Review

Zheqi Zhou, Sanling Huang, Fangying Fan, Yan Xu, Casey Moore, Sirui Li* and Chuanhui Han*

The multiple faces of cGAS-STING in antitumor immunity: prospects and challenges

Abstract: As a key sensor of double-stranded DNA (dsDNA), cyclic GMP-AMP synthase (cGAS) detects cytosolic dsDNA and initiates the synthesis of 2′3′ cyclic GMP-AMP (cGAMP) that activates the stimulator of interferon genes (STING). This finally promotes the production of type I interferons (IFN-I) that is crucial for bridging innate and adaptive immunity. Recent evidence show that several antitumor therapies, including radiotherapy (RT), chemotherapy, targeted therapies and immunotherapies, activate the cGAS-STING pathway to provoke the antitumor immunity. In the last decade, the development of STING agonists has been a major focus in both basic research and the pharmaceutical industry. However, up to now, none of STING agonists have been approved for clinical use. Considering the broad expression of STING in whole body and the direct lethal effect of STING agonists on immune cells in the draining lymph node (dLN), research on the optimal way to activate STING in tumor microenvironment (TME) appears to be a promising direction. Moreover, besides enhancing IFN-I signaling, the cGAS-STING pathway also plays roles in senescence, autophagy, apoptosis, mitotic arrest, and DNA repair, contributing to tumor development and metastasis. In this review, we summarize the recent advances on cGAS-STING pathway's response to antitumor therapies and the strategies involving this pathway for tumor treatment.

*Corresponding authors: Chuanhui Han, Peking University International Cancer Institute, Peking University Cancer Hospital and Institute, Health Science Center, Peking University, Beijing 100143, China,
E-mail: chuanhui.han@bjmu.edu.cn. https://orcid.org/0000-0002-7634-8471;
and **Sirui Li**, Department of Genetics, Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA,
E-mail: lsr519@ad.unc.edu. https://orcid.org/0000-0002-1645-6868
Zheqi Zhou, Sanling Huang and Yan Xu, Peking University International Cancer Institute, Peking University Cancer Hospital and Institute, Health Science Center, Peking University, Beijing, China
Fangying Fan, Department of Interventional Ultrasound, Chinese PLA General Hospital, Beijing, China
Casey Moore, Departments of Immunology, Pathology, UT Southwestern Medical Center, Dallas, Texas, USA

Keywords: cGAS; STING agonist; IFN-I; tumor

Introduction

Despite cancer remaining a significant contributor to global mortality [1], the past few decades have witnessed new milestones in clinical antitumor therapies which substantially increase tumor survival. Notably, the emergence of immune checkpoint blocking therapy (ICB) and chimeric antigen receptor (CAR)-T cell therapy have ushered in a new era in the field of clinical cancer treatment. Unfortunately, immunotherapies yield tumor remission for only a small fraction of patients. Therefore, it is imperative to increase the response rate of ICB therapy and resolve the bottleneck issue of CAR-T therapy in solid tumor treatment [2, 3].

The activation of innate immune sensing serves as a crucial mechanism for inducing adaptive antitumor immune responses inside the tumor microenvironment (TME); therefore, the therapeutic effect heavily relies on innate immune sensing pathways. Mechanistically, innate immune sensing, generally initiating in antigen-presenting cells (APCs) or macrophages, facilitating antigen processing and presentation to prime tumor-specific CD8$^+$ T cells by promoting the production of type I interferons (IFN-I) and other proinflammatory cytokines [4–9]. Moreover, innate immune sensing could also recruit APCs and CD8$^+$ T cells to the TME and activate CD8$^+$ T cells or natural killer (NK) cells to eliminate tumor cells. Activation of innate immune sensing pathways has consistently been linked to better prognosis and improved overall survival in cancer patients receiving conventional therapies or immunotherapies [10–12]. Up to now, several tactics have been devised with the objective of activating intrinsic sensing pathways, and certain approaches have demonstrated the potential to enhance the efficacy of conventional anticancer treatments. However, innate immune sensing pathways are intended to be the first line of defense against pathogen invasion and thus are broadly expressed across the whole body. This not only predicts how toxicity is induced, but also highlights the significance of localizing the innate immune sensing activation in TME.

Innate immune sensing is mediated by pattern-recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs) releasing from treated tumor tissues to trigger the innate and adaptive antitumor immunity [9, 13–15]. In the past decade, cytoplasmic double-stranded DNA (dsDNA) sensing pathway via cyclic GMP-AMP synthase (cGAS) stimulator of interferon genes (STING) has received more and more attention in basic research and clinical practice. Numerous studies have uncovered the critical role of cGAS-STING in traditional antitumor therapies and immunotherapy [16–20]. The cGAS, as a cytoplasmic dsDNA sensor, recognizes various exogenous dsDNA and also self-derived dsDNA to resist pathogen infection and reject cancer, also contributes to autoimmune disorders [21–28]. Following its recognition of dsDNA, cGAS assembles into a dimer or oligomer ladder to synthesize cyclic GMP-AMP (cGAMP), which binds to STING in the endoplasmic reticulum (ER), and translocates to endoplasmic reticulum (ER)-Golgi intermediate compartment (ERGIC) and Golgi [29]; furthermore, activated STING will activate downstream signaling. During STING transport activation, armadillo-like helical domain-containing protein 3 (ARMH3) recruits phosphatidylinositol 4-kinase beta (PI4KB) to synthesize phosphatidylinositol 4-phosphate (PI4P), which directs STING Golgi-to-endosome trafficking, and helps STING activate more stably and efficiently [30]. This will lead to production of pro-inflammatory cytokines, especially IFN-I and interleukin (IL)-1β, to initiate inflammatory responses and activate CD8$^+$ T cells [4–7, 31–33]. In the classical framework of tumor immunology, radiotherapy (RT) and immunotherapy activate innate immune sensing pathways, particularly the cGAS-STING pathway, controlling tumors through immune activation beyond the direct killing effect [15, 16, 33–46]. Nevertheless, recent works provided evidence supporting that cGAS-STING could also trigger the initiation of autophagy and suppress the RT-mediated repair of DNA damage to diminish the RT-induced antitumor immunity [47, 48]. Additionally, persistent activation of cGAS-STING signaling leads to a pro-tumor effect by reshaping an immunosuppressive TME, thus helping tumor evade immunosurveillance and promoting metastasis [49, 50]. Therefore, the cGAS-STING pathway exhibits dual functionality in the TME: one to be hijacked by tumor cells to resist therapeutic interventions and facilitate tumor progression, the other to provoke anti-tumor immunity by innate immune sensing. Here, we review the advances of cGAS-STING mediated dsDNA sensing in tumor study and emphasize the significance of local activation of cGAS-STING signaling in TME.

Regulatory mechanisms of the cGAS-STING pathway

As one of the primary cytosolic DNA sensors, cGAS exhibits a particular ability to detect cytosolic dsDNA [51] or RNA-DNA hybrids [52] in a sequence independent manner and catalyzes the conversion of GTP and ATP to 2′3′-cGAMP. The activation of STING by 2′3′-cGAMP triggers an inflammatory response via the TANK-binding kinase 1 (TBK1)-Interferon Regulatory Factor 3 (IRF3) pathway, ultimately leading to the production of IFN-I [5, 23]. Importantly, the IFN-I further bridges the innate and adaptive immune response by promoting the maturation, activation and migration of DC, T cells and NK cells, while also impeding regulatory T (Treg) cells and inhibitory macrophage differentiation [4, 6, 53]. Intriguingly, both cGAS and STING are IFN-stimulated genes (ISGs), indicating a positive feedback loop in this pathway. Persisting cytosolic dsDNA will thereafter augment IFN-I response by boosting local cGAS production and subsequent cGAS activation via positive feedback [54]. In parallel, STING also activates I kappa B kinase (IKK) to mediate the induction of Nuclear factor Kappa-B (NF-κB)-driven pro-inflammatory cytokines production, such as tumor necrosis factor-α (TNF-α), IL-1β and IL-6 [8, 9, 55].

Mutual regulation of cGAS-STING and cell death pathways

The cGAS-STING pathway not only enhances the production of proinflammatory cytokines, but it also plays a crucial role in initiating cell death through many mechanisms. Intact cGAS-STING pathway is activated by treatment to regulate tumor cell growth, senescence and immune surveillance [55]. Therefore, in multiple cancers, cancer cells often exhibit abnormalities in the cGAS-STING pathway as a result of epigenetic hypermethylation [56, 57]. Additionally, cell death pathways can also be hijacked by tumor cells or pathogens to suppress the activation of cGAS-STING pathway and diminish the immune response [58, 59] (Figure 1).

Persistent stimulation of cGAS-STING signaling can directly induce cell death in a variety of machineries. Importantly, STING-mediated IRF-3 activation enhances the interaction between IRF-3 and the pro-apoptotic protein Bcl2-associated X gene (Bax) and thereby triggers the mitochondrial apoptotic pathway [60]. In addition, IFN-I could upregulate apoptosis inducers, such as TNF-related apoptosis-inducing ligand (TRAIL), initiating cell death [61–63]. Furthermore, STING can also activate autophagy through a mechanism independent of TBK1 and IFN-I [47, 64], which also
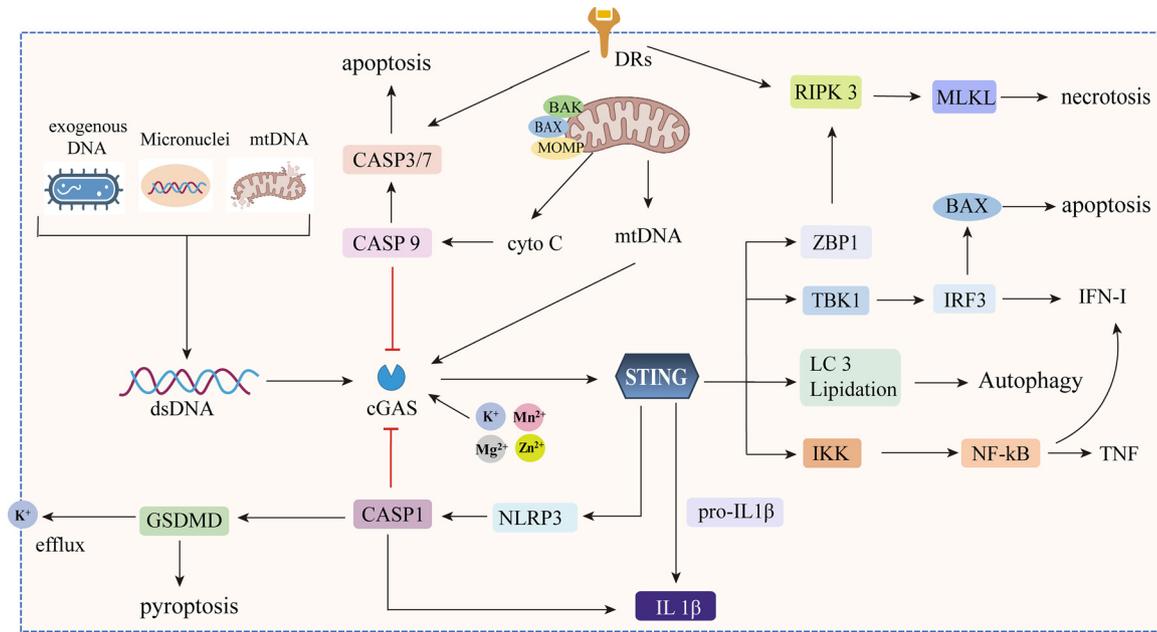
Figure 1: cGAS-STING and cell death pathways. cGAS recognizes cytosolic dsDNA released from the nucleus, mitochondria or exogenous pathogens, and then catalyzes the conversion of GTP and ATP to cGAMP. cGAMP initiates an inflammatory response via the SITNG-TBK1-IRF3-IFN and IKK-NF-κB pathways. cGAS-STING also involves in regulating cell death pathways. Chronic activation of STING increases the mitochondrial apoptotic pathway by enhancing the interaction of IRF-3 and Bax. STING also activates pyroptosis by initiating potassium efflux and upregulating and maintaining NLRP3. On the contrary, activated CASP9/3/7/1 will suppress the activation of cGAS-STING pathway and avoid to awaking the antitumor immunity. Moreover, STING also regulates necroptosis through ZBP1-RIPK3-MLKL axis. Furthermore, STING also activate autophagy independent of TBK1 and IFN by translocating to ERGIC and Golgi, which leads to LC3 lipidation and autophagosome formation. cGAS, GMP-AMP synthase; dsDNA, double-stranded DNA; cGAMP, cyclic GMP-AMP; SITNG, stimulator of interferon genes; TBK1, TANK-binding kinase 1; IRF3, Interferon Regulatory Factor 3; IFN, interferons; IKK, I kappa B kinase; NF-κB, nuclear factor Kappa-B; CASP, caspase; ZBP1, Z-DNA binding protein 1; MLKL, mixed-linage kinase domain like; ERGIC, endoplasmic reticulum-Golgi intermediate compartment.

involves in negatively regulating the activation of cGAS-STING pathway and cell death. Upon binding cGAMP, STING translocates to the ERGIC and Golgi resulting in the lipidation of microtubule-associated protein 1 light chain 3 (MAP1LC3, hereafter referred to as LC3) and the fomation of autophagosomes, to defend against viral infection or degradation of damaged organelles [47]. Moreover, the cGAS-STING pathway activates receptor-interacting protein kinase 3 (RIPK3) to initiate necroptosis, which is coordinated by the actions of with IFN-I and TNF-α [65]. Meanwhile, cGAS-STING also regulates necroptosis by maintaining the expression of the key necroptosis executor mixed-linage kinase domain like (MLKL) [66], or through the Z-DNA binding protein 1 (ZBP1)-RIPK3 axis [65, 67–69]. Recent studies have also shown that STING could facilitate the activation of pyroptosis by initiating potassium efflux and upregulating and maintaining expression of NLR family pyrin domain containing 3 (NLRP3) [70, 71].

Caspase 9 (CASP9) is critical to initiate intrinsic apoptosis. In 2014, Richard A. Flavell and Benjamin T. Kile groups separately reported that viral infection or stress-induced cell apoptosis could trigger mitochondrial membrane permeabilization, thereby resulting in mitochondrial DNA

(mtDNA) release into the cytosol and activation of CASP9 signaling. However, the activation of CASP9/3/7 further suppresses the mtDNA-mediated cGAS-STING activation [72, 73]. It was later discovered that CASP3 can cleave cGAS and other key factors to limit cGAS-mediated IFN-I production [74]. However, tumor cells seem to utilize a different mechanism to hijack apoptosis and suppress tumor-intrinsic cGAS-STING activation. We previously reported that blocking CASP9 signaling with Emricasan or Q-VD-OPh could facilitate mtDNA mediated cGAS-STING activation after radiation treatment; however, blocking caspases does not further raise cGAS-STING axis protein expression level, indicating that cleavage of cGAS may not be the major mechanism of caspase mediated cGAS-STING inhibition in tumor cells [58]. Furthermore, Jiang et al. demonstrated that the activation of key enzymes in pyroptosis, caspase-1, -4, -5, and -11 cleaved cGAS, reduces cGAMP levels, and inhibits IFN-I production [75]; Rathinam et al. reported that gasdermin D (GSDMD) forms membrane pores after inflammasome activation depletes cytosolic potassium that is essential for activation of cGAS-STING signaling [76]. However, unlike pyroptosis, MLKL mediated necroptosis seems to trigger cGAS-STING activation by

facilitating the release of mtDNA into the cytosol [77]. Considering the critical role of caspases in regulating the cell death, the regulatory network between caspases and cGAS-STING signaling still needs to be further validated. Thus, cGAS-STING bridges the innate immune sensing pathways and the cell death pathways. However, tumor could hijack the cell death pathways to regulate the activation of cGAS-STING pathway and influence antitumor immunity.

Additionally, some other variables affecting the activation of the cGAS-STING pathway have also been recently identified. These factors are related to cytoplasmic accumulation of mitochondrial DNA. DNA methyltransferase 3 alpha (DNMT3A) and TET methylcytosine dioxygenase 2 (TET2) maintain mtDNA integrity by regulating the expression of transcription factor A mitochondria (TFAM), and deletion or mutation of both causes damage to mtDNA integrity and activation of cGAS signaling [78]. Oxidized DNA in the mitochondria is repaired by 8-oxoguanine DNA glycosylase (OGG1), or cleaved into fragments by endonuclease flap endonuclease (FEN1). The fragment of mtDNA leaks into cytosol via mitochondrial permeability transition pore (mPTP)- and voltage dependent anion channel (VDAC)-dependent channels to initiate cGAS-STING activation [79]. Instability in the mitochondrial genome also promotes the accumulation of Z-type DNA, upregulation of ZBP1 expression, and nucleates a cytosolic complex containing cGAS, RIPK1, and RIPK3 to sustain IFN-I signaling [80]. In addition, the loss of fumarate hydratase (FH1) also leads to the release of mtDNA into the cytoplasm through mitochondrial derived vesicles (MDVs), promoting the activation of the cGAS-STING pathway [81]; however, whether the apoptosis pathway is involved in the cGAS-STING activation in FH1 deficient cells remains unclear.

Posttranslational modifications of cGAS-STING

Posttranslational modifications (PTMs), including phosphorylation, ubiquitination, SUMOylation and others, can also
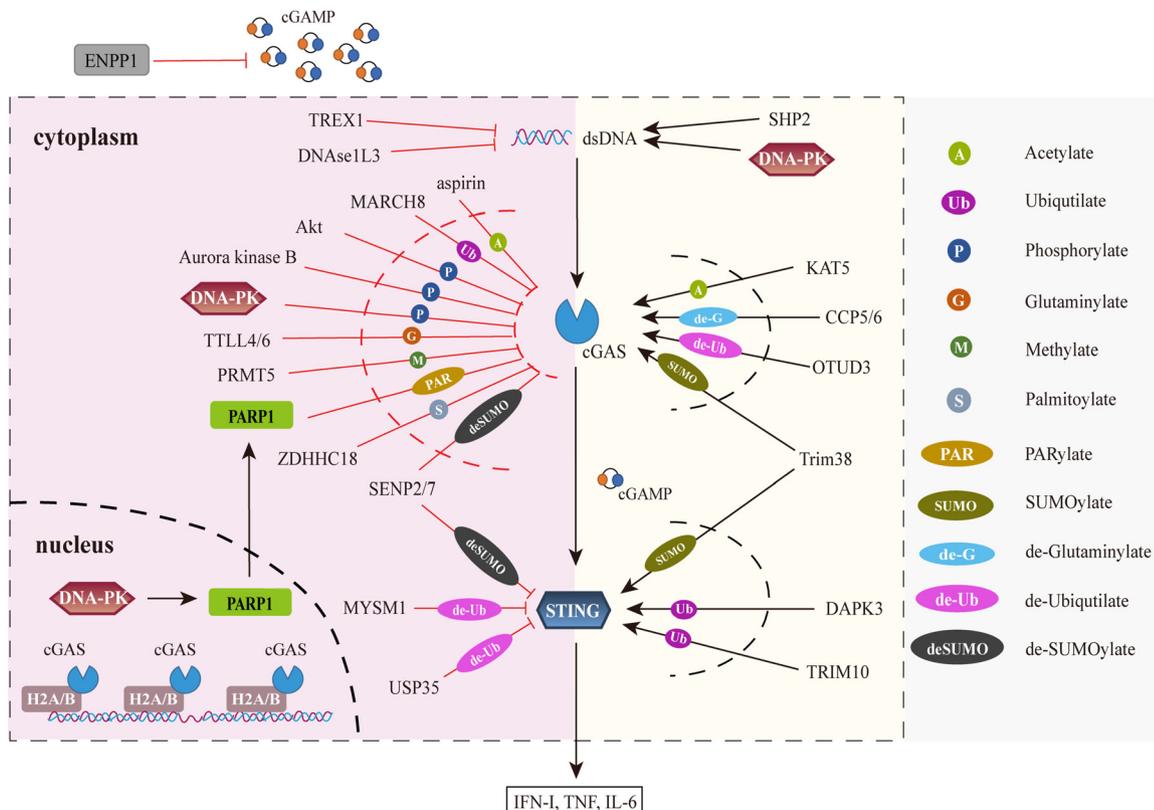

Figure 2: Regulation of cGAS-STING signaling pathway. Different PTMs of cGAS-STING, including ubiquitylation, palmitoylation, acetylation, can directly trigger the function of cGAS-STING. DNA-PK, as a DNA sensor, has multiple roles in regulating the cGAS-STING pathway. In addition to direct regulation, DNA-PK can promote cytoplasmic translocation of PARP1, and inhibits cGAS-DNA-binding ability through PARylating cGAS. TREX1 and DNAse1L3 degrade the dsDNA, and ENPP1 hydrolyzes extracellular cGAMP, thereby all inhibit the activation of downstream STING. Nucleosome DNA can also inhibit the cGAS activation through H2A-B binding to the cGAS and maintain cGAS in a monomeric state. PTMs, posttranslational modifications; cGAS, GMP-AMP synthase; SITNG, stimulator of interferon genes; DNA-PK, DNA-dependent protein kinase; PARP1, polymerase 1; TREX1, 3′ repair exonuclease 1; ENPP1, ecto-nucleotide pyrophosphatase phosphodiesterase 1.

directly regulate cGAS-STING signaling (Figure 2). The E3 ubiquitin ligase membrane associated ring-CH-type finger 8 (MARCH8) ubiquitinates cGAS at Lys411, inhibiting the binding of cGAS to DNA, thereby reducing the production of cGAMP and IFN-I [82]. Moreover, ovarian-tumor-domain-containing deubiquitinase 3 (OTUD3) binds to cGAS and targets Lys279 to deubiquitinate K48-linked ubiquitination, thereby enhancing the stability of the cGAS protein and its DNA-binding ability [83]. Similarly, Death-associated protein kinase 3 (DAPK3) facilitates the K63-linked ubiquitination and stabilizes the interaction of STING and TBK1 [84]. Tripartite motif 10 (TRIM10) binds to STING, catalyzing the polyubiquitination of STING at K289 and K370 sites at K27- and K29-, thereby promoting the Golgi transport and activation of STING [85]. On the contrary, myb-like SWIRM and MPN domains 1 (MYSM1) and ubiquitin specific peptidase 35 (USP35) suppress the activation of STING by cleaving STING K63-linked ubiquitination [86, 87]. Besides, phosphorylation, SUMOylation, methylation, glutamylation, acetylation and palmitoylation can also regulate the activities of cGAS-STING. It has been reported that the epidermal growth factor receptor (EGFR) and Akt (protein kinase B, PKB) axis inhibits cGAS activity by phosphorylating S291 or S305 residues [88]. Phosphorylation also involves in regulating cGAS sensing genomic DNA. As a cytosolic dsDNA sensor, it is not surprising that cGAS recognizes cytosolic DNA, including micronuclei DNA in cytoplasm. However, how genomic DNA avoids activating cGAS during nuclear envelope disappearing in mitosis has attracted more attention. In 2019, the Funabiki lab reported that nucleosome DNA traps more cGAS during mitotic envelope breakdown; unlike naked dsDNA, nucleosome DNA inhibits cGAS activation through H2A-B binding to cGAS [89]. However, during mitotic arrest, cGAS is slightly activated and promotes the aggregation of IRF3 to initiate cell apoptosis [89]. Later, multiple studies uncovered the structure of nucleosome-cGAS and revealed that nucleosome traps the cGAS in H2A-B to block dsDNA binding and maintains cGAS in a monomeric state [90–94]. Besides, cGAS could also be hyperphosphorylated by mitotic kinases, including Aurora kinase B and DNA-dependent protein kinase (DNA-PK), to suppress genomic DNA recognition [95, 96]. Notably, TBK1 is also involved in regulating STING by through phosphorylation. TBK1 phosphorylates the Ser366 residues of STING in a conserved pLxIS motif that is also necessary for STING activity [97, 98].

SUMOylation is also vital to the activation of cGAS. Viral infection first facilitates the SUMOylation of both cGAS and STING by tripartite motif protein 38 (TRIM38), increasing the stability of cGAS and STING. However, in the late phase of viral infection, cGAS and STING are deSUMOylated by Sentrin/SUMO-specific protease 2 (SENP2) [99] and SENP7 [100], leading to their degradation. Similarly, glutaminylation is also reversibly regulated by multiple

enzymes to balance the activation of cGAS. Tubulin tyrosine ligase-like 6 (TTLL6) and TTLL4 inhibit the activity of cGAS by glutaminylation, whereas cytosolic carboxypeptidase 6 (CCP6) and CCP5 remove the glutaminylation of cGAS, jointly regulating the activation of cGAS [101]. Acetylation of cGAS in different domains has different effects on its activity. Acetylation of the C-terminal catalytic domain (CCD) of cGAS is regulated by multiple PTMs to influence its enzymatic activity; for example, aspirin could also promote the acetylation of the C-terminal of cGAS and inhibit its function [24]. Meanwhile, acetylation of N-terminal unstructured domain (NUD) by lysine acetyltransferase 5 (KAT5) enhances the binding ability of cGAS to DNA [24, 102]. Besides, other kinds of PTMs also involve in cGAS regulation. Protein arginine methyltransferase 5 (PRMT5) was reported to methylate cGAS, thereby blocking its DNA-binding ability [103]. More recently, Yin et al. also found that palmitoyltransferase ZDHHC18 negatively regulates the function of cGAS through palmitoylation [104]. In contrast, palmitoylation of STING at Cys residues (Cys88/91) promotes its activation [105].

The DNA repair system is not only involve in DNA generation, but also participates in regulating the cGAS pathway. Recently, it has been found that DNA virus infection facilitates the activation of DNA-PK, which promotes cytoplasmic translocation of poly (ADP-ribose) polymerase 1 (PARP1). PARP1 thereafter inhibits cGAS-DNA-binding ability through PARylating cGAS [106]. However, the role of DNA-PK in regulating cGAS-STING signaling is complicated; DNA-PK, itself as a DNA sensor [107], has been reported to trigger cGAS-STING activation, but in some case also inhibits cGAS-STING [96, 108]. Besides, the degradation of cGAMP also represents a form of negative regulation that suppresses cGAS-STING signaling. Ecto-nucleotide pyrophosphatase phosphodiesterase 1 (ENPP1) hydrolyzes extracellular cGAMP and thus prevents extensive spread, thereby inhibiting the activation of downstream STING [109].

Metalloimmunotherapy and cellular localization regulates the function of cGAS-STING pahtway

The activity of cGAS-STING pathway can also be regulated by metal ions. Mg^{2+} is revealed to be a cofactor for cGAS enzyme [110]; later, the Jiang lab reported that Mn^{2+} releases into the cytosol and promotes the cGAS-STING activation during virus infection [111]; furthermore, they also validated that Mn^{2+} activates the cGAS-STING in a dsDNA-independent manner to potentiate the immunotherapies [112, 113]. Moreover, the Chen lab revealed that binding of dsDNA also induces a cGAS liquid phase separation, which is critical to cGAS

synthesizing cGAMP, while zinc ions promote DNA-induced phase separation to activate the cGAS [114]. Notably, stimulation with cGAMP or Mn^{2+} also promotes the STING to form a liquid–liquid phase-separation (LLPS) and a gel-like transition; however, condensation of ER-resident STING could form a puzzle-like structure to limit the overactivation of STING-TBK1 signaling [115]. Additionally, potassium is also required for the full activation of cGAS-STING pathway; therefore, inflammasome pathway could trigger the cytosolic potassium efflux to block the activation of cGAS-STING signaling [70, 76].

In addition to directly regulating the function of cGAS or STING, the activation of cGAS-STING pathway could also be regulated by adjusting the formation of cytosolic dsDNA [116]. DNA 3′ repair exonuclease 1 (TREX1) is a 3′-5′ single strand exonuclease with Mg^{2+} as a cofactor that is reported to degrade dsDNA and DNA-RNA hybrid in cases where it exhibits helicase activity [117, 118]. It is reported that TREX1 can degrade damaged cytosolic DNA, inhibiting the activation of cGAS [119, 120]. However, the activated cGAS-dsDNA complex could resist TREX1-mediated degradation by forming the phase separation [121]. Moreover, it is found that p53 induces degradation of TREX1, resulting in cytosolic dsDNA accumulation, then engages the cGAS/STING cytosolic DNA sensing pathway for tumor suppression [122]. Besides TREX1, DNase1L3 is also reported to degrade the serum vascular dsDNA and prevent the self-DNA mediated autoimmune disease [123–125]. However, the role of other nucleases is also under evaluation.

In addition to molecules that regulate the release of mtDNA, the amino terminal (N terminal) of cGAS also has an influence on the DNA reactivity of the catalytic domain. The activity of the human cGAS catalytic domain was inhibited by the N-terminal. In contrast, the N-terminal of mouse cGAS promotes its own DNA reactivity [126]. And there are other molecules that affect the stability of STING. Adaptor protein complex 1 (AP-1) sorted phosphorylated STING into clathrin-coated transport vesicles for delivery to the endolysosomal system, leading to degradation of STING, and thereby inhibiting long-term activation of STING [127]. STING has also recently been reported to act as a proton channel to mediate the outflow of hydrogen ions in Golgi, resulting in an increase in the pH value of the Golgi apparatus, induction of LC3B lipidation and autophagy formation, and ultimately degradation of STING [128].

cGAS-STING, a double-edged sword for cancer treatments

Unlike host immune cells, tumor cells seem to hijack plenty of pathways to suppress intrinsic cGAS-STING activation. Even

though some previous studies reported that antitumor treatments could induce the slight increase of tumor-derived IFN-I and provoke the antitumor effect in a tumor-intrinsic cGAS-STING dependent manner [46, 84, 129–131]. However, considering the opposite role of chronic and acute production of IFN-I in antitumor immunity, the treatment slight increasing of IFN-I could blur the clinical outcome [16]. Therefore, it is still under debate whether tumor-intrinsic cGAS-STING signaling provokes antitumor immunity by increasing IFN-I or other cell stress related pathways. Unexpectedly, chromosomal instability (CIN) can contribute to the aggregation of cytosolic dsDNA and activate cGAS in tumor cells [49, 132, 133]; similarly, the aggregation of cytosolic dsDNA is also vital to provoking antitumor immunity in DNA mismatch repair deficiency (dMMR) tumor models responding to ICB [130]. Tumor cells with CIN typically undergo chromosome missegregation during cell division, resulting in chromosomes fragments are enveloped by primitive nuclear membranes to form micronuclei. Micronuclear membranes readily decompose and release dsDNA contained therein [134] and trigger the cGAS-STING pathway [135]. However, the impact of tumor-intrinsic cGAS-STING pathway varies across different tumor types. Clinical data showed that low expression of STING in tumor tissues is associated with poor prognosis of gastric cancer [136]. Multiple studies add proofs of the positive role of tumor-intrinsic cGAS-STING in strengthening antitumor immunity [18, 137, 138]. However, other groups have also showed that upregulated cGAS-STING is negatively correlated with immune cell infiltration in some tumors and also correlates with poor prognosis [139], thereby promotes the tumor metastasis [49]. In summary, the cGAS-STING pathway exhibits dual roles in cancer, acting both as a suppressor and as a promoter of oncogenic processes. Therefore, identifying specific biomarkers to delineate the role of the cGAS-STING pathway in the TME warrants further investigation.

Activation of the cGAS-STING pathway in the TME can promote infiltration of immune cells and antitumor immunity. Tumor cells or tumor cell-derived vesicles, including exosomes and macrovesicles, can be captured by phagocytes and antigen presenting cells. The phagocytosed dsDNA is released into the cytosol of immune cells and activates the cGAS-STING signaling [140, 141]. This will further enhance the secretion of IFN-I, the presentation of tumor antigens, and the recruitment of tumor-specific $CD8^+$ T cells to exert antitumor effects [16, 18]. In addition, IFN-I also upregulates the expression of multiple chemokines such as C-X-C motif chemokine ligand 9 (CXCL9) and CXCL10, which promote the infiltration of APCs and effector T (Teff) cells [32, 142, 143]. Interestingly, tumor-derived cGAMP is reportedly secreted into the TME, possibly via ATP binding cassette subfamily C member 1 (ABCC1) transporter, and enters immune cells via the folate transporter solute carrier family 19 member 1

(SLC19A1) [144, 145]. Delaying dead clearance by blocking MerTK will enhance the extracellular ATP that could trigger the macrophage capture tumor-derived cGAMP to enhance antitumor immunity [138]. Tumor-derived cGAMP also triggers STING-mediated interferon responses in non-tumor cells to activate an NK cell mediated antitumor effect [137]. However, activation of the cGAS-STING pathway in the TME does not always indicate a better prognosis. The Ge lab reported that DNA damage could promote cGAS translocation into the nucleus and inhibit homologous recombination by preventing the PARP1-Timeless complex formation [146]. Moreover, CIN chronically activates STING-dependent noncanonical NF-κB signaling to drive secretion of proinflammatory cytokines and metastasis formation, thereby contributing to CIN maintenance [49]. CIN tumors can also activate cell survival signals and form a metastasis-promoting tumor microenvironment through NF-κB-IL-6-STAT3 pathway and endoplasmic reticulum stress [147, 148]. Known for its role in RNA editing, APOBEC3A also promotes CIN and triggers the formation of micronuclei to activate cGAS-STING in tumor cells; this helps tumors to develop and metastasize [149, 150]. Another study showed that cGAS anchored to the mitochondrial outer membrane can promote dynamin-related protein 1 (DRP1) oligomerization, inhibiting mitochondrial ROS accumulation and ferroptosis, and further promoting tumor progression [151]. Therefore, it is important to avoid promoting the pro-tumorigenesis functions of cGAS-STING while endeavoring to promote antitumor immunity.

Over the past decade, immunotherapy has heralded a new era in cancer treatment. This inspiration has led researchers to progressively uncover the role of the immune responses in traditional antitumor therapies, such as radiotherapy, chemotherapy, and targeted therapy. Besides directly killing tumor cells, traditional antitumor therapies also activate innate immune sensing pathways, especially the cGAS-STING pathway, to provoke anti-tumor immunity in the TME [7, 15, 34–38]. However, the antitumor immunity induced by RT and chemotherapy often proves insufficient to maintain long-term antitumor effects; relapse remains one of the major challenges in clinical cancer treatment [152]. Therefore, identifying ways to further enhance therapy-induced antitumor immunity remains a crucial area of research to synergize with traditional cancer therapy and immunotherapy [153].

Radiation

The traditional dogma was that RT functions only by inducing DNA double strand breaks (DSB) to kill tumor cells [154]. The current radioimmunology theory highlights the vital role of antitumor immunity induced by RT. Irradiated tumor cells can activate the cGAS-STING pathway in DCs, which then produce IFN-I to provoke the adaptive antitumor immunity [16, 140, 155, 156]. Meanwhile, several studies also revealed that in certain settings, tumor-intrinsic cGAS could also be activated by RT to contribute to awaking the function of CD8$^+$ T cells [108, 129, 140, 156, 157]. However, the slight increase in irradiated tumor-derived IFN-I suggests that tumor infiltrated immune cells should be the major cGAS-STING acting cells [16]. Generally, irradiated tumor cells fail to fully activate endogenous cGAS-STING signaling. We previously identified that RT-induced intrinsic apoptosis signaling is hijacked by tumor cells to suppress cGAS activation. Knocking out intrinsic apoptosis signaling switches tumor cells to be the major source of IFN-I in tumor tissue and inhibits tumor relapse after radiation [58]. Tumor-intrinsic TREX1 and autophagy were also reported to limit cGAS-STING activation after RT [129, 140]. Notably, given the high heterogeneity of tumors, they can hijack various negative cGAS-STING regulators to suppress the endogenous innate immune sensing to avoid strengthening the antitumor immunity. Therefore, it is valuable to map the network of tumor-intrinsic innate immune sensing negative regulators, which is critical to develop the new strategies to synergize with RT and immunotherapy. On the other hand, RT-induced cGAS-STING activation not only provokes the anti-tumor immune response, but also induces immunosuppressive factors to limit excessive activation of the immune response. For example, RT-triggered STING activation promotes innate immunosuppression by recruiting monocyte-derived suppressor cells (MDSC) into the TME, thus playing a pro-tumor role [158]. Meanwhile, in contrast to the antitumor effect of the canonical NF-κB pathway, the activation of the non-canonical NF-κB pathway in DCs inhibits the production of radiation-induced, STING-mediated IFN-I and inhibited the therapeutic effect of radiotherapy [159].

Generally, high dose of RT is effective in exterminating tumor lesions; however, when a tumor is adjacent to vital organs, such high doses and subsequent adaptive irradiation may not fully control tumor cells growth. Therefore, tumor relapse and the extremely rare abscopal effect remain the two major challenges of RT in clinic. Previous studies have revealed that blocking T cell infiltration after RT does not reduce the antitumor effect of RT, indicating the pre-existing CD8$^+$ T cells are vital to RT [160, 161]. Consistently, a more recent study reported that RT-induced neutrophil NETosis blocks CD8$^+$ T cell infiltration into the tumor area [162]. Meanwhile, combining RT with ICB can enhance therapeutic effects, yet it still fails to completely eradicate metastases [153, 163]. Our research demonstrated that irradiating CASP9-deficient tumors significantly

trigger the activation of cGAS-STING to provoke CD8^{+} T cell mediated antitumor effect, and the CASP9-deficient tumor disappeared after RT without relapse. However, irradiated CASP9-deficient tumors failed to induce systemic antitumor immunity to reject the distal nonirradiated tumor tissue; combining with ICB induced an abscopal effect but still failed to totally reject the distal nonirradiated tumors [58]. Moreover, previous studies have reported that DCs activated by local radiation mediates the activation of CD8^{+} T cells and contribute to tumor control by RT [156]. It is suggested the crosstalk between TME and peripheral immunity seems to be interfered after RT. To date, further research is needed to elucidate the mechanisms by which radiotherapy seldom induces an abscopal response. Considering the technological advancements in RT delivery, it is now more feasible to specifically irradiate tumor tissues without damaging adjacent healthy tissues. Therefore, there is an urgent need to refine current radio-immunology theories and develop novel strategies that effectively harness systemic antitumor immunity.

Chemotherapy

Chemotherapy functions to inhibit tumor cells proliferation and directly kill the tumor cells. Similar to RT, plenty of chemotherapeutic drugs, including anthracyclines, oxaliplatin, doxorubicin, and teniposide, topotecan, 6-thio-dG and others, can induce DNA DSBs and activate the cGAS-STING pathway to provoke the anti-tumor immunity [19, 32, 43, 164–167]. Following DNA damage, tumor cells engage the DNA damage response (DDR) mechanisms to maintain genomic integrity [168, 169]. One of the earliest events in the DDR is recruitment of PARP1 to various kinds of DNA lesions [170, 171]. Drugs that target DDR proteins, including PARP inhibitors, and checkpoint kinase 1 (CHK1) inhibitors, have been utilized in anti-tumor therapies. These targeted therapeutics have been shown to amplify DNA damage in tumor cells, further activate the cGAS-STING pathway, regulate the immune microenvironment, and improve the efficacy of anti-PD-1 therapy when used in combination [45, 46, 120, 172]. Ataxia telangiectasia mutated (ATM) is also a component of the DDR; thus, inhibition of ATM can further promote the activation of cGAS-STING signaling, paving the road for synergizing with ICB therapy [173, 174]. Notably, the Liu lab recently revealed that the chemotherapy paclitaxel diminishes CXCL13^{+} CD8^{+} T cells to limit the synergistic effect of chemotherapy and ICB [175], indicating potential risks in using chemotherapies to awaken anti-tumor immunity.

Immunotherapy

Immunotherapies function by awakening the antitumor immunity; therefore, immunotherapies show a great potential to synergize with RT and other antitumor therapies. ICB therapies, as the most famous immunotherapies, block immunosuppressive receptors on the cell surface, including programmed cell death-1 (PD-1)/programmed cell death ligand 1 (PD-L1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), and specifically provoke adaptive immunity. Therefore, the effectiveness of immunotherapies depends on the adaptive antitumor immunity, most notably the recognition of tumor antigens and generation of tumor-specific cytotoxic T cells (CTLs). The Chen lab first reported that cGAS-deficient mice show less tumor antigen-specific CD8^{+} T cells infiltration and respond worse to PD-L1 blockade, suggesting that cGAS is essential for the antitumor effects of immune checkpoint blockade [20]. In addition, Fu lab demonstrated that the anti-tumor effect of CD47 blockade also requires DC-intrinsic, STING-mediated T cell activation [41, 176]. In some cases, endogenous tumor STING signaling can also contribute to the response to ICB therapy. The Greenberg lab revealed that irradiated B16-STING knockout cells, as a vaccine, reduced tumor sensitivity to anti-CTLA4 treatment [108]. Notably, considering the role of cGAS in DNA damage repair and mitotic arrest, the mechanism of how tumor-intrinsic factors influence to ICB therapy is still under-evaluated. On the other hand, cGAS-STING generally functions through IFN-I; however, the Zitvogel lab also observed that IFNαR1-deficient mice respond better to anti-PD1 therapy [177], indicating that an IFN-I-independent role of cGAS-STING pathway may also play a role in the effectiveness of immunotherapies.

Notably, although radiotherapy and chemotherapy can activate the tumor-intrinsic cGAS-STING pathway in some certain tumor cell lines or experimental setting, the activation is often not strong enough to fully awaken antitumor immunity, and the host cGAS-STING signaling still seems to play the major role post antitumor treatment. This is mainly caused by the complicated negative regulation network in tumor cells. Some factors, such as caspases, TREX1 and autophagy, induced by antitumor therapies to initiate cell death, can also be hijacked by tumor cells to evade immunosurveillance [129, 140, 178–180]. Interestingly, interfering with the major negative regulators successfully switch tumor cells to become the major IFN-I source in the TME and play a vital role in enhancing antitumor immunity [32, 58]. These findings point out a new direction for novel antitumor strategies development (Figure 3).
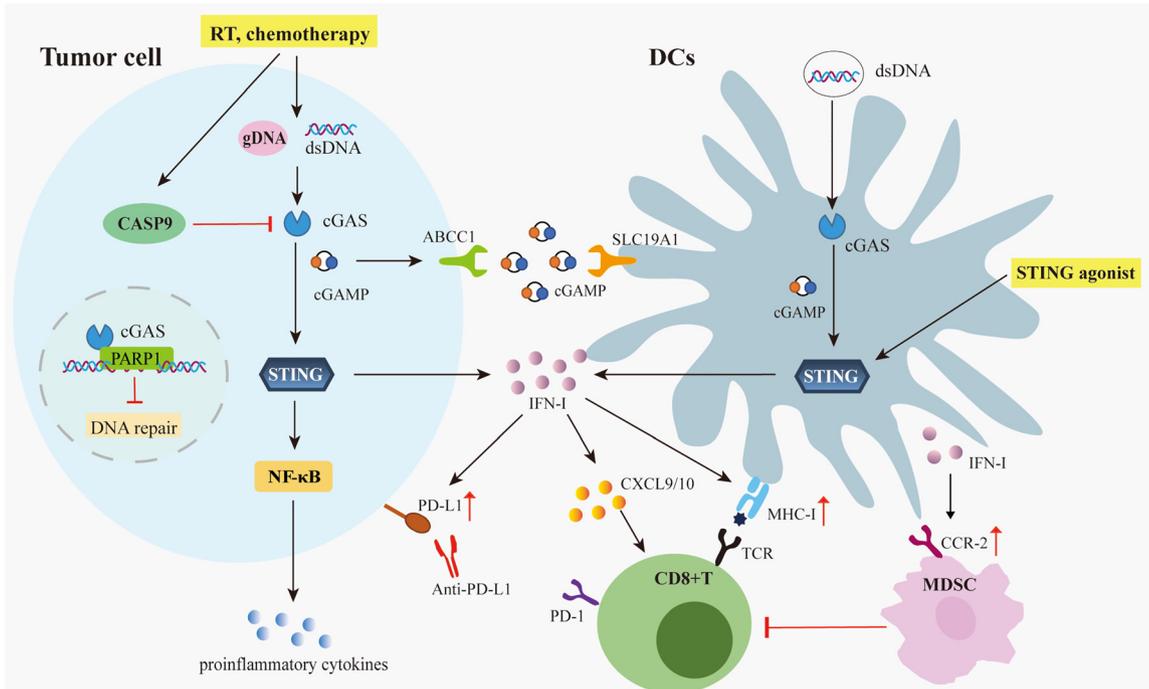
Figure 3: Antitumor therapies awake the cGAS-STING signaling to accelerate the antitumor immunity. Features of the tumor microenvironment like chromatin instability and growth stress can cause cytoplasmic or extracellular accumulation of dsDNA to activate the cGAS-STING pathway. Similarly, antitumor therapies such as radiotherapy and chemotherapy can also cause accumulation of genomic or mitochondria-derived dsDNA in the cytosol. Tumor-derived cGAMP can also secrete into the TME, and enter immune cells via the folate transporter SLC19A1. However, DNA damage recruits the cGAS translocating to nuclear and inhibits the homologous recombination by interfering the PARP1-timeless complex formation. On the other hand, antitumor therapies also upregulate negative regulators of cGAS-STING, as CASP9 signaling, to suppress the activation of the intrinsic cGAS-STING pathway. Therefore, targeting such negative regulators or delivery of STIGN agonists could specifically activate the cGAS-STING signaling in the TME. This will help to upregulate the expression of Th1 chemokines (CXCL9 and CXCL10) and antigen presenting molecules (MHCI), recruit CD8+T cells, and activate innate immune cells. However, the increase of IFN secretion may also enhance the infiltration of MDSC and upregulate PD-L1 and other immunosuppressive factors to exert an inhibitory effect. dsDNA, double-stranded DNA; cGAS, GMP-AMP synthase; SITNG, stimulator of interferon genes; cGAMP, cyclic GMP-AMP; CASP, caspase; TME, tumor microenvironment; MDSC, monocyte-derived suppressor cells.

cGAS-STING based agonists in cancer therapies

Unlike other innate immune sensing platforms, the cGAS-STING pathway is broadly expressed and can be activated by small molecular cGAMP and its analogues; therefore, multiple STING agonists were developed and validated in preclinical models and clinical practice. However, considering the widespread expression of STING, high-dose local treatment or systemic administration of STING agonists might induce immune cells' death to diminish the enhanced antitumor immunity; therefore, how to specifically activate STING in TME should be further considered as critical next-generation STING agonist-based strategies.

STING agonist

Cytosolic dsDNA triggers cGAS to synthesize cGAMP, a cyclic dinucleotide (CDN), to bind and activate STING. Besides cGAMP, plenty of natural CDNs have been reported in other organisms [181–183], indicating the conserved role of this axis. Antitumor modulation of CDNs was revealed that c-di-GMP could inhibit tumor cell proliferation [184]. The intratumoral injection of cGAMP has been reported to limit tumor growth and promote the CD8+ T cell infiltration, and this ability could be further synergized with ICB or RT [16, 20, 185]. Beyond naturally derived CDNs, synthetic CDNs with better properties have been developed, including ADU-S100 and MK-1454, which have entered phase II clinical trials for the treatment of head and neck tumors in combination with pembrolizumab [186]. Especially, MK-1454 was developed by Merck as a STING agonist for various tumors with an enzymatic cascade system [187] (Table 1).

Dimethyloxoxanthenyl acetic acid (DMXAA) is the first non-CDN STING agonist, but DMXAA and its analogous 10-carboxymethyl-9-acridanone (CMA), fails in clinical trials. Later, it is revealed that they can only activate mouse STING, but not human STING [188–191]. The natural compound α-mangostin is a bioflavonoid-like molecule with antiviral activity and can activate hSTING [192]. A series of

Table 1: STING agonists.

STING agonist	Agents		Targets	Phase	Clinical trial ID/PMID
Direct	ADU-S100(MIW815)	ADU-S100	STING	Phase 2	NCT03937141
		ADU-S100+/−Ipilimumab	STING+/−CTLA-4	Phase 1	NCT02675439
		ADU-S100 + PDR001	STING + PD-1	Phase 1	NCT03172936
	E7766	E7766	STING	Phase 1	NCT04109092
		E7766	STING	Phase 1	NCT04144140
	IMSA101 (GB492)	IMSA101+/−PULSAR-ICI	STING+/−PD-1	Phase 2	NCT05846659
					NCT05846646
	GSK3745417	GSK3745417	STING	Phase 1	NCT03843359
	MK-1454	MK-1454	STING	Phase 1	NCT03010176
		MK-1454+/−pembrolizumab	STING + PD-1	Phase 2	NCT04220866
	MK2118	MK-2118+/− pembrolizumab	STING + PD-1	Phase 1	NCT03249792
	BMS-986301	BMS-986301	STING	Phase 1	NCT03956680
	SB 11285	SB 11285	STING	Phase 1	NCT04096638
	SNX281	SNX281+/−pembrolizumab	STING+/−PD-1	Phase 1	NCT04609579
	TAK-500	TAK-500+/−pembrolizumab	STING+/−PD-1	Phase 1	NCT05070247
	c-di-GMP	c-di-GMP	STING	Pre-clinical	PMID: 15721270
	MSA-2	MSA-2	STING	Pre-clinical	PMID: 32820094
	SR-717	SR-717	STING	Pre-clinical	PMID: 32820126
	SYNB1891	SYNB1891+/−Atezolizumab	STING+/−PD-L1	Phase 1	NCT04167137
	DMXAA (ASA404)	DMXAA	STING	Phase 3	NCT00738387
	CMA (Cridanimod)	CMA + progestin	STING	Phase 2	PMID: 23604073
	α-Mangostin	α-Mangostin	STING	Pre-clinical	PMID: 30079976
	CRD3874-SI	CRD3874-SI	STING	Phase 1	NCT06021626
	Compound 12b	Compound 12b	STING	Pre-clinical	PMID: 31927317
	C53	C53	STING	Pre-clinical	PMID: 35388221
	G10	G10	STING	Pre-clinical	PMID: 32911484
	C11	C11	STING	Pre-clinical	PMID: 29263267
Indirect	Emricasan	Emricasan + anti-PD-L1	Caspases + PD-L1	Pre-clinical	PMID: 32231300
	prexasertib	prexasertib	CHK1	Phase 2	PMID: 30777870
	Olaparib	Olaparib	PARP1	Phase 2	PMID: 30540933
	Rucaparib	Rucaparib	PARP1	Phase 2	PMID: 30589644
	Talazoparib	Talazoparib	PARP1	Phase 2	PMID: 33495297
	Topotecan	Topotecan	Topoisomerase I	Phase 2	PMID: 28069806
	MM-398	MM-398	Topoisomerase I	Phase 1	PMID: 29267866
	Doxorubicin	Doxorubicin	Topoisomerase II	Phase 2	PMID: 25344738
	Teniposide	Teniposide	Topoisomerase II	Phase 2	PMID: 31408442
	Cisplatin	Cisplatin	DNA replication	Phase 2	PMID: 33423764
					PMID: 29658856
	6-thio-dG	6-thio-dG	Telomerase	Phase 2	PMID: 32619407
					NCT05208944
	AZD1390	AZD1390	ATM	Phase 1	PMID: 33290271
					NCT03215381

oxoacridinyl acetic acid derivatives with the same skeleton as CMA were also synthesized, which could be used as hSTING agonists, but their antitumor effects are still being evaluated. Thereby, several groups aim to develop systemically available STING agonists that can circumvent these issues. Amidobenzimidazoles (ABZI), a novel STING agonist designed for patients through systemic administration, has significantly enhanced binding affinity using the 4-carbon butane linker (di-ABZI) for dimerization; however, its safety remains to be considered and the hemodynamics of patients using these drugs need to be closely monitored [193, 194]. Furthermore, another non-nucleotide cGAMP mimetic small-molecule STING agonist, SR-717, was developed for oral administration [195]. SR-717 promotes the activation of CD8$^+$ T cells, dendritic cells and limited tumor growth, but failed to synergize with ICB in pre-clinical models [195]. SR-717 also induces the expression of clinically relevant targets, including PD-L1, in a STING-dependent manner [195].

MSA-2, another orally available non-nucleotide STING agonist with antitumor activity, is also moderately or poorly synergistic with PD-1 blockade; combinations of MSA-2 and anti-PD-1 antibody were superior in inhibiting tumor growth and prolonging survival over monotherapy [196]. Notably, most STING agonists only trigger the activation of host-derived, but not tumor-intrinsic, STING signaling. STING-deficient mice fail to increase IFN-I levels and resisted STING agonist treatment, even in tumor models that have an intact cGAS-STING axis [195, 197]. This indicates that tumor cells could hijack some negative regulators to suppress STING signaling. Therefore, in future studies, it will be worth to continue to explore the activation and inhibition mechanisms of the cGAS-STING pathway in the TME, as well as the mechanism of action on tumors, to develop better drugs targeting this pathway, reduce drug toxicity and improve bioavailability. Meanwhile, the mechanism of combined action of STING agonists and existing immunotherapy methods must be actively explored to find the best combined treatment strategy for different tumors.

Although STING agonists have shown promising clinical benefit, most of them can only be delivered by intratumoral administration, in case of their poor cell membrane permeability, low bioavailability, or high toxicity [197–199]. Systemic administration of STING agonists also induced the occurrence of Breg (regulatory B cells), secreted IL-35, and inhibited the NK cell-mediated anti-tumor response [200]. Notably, MSA-2 and SR-717 were available as STING agonists by oral administration, but toxicity could not be ignored when systemically administered in mice [195, 196]. Therefore, preferred activation of STING in the TME is critical for clinical benefit. The design of systemically administered STING agonists will be crucial, since activation of STING in normal tissues may induce an inflammatory response, leading to side effects. For example, diABZI, administered by intratracheal injection, induces a neutrophil response in the bronchoalveolar space, leading to STING-dependent acute respiratory distress syndrome (ARDS) [194]. However, MSA-2 takes advantage of the weak acidity of the TME to synergize with ICB [196]. At present, there are some strategies for STING agonist targeted activation. For example, incorporation of cGAMP into non-infectious enveloped virus-like particles (VLPs) can selectively activate STING in APC and reduce the production of Treg [201]. In addition, bacterial-derived cyclic dimeric adenosine monophosphate (CDA) encapsulated in nanoscale coordination polymer to form Zinc cyclic di-AMP nanoparticles, intravenous injection of ZnCDA can enhance tumor accumulation by destroying endothelial cells in the tumor vasculature, with priority targeting tumor-associated macrophages, and then enhance anti-tumor immune response [202]. The effective penetration of STING agonists into the tumor can also be achieved by delivering STING agonists by tumor-penetrating PEG-lipid nanodiscs [203]. However, these strategies still activate STING pathways in immune cells in the tumor microenvironment, and cannot avoid the death of immune cells caused by overactivation of STING. Therefore, novel strategies should be considered to achieve the specific activation of STING in tumor tissues when STING agonists are used alone or in combination with other tumor immunotherapies.

STING agonist combined with radiation, chemotherapy and targeted therapy

Although traditional antitumor therapies can partially activate cGAS-STING in the TME, combining these therapies with STING agonists can strengthen anti-tumor immunity. Fu lab previously demonstrated that cGAMP could synergize with RT [16]. STING agonist, diABZI, could also significantly promote apoptosis in irradiated non-small cell lung cancer (NSCLC) cells, and enhance radiosensitivity of NSCLC cells [204]. ADU-S100 has the similar effect of enhancing radiotherapy sensitivity in esophageal cancer models [205]. Besides RT, STING agonists could also synergize with chemotherapy. Compared with carboplatin single treatment, carboplatin combined with STING agonist significantly prolonged survival of mice with high-grade serious ovarian cancer [206]; furthermore, carboplatin combined with STING agonist and anti-PD-1 antibody had better therapeutic effects [206]. In addition, cGAMP could also synergize with AKT 1/2 inhibitor to induce extensive apoptosis of tumor endothelial cells and reject spontaneous tumors [207].

STING agonist combined with CAR-T therapy

CAR-T cells recognize a specific antigen on the surface of tumor cells through their engineered single-chain variable fragment domain [208, 209]. CAR-T cell therapy has shown substantial activity against human CD19 or B-cell maturation antigen (BCMA)-expressing B cell malignancies [210, 211], but has been less successful in solid tumors [212], mainly due to the suboptimal migration, impaired function mediated by the immunosuppressive TME and CAR-T cell exhaustion. The search for ways to enhance adoptive T cells in solid tumors continues. Studies have shown that, compared with CAR-T alone, the combination of STING agonist c-di-GMP and CAR-T

cells can significantly activate host APCs and lymphocyte responses, further enhancing the tumor treatment effect, and prolonging survival in mice [213]; however, considering the role of STING in cell death, it is still under debate whether STING agonist-based immunotherapy could synergize with other cancer therapies in clinical. In addition, CAR-T cells generated from Th/cytotoxic T (Tc) 17 cells given with the STING agonists DMXAA or cGAMP also greatly enhanced tumor control, allowing for more CAR-T cell infiltration in the TME [214].

STING agonist combined with ICB treatment

Although immune checkpoint inhibitors (ICIs) has become a pillar of cancer therapy, the actual clinical application is also limited by low response rate. For example, cancer patients' overall response rate to ICIs is about 20–30 %, which seems to be associated with a pre-existing pro-inflammatory TME with higher immune cell infiltration or PD-L1 expression. Indeed, "immune desert" or "cold" tumors appear to be less responsive to ICIs [215–217]. Given the critical role of the cGAS-STING pathway in the TME immune response, the anti-tumor potential of combination therapy with STING agonists and ICIs is currently being actively evaluated.

STING agonists hold the promise to switch "cold tumors" to "hot tumors" and promote T cell infiltration, increase the IFN-I mediated expression of antigen presenting molecules, and improve the sensitivity of tumor cells to NK and CTL immune killing. On the other hand, activated cGAS-STING is accompanied by up-regulation of PD-L1 expression [218], and anti-PD-1/PD-L1 treatment could neutralize the immuno-suppressive effect of STING agonists. STING agonists combined with ICB therapy have achieved remarkable results in many clinically refractory tumor models, including c-di-GMP and low dose of ADU-S100 [219–221]. In anti-CTLA-4 treatment, the combination of STING agonists can also reduce the activation threshold of T cells and amplify the tumor-specific immune response [222].

Notably, as mentioned above, activation of STING signaling leads to cell death of immune cells, especially for T cells and DCs. Consistently, high dose of ADU-S100 i.t. injection could regress tumors in a CD8^{+} T cells independent manner and induce immune cell death in tumor draining lymph nodes; however, low-dose ADU-S100 treatment could increase the cytotoxicity of CD8^{+} T cells and synergize with ICB [197]. This indicates that adjustment of STING agonist dose based on the activity is essential to avoid induction of the immune cell death and synergize with other antitumor therapies.

STING agonist: cancer vaccine adjuvant

Cancer vaccines are generally made up of cancer cells, parts of cells, or pure antigens (tumor-associated antigens, TAAs). Due to central and peripheral tolerance, the immunogenicity of TAAs is always very weak [223, 224]. Appropriate adjuvants are essential to overcome tolerance and enhance tumor-specific immunity. Therefore, adjuvant substances have attracted great attention from cancer vaccinologists. Considering the role of STING in innate immune sensing, STING agonists could be an ideal cancer vaccine adjuvant.

The first STING agonist-based cancer vaccine, STING-VAX, which consisted of CDNs and granulocyte-macrophage colony-stimulating factor (GM-CSF)-secreting cancer cells, has *in vivo* antitumor efficacy in several established cancer models, and can cure established tumors that resist PD-1 blockade [225]. Compared with CDN-free GM-CSF secreting cancer cell vaccines, STINGVAX treatment enhanced the IFNγ response and recruited more CD8^{+} T cells in TME, overall enhancing antitumor efficacy [225]. Meanwhile, after STINGVAX treatment, the expression of PD-L1 increased, enhancing the efficacy of anti-PD-1 therapy, and all established tumors in the CT26 model subsided after combination therapy [225]. In subsequent studies, the feasibility of using different STING agonists as cancer vaccine adjuvants has also been repeatedly verified [226, 227], indicating the promising function of STING agonists as adjuvants for tumor vaccines.

Concluding remarks

The cGAS-STING pathway, as a crucial intrinsic sensing platform, has garnered significant attention in academic research and plays a pivotal role in many anticancer therapeutic approaches. At now, there have been several STING agonists that have been produced for the purpose of treating tumors, exhibiting positive clinical therapeutic outcomes. Simultaneously, the STING agonists also synergize with immunotherapies, such as CAR-T and ICB. Nevertheless, STING activation can lead to the over-expression of immune checkpoints and induce immune cell death at higher concentrations, potentially resulting in pro-tumor consequences. Hence, effectively and specifically stimulating the cGAS-STING pathway in TME holds promise as a therapeutic strategy for tumor treatment, especially when combined with cancer immunotherapy in future endeavors.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Ca – Cancer J Clin 2021;71:209–49.

2. Ribas A. Adaptive immune resistance: how cancer protects from immune attack. Cancer Discov 2015;5:915–9.

3. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, adaptive, and acquired resistance to cancer immunotherapy. Cell 2017;168:707–23.

4. Fuertes MB, Woo SR, Burnett B, Fu YX, Gajewski TF. Type I interferon response and innate immune sensing of cancer. Trends Immunol 2013;34:67–73.

5. Sun LJ, Wu JX, Du FH, Chen X, Chen ZJJ. Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. Science 2013;339:786–91.

6. Diamond MS, Kinder M, Matsushita H, Mashayekhi M, Dunn GP, Archambault JM, et al. Type I interferon is selectively required by dendritic cells for immune rejection of tumors. J Exp Med 2011;208: 1989–2003.

7. Fuertes MB, Kacha AK, Kline J, Woo SR, Kranz DM, Murphy KM, et al. Host type I IFN signals are required for antitumor CD8(+) T cell responses through CD8 alpha(+) dendritic cells. J Exp Med 2011;208: 2005–16.

8. Ishikawa H, Barber GN. STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. Nature 2008;455:674–8.

9. Liu Z, Han C, Fu YX. Targeting innate sensing in the tumor microenvironment to improve immunotherapy. Cell Mol Immunol 2020;17:13–26.

10. Corrales L, Matson V, Flood B, Spranger S, Gajewski TF. Innate immune signaling and regulation in cancer immunotherapy. Cell Res 2017;27:96–108.

11. Pages F, Galon J, Dieu-Nosjean MC, Tartour E, Sautes-Fridman C, Fridman WH. Immune infiltration in human tumors: a prognostic factor that should not be ignored. Oncogene 2010;29:1093–102.

12. Weichselbaum RR, Liang H, Deng L, Fu YX. Radiotherapy and immunotherapy: a beneficial liaison? Nat Rev Clin Oncol 2017;14:365–79.

13. Shi Y, Zheng W, Yang K, Harris KG, Ni K, Xue L, et al. Intratumoral accumulation of gut microbiota facilitates CD47-based immunotherapy via STING signaling. J Exp Med 2020;217:e20192282.

14. Li X, Liu Z, Zhang A, Han C, Shen A, Jiang L, et al. NQO1 targeting prodrug triggers innate sensing to overcome checkpoint blockade resistance. Nat Commun 2019;10:3251.

15. Liu Z, Han C, Dong C, Shen A, Hsu E, Ren Z, et al. Hypofractionated EGFR tyrosine kinase inhibitor limits tumor relapse through triggering innate and adaptive immunity. Sci Immunol 2019;4:eaav6473.

16. Deng L, Liang H, Xu M, Yang X, Burnette B, Arina A, et al. STING-dependent cytosolic DNA sensing promotes radiation-induced type I interferon-dependent antitumor immunity in immunogenic tumors. Immunity 2014;41:843–52.

17. Ishikawa H, Ma Z, Barber GN. STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity. Nature 2009;461:788–92.

18. Woo SR, Fuertes MB, Corrales L, Spranger S, Furdyna MJ, Leung MY, et al. STING-dependent cytosolic DNA sensing mediates innate immune recognition of immunogenic tumors. Immunity 2014;41: 830–42.

19. Wang Z, Chen J, Hu J, Zhang H, Xu F, He W, et al. cGAS/STING axis mediates a topoisomerase II inhibitor-induced tumor immunogenicity. J Clin Invest 2019;129:4850–62.

20. Wang H, Hu S, Chen X, Shi H, Chen C, Sun L, et al. cGAS is essential for the antitumor effect of immune checkpoint blockade. Proc Natl Acad Sci USA 2017;114:1637–42.

21. Cheng ZL, Dai T, He XL, Zhang ZK, Xie F, Wang S, et al. The interactions between cGAS-STING pathway and pathogens. Signal Transduct Targeted Ther 2020;5:501–21.

22. Kwon J, Bakhoum SF. The cytosolic DNA-sensing cGAS-STING pathway in cancer. Cancer Discov 2020;10:26–39.

23. Wan DS, Jiang W, Hao JW. Research advances in how the cGAS-STING pathway controls the cellular inflammatory response. Front Immunol 2020;11:615.

24. Dai J, Huang YJ, He X, Zhao M, Wang X, Liu ZS, et al. Acetylation blocks cGAS activity and inhibits self-DNA-induced autoimmunity. Cell 2019; 176:1447–60 e14.

25. Decout A, Katz JD, Venkatraman S, Ablasser A. The cGAS-STING pathway as a therapeutic target in inflammatory diseases. Nat Rev Immunol 2021;21:548–69.

26. Zhang Q, Tang Z, An R, Ye L, Zhong B. USP29 maintains the stability of cGAS and promotes cellular antiviral responses and autoimmunity. Cell Res 2020;30:914–27.

27. Gao D, Li T, Li XD, Chen X, Li QZ, Wight-Carter M, et al. Activation of cyclic GMP-AMP synthase by self-DNA causes autoimmune diseases. Proc Natl Acad Sci USA 2015;112:E5699–705.

28. Xiao N, Wei J, Xu S, Du H, Huang M, Zhang S, et al. cGAS activation causes lupus-like autoimmune disorders in a TREX1 mutant mouse model. J Autoimmun 2019;100:84–94.

29. Chen Q, Sun L, Chen ZJ. Regulation and function of the cGAS-STING pathway of cytosolic DNA sensing. Nat Immunol 2016;17:1142–9.

30. Fang R, Jiang Q, Jia X, Jiang Z. ARMH3-mediated recruitment of PI4KB directs Golgi-to-endosome trafficking and activation of the antiviral effector STING. Immunity 2023;56:500–15.e6.

31. Wu JX, Sun LJ, Chen X, Du FH, Shi HP, Chen C, et al. Cyclic GMP-AMP is an endogenous second messenger in innate immune signaling by cytosolic DNA. Science 2013;339:826–30.

32. Han C, Zhang A, Liu Z, Moore C, Fu YX. Small molecular drugs reshape tumor microenvironment to synergize with immunotherapy. Oncogene 2021;40:885–98.

33. Han C, Godfrey V, Liu Z, Han Y, Liu L, Peng H, et al. The AIM2 and NLRP3 inflammasomes trigger IL-1-mediated antitumor effects during radiation. Sci Immunol. 2021;6. https://doi.org/10.1126/sciimmunol. abc6998.

34. Lee YJ, Auh SL, Wang YG, Burnette B, Wang Y, Meng YR, et al. Therapeutic effects of ablative radiation on local tumor require CD8(+) T cells: changing strategies for cancer treatment. Blood 2009; 114:589–95.

35. Burnette BC, Liang H, Lee Y, Chlewicki L, Khodarev NN, Weichselbaum RR, et al. The efficacy of radiotherapy relies upon induction of type I interferon-dependent innate and adaptive immunity. Cancer Res 2011;71:2488–96.

36. Ahn J, Xia TL, Konno H, Konno K, Ruiz P, Barber GN. Inflammation-driven carcinogenesis is mediated through STING. Nat Commun 2014; 5:5166.

37. Iida N, Dzutsev A, Stewart CA, Smith L, Bouladoux N, Weingarten RA, et al. Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. Science 2013;342:967–70.

38. Apetoh L, Ghiringhelli F, Tesniere A, Obeid M, Ortiz C, Criollo A, et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. Nat Med 2007;13: 1050–9.

39. Park S, Jiang ZJ, Mortenson ED, Deng LF, Radkevich-Brown O, Yang XM, et al. The therapeutic effect of anti-HER2/neu antibody depends on both innate and adaptive immunity. Cancer Cell 2010;18: 160–70.

40. Li W, Lu L, Lu J, Wang X, Yang C, Jin J, et al. cGAS-STING-mediated DNA sensing maintains CD8(+) T cell stemness and promotes antitumor T cell therapy. Sci Transl Med 2020;12. https://doi.org/10.1126/scitranslmed.aay9013.

41. Liu X, Pu Y, Cron K, Deng L, Kline J, Frazier WA, et al. CD47 blockade triggers T cell-mediated destruction of immunogenic tumors. Nat Med 2015;21:1209–15.

42. Heidegger S, Wintges A, Stritzke F, Bek S, Steiger K, Koenig PA, et al. RIG-I activation is critical for responsiveness to checkpoint blockade. Sci Immunol. 2019;4:eaau8943.

43. Mender I, Zhang A, Ren Z, Han C, Deng Y, Siteni S, et al. Telomere stress potentiates STING-dependent anti-tumor immunity. Cancer Cell 2020;38:400–11 e6.

44. Segovia M, Russo S, Jeldres M, Mahmoud YD, Perez V, Duhalde M, et al. Targeting TMEM176B enhances antitumor immunity and augments the efficacy of immune checkpoint blockers by unleashing inflammasome activation. Cancer Cell 2019;35:767–81 e6.

45. Kim C, Wang XD, Yu Y. PARP1 inhibitors trigger innate immunity via PARP1 trapping-induced DNA damage response. Elife 2020;9:e60637.

46. Chabanon RM, Muirhead G, Krastev DB, Adam J, Morel D, Garrido M, et al. PARP inhibition enhances tumor cell-intrinsic immunity in ERCC1-deficient non-small cell lung cancer. J Clin Invest 2019;129: 1211–28.

47. Gui X, Yang H, Li T, Tan XJ, Shi PQ, Li MH, et al. Autophagy induction via STING trafficking is a primordial function of the cGAS pathway. Nature 2019;567:262–6.

48. Yamazaki T, Kirchmair A, Sato A, Buque A, Rybstein M, Petroni G, et al. Mitochondrial DNA drives abscopal responses to radiation that are inhibited by autophagy. Nat Immunol 2020;21:1160–71.

49. Bakhoum SF, Ngo B, Laughney AM, Cavallo JA, Murphy CJ, Ly P, et al. Chromosomal instability drives metastasis through a cytosolic DNA response. Nature 2018;553:467–72.

50. Zheng Z, Jia S, Shao C, Shi Y. Irradiation induces cancer lung metastasis through activation of the cGAS-STING-CCL5 pathway in mesenchymal stromal cells. Cell Death Dis 2020;11:326.

51. Civril F, Deimling T, de Oliveira Mann CC, Ablasser A, Moldt M, Witte G, et al. Structural mechanism of cytosolic DNA sensing by cGAS. Nature 2013;498:332–7.

52. Crossley MP, Song C, Bocek MJ, Choi JH, Kousorous J, Sathirachinda A, et al. R-loop-derived cytoplasmic RNA-DNA hybrids activate an immune response. Nature 2023;613:187–94.

53. Zitvogel L, Galluzzi L, Kepp O, Smyth MJ, Kroemer G. Type I interferons in anticancer immunity. Nat Rev Immunol 2015;15:405–14.

54. Ma F, Li B, Liu SY, Iyer SS, Yu Y, Wu A, et al. Positive feedback regulation of type I IFN production by the IFN-inducible DNA sensor cGAS. J Immunol 2015;194:1545–54.

55. Zhang X, Bai XC, Chen ZJ. Structures and mechanisms in the cGAS-STING innate immunity pathway. Immunity 2020;53:43–53.

56. Xia T, Konno H, Ahn J, Barber GN. Deregulation of STING signaling in colorectal carcinoma constrains DNA damage responses and correlates with tumorigenesis. Cell Rep 2016;14:282–97.

57. Falahat R, Berglund A, Putney RM, Perez-Villarroel P, Aoyama S, Pilon-Thomas S, et al. Epigenetic reprogramming of tumor cell-intrinsic STING function sculpts antigenicity and T cell recognition of melanoma. Proc Natl Acad Sci USA 2021;118:e2013598118.

58. Han C, Liu Z, Zhang Y, Shen A, Dong C, Zhang A, et al. Tumor cells suppress radiation-induced immunity by hijacking caspase 9 signaling. Nat Immunol 2020;21:546–54.

59. Rodriguez-Ruiz ME, Buque A, Hensler M, Chen J, Bloy N, Petroni G, et al. Apoptotic caspases inhibit abscopal responses to radiation and identify a new prognostic biomarker for breast cancer patients. OncoImmunology 2019;8:e1655964.

60. Chattopadhyay S, Marques JT, Yamashita M, Peters KL, Smith K, Desai A, et al. Viral apoptosis is induced by IRF-3-mediated activation of Bax. EMBO J 2010;29:1762–73.

61. Zhu Q, Man SM, Karki R, Malireddi RKS, Kanneganti TD. Detrimental type I interferon signaling dominates protective AIM2 inflammasome responses during francisella novicida infection. Cell Rep 2018;22: 3168–74.

62. Herzer K, Hofmann TG, Teufel A, Schimanski CC, Moehler M, Kanzler S, et al. IFN-alpha-induced apoptosis in hepatocellular carcinoma involves promyelocytic leukemia protein and TRAIL independently of p53. Cancer Res 2009;69:855–62.

63. Kotredes KP, Gamero AM. Interferons as inducers of apoptosis in malignant cells. J Interferon Cytokine Res 2013;33:162–70.

64. Liu D, Wu H, Wang C, Li Y, Tian H, Siraj S, et al. STING directly activates autophagy to tune the innate immune response. Cell Death Differ 2019;26:1735–49.

65. Brault M, Olsen TM, Martinez J, Stetson DB, Oberst A. Intracellular nucleic acid sensing triggers necroptosis through synergistic type I IFN and TNF signaling. J Immunol 2018;200:2748–56.

66. Sarhan J, Liu BC, Muendlein HI, Weindel CG, Smirnova I, Tang AY, et al. Constitutive interferon signaling maintains critical threshold of MLKL expression to license necroptosis. Cell Death Differ 2019;26:332–47.

67. Zhang X, Wu J, Liu Q, Li X, Li S, Chen J, et al. mtDNA-STING pathway promotes necroptosis-dependent enterocyte injury in intestinal ischemia reperfusion. Cell Death Dis 2020;11:1050.

68. Schock SN, Chandra NV, Sun Y, Irie T, Kitagawa Y, Gotoh B, et al. Induction of necroptotic cell death by viral activation of the RIG-I or STING pathway. Cell Death Differ 2017;24:615–25.

69. Chen D, Tong J, Yang L, Wei L, Stolz DB, Yu J, et al. PUMA amplifies necroptosis signaling by activating cytosolic DNA sensors. Proc Natl Acad Sci USA 2018;115:3930–5.

70. Gaidt MM, Ebert TS, Chauhan D, Ramshorn K, Pinci F, Zuber S, et al. The DNA inflammasome in human myeloid cells is initiated by a STING-cell death program upstream of NLRP3. Cell 2017;171:1110–24.

71. Wang W, Hu D, Wu C, Feng Y, Li A, Liu W, et al. STING promotes NLRP3 localization in ER and facilitates NLRP3 deubiquitination to activate the inflammasome upon HSV-1 infection. PLoS Pathog 2020;16: e1008335.

72. Rongvaux A, Jackson R, Harman CC, Li T, West AP, de Zoete MR, et al. Apoptotic caspases prevent the induction of type I interferons by mitochondrial DNA. Cell 2014;159:1563–77.

73. White MJ, McArthur K, Metcalf D, Lane RM, Cambier JC, Herold MJ, et al. Apoptotic caspases suppress mtDNA-induced STING-mediated type I IFN production. Cell 2014;159:1549–62.

74. Ning XH, Wang YT, Jing M, Sha MY, Lv MZ, Gao PF, et al. Apoptotic caspases suppress type I interferon production via the cleavage of cGAS, MAVS, and IRF3. Mol Cell 2019;74:19–31.

75. Wang YT, Ning XH, Gao PF, Wu SX, Sha MY, Lv MZ, et al. Inflammasome activation triggers caspase-1-mediated cleavage of cGAS to regulate responses to DNA virus infection. Immunity 2017;46:393–404.

76. Banerjee I, Behl B, Mendonca M, Shrivastava G, Russo AJ, Menoret A, et al. Gasdermin D restrains type I interferon response to cytosolic DNA by disrupting ionic homeostasis. Immunity 2018;49:413–26.

77. Yang Y, Wu M, Cao D, Yang C, Jin J, Wu L, et al. ZBP1-MLKL necroptotic signaling potentiates radiation-induced antitumor immunity via intratumoral STING pathway activation. Sci Adv 2021;7:eabf6290.

78. Cobo I, Tanaka TN, Chandra Mangalhara K, Lana A, Yeang C, Han C, et al. DNA methyltransferase 3 alpha and TET methylcytosine dioxygenase 2 restrain mitochondrial DNA-mediated interferon signaling in macrophages. Immunity 2022;55:1386–401.e10.

79. Xian H, Watari K, Sanchez-Lopez E, Offenberger J, Onyuru J, Sampath H, et al. Oxidized DNA fragments exit mitochondria via mPTP- and VDAC-dependent channels to activate NLRP3 inflammasome and interferon signaling. Immunity 2022;55: 1370–85.e8.

80. Lei Y, VanPortfliet JJ, Chen YF, Bryant JD, Li Y, Fails D, et al. Cooperative sensing of mitochondrial DNA by ZBP1 and cGAS promotes cardiotoxicity. Cell 2023;186:3013–32.e22.

81. Zecchini V, Paupe V, Herranz-Montoya I, Janssen J, Wortel IMN, Morris JL, et al. Fumarate induces vesicular release of mtDNA to drive innate immunity. Nature 2023;615:499–506.

82. Yang X, Shi C, Li H, Shen S, Su C, Yin H. MARCH8 attenuates cGAS-mediated innate immune responses through ubiquitylation. Sci Signal 2022;15:eabk3067.

83. Cai X, Zhou Z, Zhu J, Liu X, Ouyang G, Wang J, et al. Opposing effects of deubiquitinase OTUD3 in innate immunity against RNA and DNA viruses. Cell Rep 2022;39:110920.

84. Takahashi M, Lio CJ, Campeau A, Steger M, Ay F, Mann M, et al. The tumor suppressor kinase DAPK3 drives tumor-intrinsic immunity through the STING-IFN-beta pathway. Nat Immunol 2021;22:485–96.

85. Kong L, Sui C, Chen T, Zhang L, Zhao W, Zheng Y, et al. The ubiquitin E3 ligase TRIM10 promotes STING aggregation and activation in the Golgi apparatus. Cell Rep 2023;42:112306.

86. Tian M, Liu W, Zhang Q, Huang Y, Li W, Wang W, et al. MYSM1 represses innate immunity and autoimmunity through suppressing the cGAS-STING pathway. Cell Rep 2020;33:108297.

87. Zhang J, Chen Y, Chen X, Zhang W, Zhao L, Weng L, et al. Deubiquitinase USP35 restrains STING-mediated interferon signaling in ovarian cancer. Cell Death Differ 2021;28:139–55.

88. Seo GJ, Yang A, Tan B, Kim S, Liang QM, Choi Y, et al. Akt kinase-mediated checkpoint of cGAS DNA sensing pathway. Cell Rep 2015;13: 440–9.

89. Zierhut C, Yamaguchi N, Paredes M, Luo JD, Carroll T, Funabiki H. The cytoplasmic DNA sensor cGAS promotes mitotic cell death. Cell 2019; 178:302–15.

90. Pathare GR, Decout A, Gluck S, Cavadini S, Makasheva K, Hovius R, et al. Structural mechanism of cGAS inhibition by the nucleosome. Nature 2020;587:668–72.

91. Michalski S, de Oliveira Mann CC, Stafford CA, Witte G, Bartho J, Lammens K, et al. Structural basis for sequestration and autoinhibition of cGAS by chromatin. Nature 2020;587:678–82.

92. Zhao B, Xu P, Rowlett CM, Jing T, Shinde O, Lei Y, et al. The molecular basis of tight nuclear tethering and inactivation of cGAS. Nature 2020; 587:673–7.

93. Kujirai T, Zierhut C, Takizawa Y, Kim R, Negishi L, Uruma N, et al. Structural basis for the inhibition of cGAS by nucleosomes. Science 2020;370:455–8.

94. Cao D, Han X, Fan X, Xu RM, Zhang X. Structural basis for nucleosome-mediated inhibition of cGAS activity. Cell Res 2020;30:1088–97.

95. Peitzmeier SM, Silberholz J, Gardner IH, Weinand J, Acevedo K. Time to first onset of chest binding-related symptoms in transgender youth. Pediatrics 2021;147:e20200728.

96. Sun X, Liu T, Zhao J, Xia H, Xie J, Guo Y, et al. DNA-PK deficiency potentiates cGAS-mediated antiviral innate immunity. Nat Commun 2020;11:6182.

97. Zhang C, Shang G, Gui X, Zhang X, Bai XC, Chen ZJ. Structural basis of STING binding with and phosphorylation by TBK1. Nature 2019;567: 394–8.

98. Liu S, Cai X, Wu J, Cong Q, Chen X, Li T, et al. Phosphorylation of innate immune adaptor proteins MAVS, STING, and TRIF induces IRF3 activation. Science 2015;347:aaa2630.

99. Hu MM, Yang Q, Xie XQ, Liao CY, Lin H, Liu TT, et al. Sumoylation promotes the stability of the DNA sensor cGAS and the adaptor STING to regulate the kinetics of response to DNA virus. Immunity 2016;45: 555–69.

100. Cui Y, Yu HS, Zheng X, Peng R, Wang Q, Zhou Y, et al. SENP7 potentiates cGAS activation by relieving SUMO-mediated inhibition of cytosolic DNA sensing. PLoS Pathog 2017;13:e1006156.

101. Xia PY, Ye BQ, Wang S, Zhu XX, Du Y, Xiong Z, et al. Glutamylation of the DNA sensor cGAS regulates its binding and synthase activity in antiviral immunity. Nat Immunol 2016;17:369–78.

102. Song ZM, Lin H, Yi XM, Guo W, Hu MM, Shu HB. KAT5 acetylates cGAS to promote innate immune response to DNA virus. P Natl Acad Sci USA 2020;117:21568–75.

103. Ma DP, Yang M, Wang QS, Sun CY, Shi HB, Jing WQ, et al. Arginine methyltransferase PRMT5 negatively regulates cGAS-mediated antiviral immune response. Sci Adv 2021;7:eabc1834.

104. Shi C, Yang X, Liu Y, Li H, Chu H, Li G, et al. ZDHHC18 negatively regulates cGAS-mediated innate immunity through palmitoylation. EMBO J 2022;41:e109272.

105. Mukai K, Konno H, Akiba T, Uemura T, Waguri S, Kobayashi T, et al. Activation of STING requires palmitoylation at the Golgi. Nat Commun 2016;7:11932.

106. Wang F, Zhao M, Chang B, Zhou Y, Wu X, Ma M, et al. Cytoplasmic PARP1 links the genome instability to the inhibition of antiviral immunity through PARylating cGAS. Mol Cell 2022;82:2032–49.

107. Ferguson BJ, Mansur DS, Peters NE, Ren H, Smith GL. DNA-PK is a DNA sensor for IRF-3-dependent innate immunity. Elife 2012;1:e00047.

108. Harding SM, Benci JL, Irianto J, Discher DE, Minn AJ, Greenberg RA. Mitotic progression following DNA damage enables pattern recognition within micronuclei. Nature 2017;548:466–70.

109. Li L, Yin Q, Kuss P, Maliga Z, Millan JL, Wu H, et al. Hydrolysis of 2′ 3′-cGAMP by ENPP1 and design of nonhydrolyzable analogs. Nat Chem Biol 2014;10:1043–8.

110. Lohofener J, Steinke N, Kay-Fedorov P, Baruch P, Nikulin A, Tishchenko S, et al. The activation mechanism of 2′-5′-oligoadenylate synthetase gives new insights into OAS/cGAS triggers of innate immunity. Structure 2015;23:851–62.

111. Wang C, Guan Y, Lv M, Zhang R, Guo Z, Wei X, et al. Manganese increases the sensitivity of the cGAS-STING pathway for double-stranded DNA and is required for the host defense against DNA viruses. Immunity 2018;48:675–87.

112. Zhao Z, Ma Z, Wang B, Guan Y, Su XD, Jiang Z. Mn(2+) directly activates cGAS and structural analysis suggests Mn(2+) induces a noncanonical catalytic synthesis of 2′3′-cGAMP. Cell Rep 2020;32:108053.

113. Lv M, Chen M, Zhang R, Zhang W, Wang C, Zhang Y, et al. Manganese is critical for antitumor immune responses via cGAS-STING and improves the efficacy of clinical immunotherapy. Cell Res 2020;30: 966–79.

114. Du M, Chen ZJ. DNA-induced liquid phase condensation of cGAS activates innate immune signaling. Science 2018;361:704–9.

115. Yu X, Zhang L, Shen J, Zhai Y, Jiang Q, Yi M, et al. The STING phase-separator suppresses innate immune signalling. Nat Cell Biol 2021;23: 330–40.

116. Andreeva L, Hiller B, Kostrewa D, Lässig C, de Oliveira Mann CC, Jan Drexler D, et al. cGAS senses long and HMGB/TFAM-bound U-turn DNA by forming protein-DNA ladders. Nature 2017;549:394–8.

117. Grieves JL, Fye JM, Harvey S, Grayson JM, Hollis T, Perrino FW. Exonuclease TREX1 degrades double-stranded DNA to prevent spontaneous lupus-like inflammatory disease. Proc Natl Acad Sci USA 2015;112:5117–22.

118. Yuan F, Dutta T, Wang L, Song L, Gu L, Qian L, et al. Human DNA exonuclease TREX1 is also an exoribonuclease that acts on single-stranded RNA. J Biol Chem 2015;290:13344–53.

119. Stetson DB, Ko JS, Heidmann T, Medzhitov R. Trex1 prevents cell-intrinsic initiation of autoimmunity. Cell 2008;134:587–98.

120. Pantelidou C, Sonzogni O, Taveira MD, Mehta AK, Kothari A, Wang D, et al. PARP inhibitor efficacy depends on CD8(+) T-cell recruitment via intratumoral STING pathway activation in BRCA-deficient models of triple-negative breast cancer. Cancer Discov 2019;9:722–37.

121. Zhou W, Mohr L, Maciejowski J, Kranzusch PJ. cGAS phase separation inhibits TREX1-mediated DNA degradation and enhances cytosolic DNA sensing. Mol Cell 2021;81:739–55.

122. Ghosh M, Saha S, Li J, Montrose DC, Martinez LA. p53 engages the cGAS/STING cytosolic DNA sensing pathway for tumor suppression. Mol Cell 2023;83:266–80.e6.

123. Zhao Q, Yang C, Wang J, Li Y, Yang P. Serum level of DNase1l3 in patients with dermatomyositis/polymyositis, systemic lupus erythematosus and rheumatoid arthritis, and its association with disease activity. Clin Exp Med 2017;17:459–65.

124. Sisirak V, Sally B, D'Agati V, Martinez-Ortiz W, Ozcakar ZB, David J, et al. Digestion of chromatin in apoptotic cell microparticles prevents autoimmunity. Cell 2016;166:88–101.

125. Soni C, Perez OA, Voss WN, Pucella JN, Serpas L, Mehl J, et al. Plasmacytoid dendritic cells and type I interferon promote extrafollicular B cell responses to extracellular self-DNA. Immunity 2020;52:1022–38.

126. Mosallanejad K, Kennedy SN, Bahleda KM, Slavik KM, Zhou W, Govande AA, et al. Species-specific self-DNA detection mechanisms by mammalian cyclic GMP-AMP synthases. Sci Immunol. 2023;8: eabp9765.

127. Liu Y, Xu P, Rivara S, Liu C, Ricci J, Ren X, et al. Clathrin-associated AP-1 controls termination of STING signalling. Nature 2022;610:761–7.

128. Liu B, Carlson RJ, Pires IS, Gentili M, Feng E, Hellier Q, et al. Human STING is a proton channel. Science 2023;381:508–14.

129. Vanpouille-Box C, Alard A, Aryankalayil MJ, Sarfraz Y, Diamond JM, Schneider RJ, et al. DNA exonuclease Trex1 regulates radiotherapy-induced tumour immunogenicity. Nat Commun 2017;8:15618.

130. Lu C, Guan J, Lu S, Jin Q, Rousseau B, Lu T, et al. DNA sensing in mismatch repair-deficient tumor cells is essential for anti-tumor immunity. Cancer Cell 2021;39:96–108.

131. Tian J, Zhang D, Kurbatov V, Wang Q, Wang Y, Fang D, et al. 5-Fluorouracil efficacy requires anti-tumor immunity triggered by cancer-cell-intrinsic STING. EMBO J 2021;40:e106065.

132. Crasta K, Ganem NJ, Dagher R, Lantermann AB, Ivanova EV, Pan Y, et al. DNA breaks and chromosome pulverization from errors in mitosis. Nature 2012;482:53–8.

133. Zhang W, Liu W, Jia L, Chen D, Chang I, Lake M, et al. Targeting KDM4A epigenetically activates tumor-cell-intrinsic immunity by inducing DNA replication stress. Mol Cell 2021;81:2148–65.

134. Zhang CZ, Spektor A, Cornils H, Francis JM, Jackson EK, Liu S, et al. Chromothripsis from DNA damage in micronuclei. Nature 2015;522: 179–84.

135. Mackenzie KJ, Carroll P, Martin CA, Murina O, Fluteau A, Simpson DJ, et al. cGAS surveillance of micronuclei links genome instability to innate immunity. Nature 2017;548:461–5.

136. Song S, Peng P, Tang Z, Zhao J, Wu W, Li H, et al. Decreased expression of STING predicts poor prognosis in patients with gastric cancer. Sci Rep 2017;7:39858.

137. Marcus A, Mao AJ, Lensink-Vasan M, Wang L, Vance RE, Raulet DH. Tumor-derived cGAMP triggers a STING-mediated interferon response in non-tumor cells to activate the NK cell response. Immunity 2018;49:754–63.

138. Zhou Y, Fei M, Zhang G, Liang WC, Lin W, Wu Y, et al. Blockade of the phagocytic receptor MerTK on tumor-associated macrophages enhances P2X7R-dependent STING activation by tumor-derived cGAMP. Immunity 2020;52:357–73.

139. An X, Zhu Y, Zheng T, Wang G, Zhang M, Li J, et al. An analysis of the expression and association with immune cell infiltration of the cGAS/STING pathway in pan-cancer. Mol Ther Nucleic Acids 2019;14:80–9.

140. Diamond JM, Vanpouille-Box C, Spada S, Rudqvist NP, Chapman JR, Ueberheide BM, et al. Exosomes shuttle TREX1-sensitive IFN-stimulatory dsDNA from irradiated cancer cells to DCs. Cancer Immunol Res 2018;6:910–20.

141. Zhang H, Tang K, Zhang Y, Ma R, Ma J, Li Y, et al. Cell-free tumor microparticle vaccines stimulate dendritic cells via cGAS/STING signaling. Cancer Immunol Res 2015;3:196–205.

142. Peng D, Kryczek I, Nagarsheth N, Zhao L, Wei S, Wang W, et al. Epigenetic silencing of TH1-type chemokines shapes tumour immunity and immunotherapy. Nature 2015;527:249–53.

143. Liang Y, Hannan R, Fu YX. Type I IFN activating type I dendritic cells for antitumor immunity. Clin Cancer Res 2021;27:3818–24.

144. Maltbaek JH, Cambier S, Snyder JM, Stetson DB. ABCC1 transporter exports the immunostimulatory cyclic dinucleotide cGAMP. Immunity 2022;55:1799–812.e4.

145. Ritchie C, Cordova AF, Hess GT, Bassik MC, Li L. SLC19A1 is an importer of the immunotransmitter cGAMP. Mol Cell 2019;75:372–81.

146. Liu H, Zhang H, Wu X, Ma D, Wu J, Wang L, et al. Nuclear cGAS suppresses DNA repair and promotes tumorigenesis. Nature 2018; 563:131–6.

147. Li J, Hubisz MJ, Earlie EM, Duran MA, Hong C, Varela AA, et al. Non-cell-autonomous cancer progression from chromosomal instability. Nature 2023;620:1080–8.

148. Hong C, Schubert M, Tijhuis AE, Requesens M, Roorda M, van den Brink A, et al. cGAS-STING drives the IL-6-dependent survival of chromosomally instable cancers. Nature 2022;607:366–73.

149. Bakhoum SF. APOBEC3A drives STING-dependent metastasis. Nat Can (Ott) 2021;2:1293–5.

150. Wormann SM, Zhang A, Thege FI, Cowan RW, Rupani DN, Wang R, et al. APOBEC3A drives deaminase domain-independent chromosomal instability to promote pancreatic cancer metastasis. Nat Can (Ott) 2021;2:1338–56.

151. Qiu S, Zhong X, Meng X, Li S, Qian X, Lu H, et al. Mitochondria-localized cGAS suppresses ferroptosis to promote cancer progression. Cell Res 2023;33:299–311.

152. Barcellos-Hoff MH, Park C, Wright EG. Radiation and the microenvironment – tumorigenesis and therapy. Nat Rev Cancer 2005;5:867–75.

153. Deng L, Liang H, Burnette B, Beckett M, Darga T, Weichselbaum RR, et al. Irradiation and anti-PD-L1 treatment synergistically promote antitumor immunity in mice. J Clin Invest 2014;124:687–95.

154. Cohen-Jonathan E, Bernhard EJ, McKenna WG. How does radiation kill cells? Curr Opin Chem Biol 1999;3:77–83.

155. Fang C, Mo F, Liu L, Du J, Luo M, Men K, et al. Oxidized mitochondrial DNA sensing by STING signaling promotes the antitumor effect of an irradiated immunogenic cancer cell vaccine. Cell Mol Immunol 2021; 18:2211–23.

156. Gupta A, Probst HC, Vuong V, Landshammer A, Muth S, Yagita H, et al. Radiotherapy promotes tumor-specific effector CD8(+) T cells via dendritic cell activation. J Immunol 2012;189:558–66.

157. Schadt L, Sparano C, Schweiger NA, Silina K, Cecconi V, Lucchiari G, et al. Cancer-cell-intrinsic cGAS expression mediates tumor immunogenicity. Cell Rep 2019;29:1236–48.

158. Liang H, Deng LF, Hou YZ, Meng XJ, Huang XN, Rao EY, et al. Host STING-dependent MDSC mobilization drives extrinsic radiation resistance. Nat Commun 2017;8:1736.

159. Hou Y, Liang H, Rao E, Zheng W, Huang X, Deng L, et al. Non-canonical NF-kappaB antagonizes STING sensor-mediated DNA sensing in radiotherapy. Immunity 2018;49:490–503.

160. Chen HY, Xu L, Li LF, Liu XX, Gao JX, Bai YR. Inhibiting the CD8(+) T cell infiltration in the tumor microenvironment after radiotherapy is an important mechanism of radioresistance. Sci Rep 2018;8:11934.

161. Crittenden MR, Zebertavage L, Kramer G, Bambina S, Friedman D, Troesch V, et al. Tumor cure by radiation therapy and checkpoint inhibitors depends on pre-existing immunity. Sci Rep 2018;8:7012.

162. Shinde-Jadhav S, Mansure JJ, Rayes RF, Marcq G, Ayoub M, Skowronski R, et al. Role of neutrophil extracellular traps in radiation resistance of invasive bladder cancer. Nat Commun 2021;12:2776.

163. McBride S, Sherman E, Tsai CJ, Baxi S, Aghalar J, Eng J, et al. Randomized phase II trial of nivolumab with stereotactic body radiotherapy versus nivolumab alone in metastatic head and neck squamous cell carcinoma. J Clin Oncol 2021;39:30–7.

164. Sistigu A, Yamazaki T, Vacchelli E, Chaba K, Enot DP, Adam J, et al. Cancer cell-autonomous contribution of type I interferon signaling to the efficacy of chemotherapy. Nat Med 2014;20:1301–9.

165. Zitvogel L, Kepp O, Kroemer G. Immune parameters affecting the efficacy of chemotherapeutic regimens. Nat Rev Clin Oncol 2011;8: 151–60.

166. Erdal E, Haider S, Rehwinkel J, Harris AL, McHugh PJ. A prosurvival DNA damage-induced cytoplasmic interferon response is mediated by end resection factors and is limited by Trex1. Gene Dev 2017;31:353–69.

167. Kitai Y, Kawasaki T, Sueyoshi T, Kobiyama K, Ishii KJ, Zou J, et al. DNA-containing exosomes derived from cancer cells treated with topotecan activate a STING-dependent pathway and reinforce antitumor immunity. J Immunol 2017;198:1649–59.

168. Harper JW, Elledge SJ. The DNA damage response: ten years after. Mol Cell 2007;28:739–45.

169. Jackson SP, Bartek J. The DNA-damage response in human biology and disease. Nature 2009;461:1071–8.

170. Pascal JM. The comings and goings of PARP-1 in response to DNA damage. DNA Repair 2018;71:177–82.

171. Chaudhuri AR, Nussenzweig A. The multifaceted roles of PARP1 in DNA repair and chromatin remodelling. Nat Rev Mol Cell Biol 2017;18: 610–21.

172. Sen T, Rodriguez BL, Chen LM, Della Corte CM, Morikawa N, Fujimoto J, et al. Targeting DNA damage response promotes antitumor immunity through STING-mediated T-cell activation in small cell lung cancer. Cancer Discov 2019;9:646–61.

173. Wang L, Yang L, Wang C, Zhao W, Ju Z, Zhang W, et al. Inhibition of the ATM/Chk2 axis promotes cGAS/STING signaling in ARID1A-deficient tumors. J Clin Invest 2020;130:5951–66.

174. Hu M, Zhou M, Bao X, Pan D, Jiao M, Liu X, et al. ATM inhibition enhances cancer immunotherapy by promoting mtDNA leakage and cGAS/STING activation. J Clin Invest 2021;131:e139333.

175. Zhang Y, Chen H, Mo H, Hu X, Gao R, Zhao Y, et al. Single-cell analyses reveal key immune cell subsets associated with response to PD-L1 blockade in triple-negative breast cancer. Cancer Cell 2021;39: 1578–93.

176. Xu MM, Pu Y, Han D, Shi Y, Cao X, Liang H, et al. Dendritic cells but not macrophages sense tumor mitochondrial DNA for cross-priming through signal regulatory protein alpha signaling. Immunity 2017;47: 363–73.

177. Jacquelot N, Yamazaki T, Roberti MP, Duong CPM, Andrews MC, Verlingue L, et al. Sustained type I interferon signaling as a mechanism of resistance to PD-1 blockade. Cell Res 2019;29:846–61.

178. Buque A, Rodriguez-Ruiz ME, Fucikova J, Galluzzi L. Apoptotic caspases cut down the immunogenicity of radiation. OncoImmunology 2019;8: e1655364.

179. Wang Y, Gao W, Shi X, Ding J, Liu W, He H, et al. Chemotherapy drugs induce pyroptosis through caspase-3 cleavage of a gasdermin. Nature 2017;547:99–103.

180. Chen M, Meng Q, Qin Y, Liang P, Tan P, He L, et al. TRIM14 inhibits cGAS degradation mediated by selective autophagy receptor p62 to promote innate immune responses. Mol Cell 2016;64:105–19.

181. Witte G, Hartung S, Buttner K, Hopfner KP. Structural biochemistry of a bacterial checkpoint protein reveals diadenylate cyclase activity regulated by DNA recombination intermediates. Mol Cell 2008;30: 167–78.

182. Woodward JJ, Iavarone AT, Portnoy DA. c-di-AMP secreted by intracellular Listeria monocytogenes activates a host type I interferon response. Science 2010;328:1703–5.

183. McWhirter SM, Barbalat R, Monroe KM, Fontana MF, Hyodo M, Joncker NT, et al. A host type I interferon response is induced by cytosolic sensing of the bacterial second messenger cyclic-di-GMP. J Exp Med 2009;206:1899–911.

184. Karaolis DKR, Cheng KR, Lipsky M, Elnabawi A, Catalano J, Hyodo M, et al. 3′,5′-Cyclic diguanylic acid (c-di-GMP) inhibits basal and growth factor-stimulated human colon cancer cell proliferation. Biochem Bioph Res Co 2005;329:40–5.

185. Demaria O, De Gassart A, Coso S, Gestermann N, Di Domizio J, Flatz L, et al. STING activation of tumor endothelial cells initiates spontaneous and therapeutic antitumor immunity. Proc Natl Acad Sci USA 2015;112: 15408–13.

186. Harrington KJ, Brody J, Ingham M, Strauss J, Cemerski S, Wang M, et al. Preliminary results of the first-in-human (FIH) study of MK-1454, an agonist of stimulator of interferon genes (STING), as monotherapy or

in combination with pembrolizumab (pembro) in patients with advanced solid tumors or lymphomas. Ann Oncol 2018;29:viii712.

187. McIntosh JA, Liu Z, Andresen BM, Marzijarani NS, Moore JC, Marshall NM, et al. A kinase-cGAS cascade to synthesize a therapeutic STING activator. Nature 2022;603:439–44.

188. Woon ST, Reddy CB, Drummond CJ, Schooltink MA, Baguley BC, Kieda C, et al. A comparison of the ability of DMXAA and xanthenone analogues to activate NF-kappaB in murine and human cell lines. Oncol Res 2005;15:351–64.

189. Roberts ZJ, Goutagny N, Perera PY, Kato H, Kumar H, Kawai T, et al. The chemotherapeutic agent DMXAA potently and specifically activates the TBK1-IRF-3 signaling axis. J Exp Med 2007;204:1559–69.

190. Conlon J, Burdette DL, Sharma S, Bhat N, Thompson M, Jiang Z, et al. Mouse, but not human STING, binds and signals in response to the vascular disrupting agent 5,6-dimethylxanthenone-4-acetic acid. J Immunol 2013;190:5216–25.

191. Cavlar T, Deimling T, Ablasser A, Hopfner KP, Hornung V. Species-specific detection of the antiviral small-molecule compound CMA by STING. EMBO J 2013;32:1440–50.

192. Zhang Y, Sun Z, Pei J, Luo Q, Zeng X, Li Q, et al. Identification of alpha-mangostin as an agonist of human STING. ChemMedChem 2018;13:2057–64.

193. Ramanjulu JM, Pesiridis GS, Yang JS, Concha N, Singhaus R, Zhang SY, et al. Design of amidobenzimidazole STING receptor agonists with systemic activity. Nature 2018;564:439–43.

194. Messaoud-Nacer Y, Culerier E, Rose S, Maillet I, Rouxel N, Briault S, et al. STING agonist diABZI induces PANoptosis and DNA mediated acute respiratory distress syndrome (ARDS). Cell Death Dis 2022;13:269.

195. Chin EN, Yu C, Vartabedian VF, Jia Y, Kumar M, Gamo AM, et al. Antitumor activity of a systemic STING-activating non-nucleotide cGAMP mimetic. Science 2020;369:993–9.

196. Pan BS, Perera SA, Piesvaux JA, Presland JP, Schroeder GK, Cumming JN, et al. An orally available non-nucleotide STING agonist with antitumor activity. Science 2020;369:eaba6098.

197. Sivick KE, Desbien AL, Glickman LH, Reiner GL, Corrales L, Surh NH, et al. Magnitude of therapeutic STING activation determines CD8(+) T cell-mediated anti-tumor immunity. Cell Rep 2018;25:3074–85.

198. Shae D, Becker KW, Christov P, Yun DS, Lytton-Jean AKR, Sevimli S, et al. Endosomolytic polymersomes increase the activity of cyclic dinucleotide STING agonists to enhance cancer immunotherapy. Nat Nanotechnol 2019;14:269–78.

199. Koshy ST, Cheung AS, Gu L, Graveline AR, Mooney DJ. Liposomal delivery enhances immune activation by STING agonists for cancer immunotherapy. Adv Biosyst 2017;1:1600013.

200. Li S, Mirlekar B, Johnson BM, Brickey WJ, Wrobel JA, Yang N, et al. STING-induced regulatory B cells compromise NK function in cancer immunity. Nature 2022;610:373–80.

201. Jneid B, Bochnakian A, Hoffmann C, Delisle F, Djacoto E, Sirven P, et al. Selective STING stimulation in dendritic cells primes antitumor T cell responses. Sci Immunol 2023;8:eabn6612.

202. Yang K, Han W, Jiang X, Piffko A, Bugno J, Han C, et al. Zinc cyclic di-AMP nanoparticles target and suppress tumours via endothelial STING activation and tumour-associated macrophage reinvigoration. Nat Nanotechnol 2022;17:1322–31.

203. Dane EL, Belessiotis-Richards A, Backlund C, Wang J, Hidaka K, Milling LE, et al. STING agonist delivery by tumour-penetrating PEG-lipid nanodiscs primes robust anticancer immunity. Nat Mater 2022;21:710–20.

204. Xue A, Shang Y, Jiao P, Zhang S, Zhu C, He X, et al. Increased activation of cGAS-STING pathway enhances radiosensitivity of non-small cell lung cancer cells. Thorac Cancer 2022;13:1361–8.

205. Zaidi AH, Kelly RJ, Gorbunova A, Omstead AN, Salvitti MS, Zheng P, et al. Intratumoral immunotherapy with STING agonist, ADU-S100, induces CD8^{+} T-cell mediated anti-tumor immunity in an esophageal adenocarcinoma model. Oncotarget 2021;12:292–303.

206. Ghaffari A, Peterson N, Khalaj K, Vitkin N, Robinson A, Francis JA, et al. STING agonist therapy in combination with PD-1 immune checkpoint blockade enhances response to carboplatin chemotherapy in high-grade serous ovarian cancer. Br J Cancer 2018;119:440–9.

207. Jeong SH, Yang MJ, Choi S, Kim J, Koh GY. Refractoriness of STING therapy is relieved by AKT inhibitor through effective vascular disruption in tumour. Nat Commun 2021;12:4405.

208. Eshhar Z, Waks T, Gross G, Schindler DG. Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma-subunit or zeta-subunit of the immunoglobulin and T-cell receptors. P Natl Acad Sci USA 1993;90:720–4.

209. Depil S, Duchateau P, Grupp SA, Mufti G, Poirot L. 'Off-the-shelf' allogeneic CAR T cells: development and challenges. Nat Rev Drug Discov 2020;19:185–99.

210. Neelapu SS, Locke FL, Bartlett NL, Lekakis LJ, Miklos DB, Jacobson CA, et al. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. N Engl J Med 2017;377:2531–44.

211. Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. N Engl J Med 2018;378:439–48.

212. Tchou J, Zhao YB, Levine BL, Zhang PJ, Davis MM, Melenhorst JJ, et al. Safety and efficacy of intratumoral injections of chimeric antigen receptor (CAR) T cells in metastatic breast cancer. Cancer Immunol Res 2017;5:1152–61.

213. Smith TT, Moffett HF, Stephan SB, Opel CF, Dumigan AG, Jiang XY, et al. Biopolymers codelivering engineered T cells and STING agonists can eliminate heterogeneous tumors. J Clin Invest 2017;127:2176–91.

214. Xu N, Palmer DC, Robeson AC, Shou P, Bommiasamy H, Laurie SJ, et al. STING agonist promotes CAR T cell trafficking and persistence in breast cancer. J Exp Med 2021;218:e20200844.

215. Ngiow SF, Young A, Jacquelot N, Yamazaki T, Enot D, Zitvogel L, et al. A threshold level of intratumor CD8(+) T-cell PD1 expression dictates therapeutic response to anti-PD1. Cancer Res 2015;75:3800–11.

216. Gao Q, Wang XY, Qiu SJ, Yamato I, Sho M, Nakajima Y, et al. Overexpression of PD-L1 significantly associates with tumor aggressiveness and postoperative recurrence in human hepatocellular carcinoma. Clin Cancer Res 2009;15:971–9.

217. Tang H, Wang Y, Chlewicki LK, Zhang Y, Guo J, Liang W, et al. Facilitating T cell infiltration in tumor microenvironment overcomes resistance to PD-L1 blockade. Cancer Cell 2016;29:285–96.

218. Garcia-Diaz A, Shin DS, Moreno BH, Saco J, Escuin-Ordinas H, Rodriguez GA, et al. Interferon receptor signaling pathways regulating PD-L1 and PD-L2 expression. Cell Rep 2017;19:1189–201.

219. Foote JB, Kok M, Leatherman JM, Armstrong TD, Marcinkowski BC, Ojalvo LS, et al. A STING agonist given with OX40 receptor and PD-L1 modulators primes immunity and reduces tumor growth in Tolerized mice. Cancer Immunol Res 2017;5:468–79.

220. Moore E, Clavijo PE, Davis R, Cash H, Van Waes C, Kim Y, et al. Established T cell-inflamed tumors rejected after adaptive resistance was reversed by combination STING activation and PD-1 pathway blockade. Cancer Immunol Res 2016;4:1061–71.

221. Ager CR, Reilley MJ, Nicholas C, Bartkowiak T, Jaiswal AR, Curran MA. Intratumoral STING activation with T-cell checkpoint modulation generates systemic antitumor immunity. Cancer Immunol Res 2017;5: 676–84.

222. Ok CY, Young KH. Checkpoint inhibitors in hematological malignancies. J Hematol Oncol 2017;10:103.

223. Dubensky TW, Jr., Reed SG. Adjuvants for cancer vaccines. Semin Immunol 2010;22:155–61.

224. Yi M, Qin S, Zhao W, Yu S, Chu Q, Wu K. The role of neoantigen in immune checkpoint blockade therapy. Exp Hematol Oncol 2018;7: 28.

225. Fu J, Kanne DB, Leong M, Glickman LH, McWhirter SM, Lemmens E, et al. STING agonist formulated cancer vaccines can cure established tumors resistant to PD-1 blockade. Sci Transl Med 2015;7:283ra52.

226. Wang Z, Celis E. STING activator c-di-GMP enhances the anti-tumor effects of peptide vaccines in melanoma-bearing mice. Cancer Immunol Immunother 2015;64:1057–66.

227. Kinkead HL, Hopkins A, Lutz E, Wu AA, Yarchoan M, Cruz K, et al. Combining STING-based neoantigen-targeted vaccine with checkpoint modulators enhances antitumor immunity in murine pancreatic cancer. JCI Insight 2018;3. https://doi.org/10.1172/jci. insight.122857.