and pancreatic adenocarcinoma (Baird et al. 2016, Deng et al. 2014b).

Anti-CD20 targets and eliminates B cells by phagocyte-mediated antibody-dependent cell-mediated cytotoxicity or complement activation. However, in the case of B cell lymphoma, tumor-associated macrophages display immunosuppressive signatures and express inhibitory Fcγ receptors. DMXAA treatment increased stimulatory Fcγ receptors on macrophages, allowing B cell lymphoma clearance by anti-CD20-driven phagocytosis. Priming with DMXAA prior to anti-CD20 administration significantly enhanced survival in a mouse lymphoma model, effectively curing 90% of the mice (Dahal et al. 2017).

Chimeric antigen receptor–expressing T cells (CAR T cells) are engineered to recognize tumor-associated antigens. An implanted biopolymer device delivering CAR T cells and



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c-di-GMP eradicated murine pancreatic cancers and melanomas, whereas infusions of c-di-GMP or CAR T cells alone were ineffective (Smith et al. 2017). DC maturation and an increased number of circulating tumor-specific CTLs were only detected with CAR T cell and c-di-GMP combination treatment.

The mechanism by which combination therapy yields enhanced antitumor immune responses may vary. Innate immune stimulation with STING agonists promotes tumor antigen–specific $CD8^+$ T cell priming and tumor infiltration; this influx of primed $CD8^+$ T cells synergizes with CAR T cell therapy or with immune checkpoint inhibitors. Several DAMPs, including DNA, may be released by immunogenic cell death induced by ionizing radiation, tumor-targeting monoclonal antibodies, or chemotherapy. DAMPs induced by cancer therapy or PRR ligands may amplify innate immune stimulation from STING activation.

## FUTURE PERSPECTIVES

Recent studies on the cGAS-STING pathway have significantly advanced our understanding of pathogen recognition, autoimmune diseases, senescence, and tumor immunity. However, many questions remain. For example, during mitosis, chromatin is exposed to the cytosol and cGAS binds to chromatins without pathway activation (Yang et al. 2017). One hypothesis is that cGAS does not form its catalytically active dimer structure upon binding highly condensed mitotic chromosomes (Andreeva et al. 2017). Other possibilities include the existence of a putative cGAS inhibitor during the cell cycle or posttranslational modifications of cGAS. Additional studies are needed to understand cGAS regulation in the nucleus. The common outcome of STING activation is type I IFN and proinflammatory cytokine induction, but recent studies also include autophagy, lysosomal cell death with IL-1 production, and apoptosis as STING-driven events (Collins et al. 2015, Gaidt et al. 2017, Tang et al. 2016). Investigating additional outcomes of STING activation and the cross talk between the cGAS-STING pathway and other immune pathways may provide novel insights into the innate immune system. The critical role of aberrant activation of the cGAS-STING pathway during autoinflammatory and autoimmune diseases suggests that treating these diseases using cGAS and STING inhibitors has great potential. An active area of future research will be the role of the cGAS-STING pathway in a variety of chronic inflammatory diseases and senescence- or age-related diseases.

Although activation of cGAS by tumors is evident in many studies, how tumor-derived DNA is transferred into the cytosol of phagocytes is unclear. In addition, the role of the cGAS-STING pathway within tumor cells during tumor growth and the antitumor response has not been investigated. Addressing these questions will provide a deeper understanding of pathway functionality in tumor cells and also yield knowledge about STING activation as a therapeutic target. As noted in the previous section, several STING agonists show antitumor efficacy in rodent models and activate human STING. Development of STING agonists with high affinity and stability will promote the efficacy of STING-targeting cancer immunotherapy (Corrales et al. 2015). Further developments and comparisons of STING agonists are needed in rodent models and nonhuman primates. Furthermore, the therapeutic efficacy and safety of intratumoral and systemic administration of STING agonists need to be compared.

Preclinical studies highlighted the importance of the cGAS-STING pathway in tumors and its potential in cancer immunotherapy. However, additional translational research is necessary because humans have different cancer biology and immune systems from mouse models; furthermore, individual cancer patients have clinical heterogeneity. More studies in humanized mice bearing patient-derived xenografts and clinical biopsy samples from STING agonist–treated patients will shed light on the mechanism and therapeutic efficacy of STING activation in tumors.

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Overall, several important questions will need to be addressed in the next few years as clinical trials are advancing. Will certain tumors or tumor subsets be more responsive to STING agonists? Are there predictive biomarkers for therapeutic efficacy and safety? What combination of immune-modulating agents (e.g., antibodies to PD-1, PD-L1, CTLA-4, or CAR T cells) with STING agonists will maximize clinical response while minimizing immune toxicity? Future work elucidating the interplay of innate and adaptive immunity in tumors and testing the efficacy of STING-targeting agents will lead to more effective cancer therapies.

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