



# Role of the cGAS–STING pathway in systemic and organ-specific diseases

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**Abstract** | Cells are equipped with numerous sensors that recognize nucleic acids, which probably evolved for defence against viruses. Once triggered, these sensors stimulate the production of type I interferons and other cytokines that activate immune cells and promote an antiviral state. The evolutionary conserved enzyme cyclic GMP–AMP synthase (cGAS) is one of the most recently identified DNA sensors. Upon ligand engagement, cGAS dimerizes and synthesizes the dinucleotide second messenger 2',3'-cyclic GMP–AMP (cGAMP), which binds to the endoplasmic reticulum protein stimulator of interferon genes (STING) with high affinity, thereby unleashing an inflammatory response. cGAS-binding DNA is not restricted by sequence and must only be >45 nucleotides in length; therefore, cGAS can also be stimulated by self genomic or mitochondrial DNA. This broad specificity probably explains why the cGAS–STING pathway has been implicated in a number of autoinflammatory, autoimmune and neurodegenerative diseases; this pathway might also be activated during acute and chronic kidney injury. Therapeutic manipulation of the cGAS–STING pathway, using synthetic cyclic dinucleotides or inhibitors of cGAMP metabolism, promises to enhance immune responses in cancer or viral infections. By contrast, inhibitors of cGAS or STING might be useful in diseases in which this pro-inflammatory pathway is chronically activated.

Throughout evolution, from prokaryotes to mammals, host defence mechanisms have co-evolved to limit the deleterious effects of invading organisms. One such defence mechanism comprises three main components — cyclic GMP–AMP synthase (cGAS), a cyclic dinucleotide (CDN) and stimulator of interferon genes (STING)<sup>1</sup>. The structure and function of each of these three components have evolved in different ways in diverse organisms but the essential function of the cGAS–STING pathway is to detect and limit the propagation of foreign DNA. In mammals, the most prominent functions of this pathway in response to microbial nucleic acids is to generate inflammatory and antiviral proteins, and is dominated by the production of type I interferon (IFN-I)<sup>2</sup>. However, DNA binding to cGAS is not sequence specific; therefore, in addition to responding to pathogen-derived DNA, this pathway can generate an inflammatory response to self nucleic acids. Chronic activation of cGAS–STING by self DNA underlies rare autoinflammatory diseases such as Aicardi–Goutières syndrome and might also contribute to other disease processes related to autoimmunity, neurodegeneration, ageing and cancer<sup>3</sup> (FIG. 1 and TABLE 1). For example, activation of cGAS–STING, predominantly by mitochondrial DNA (mtDNA), has been implicated in both acute and chronic kidney disease (CKD)<sup>4–7</sup>.

However, deep knowledge of the biochemistry of the cGAS–STING cassette has also enabled investigators to harness this pathway to augment immune responses against tumours and reverse cancer-associated immune suppression (reviewed in REFS<sup>8,9</sup>).

In this Review, we first introduce the cytoplasmic cGAS–STING pathway and contrast cGAS with endosomal sensors from the Toll-like receptor (TLR) family. Next, we discuss the cell biology and biochemistry of cGAS–STING and highlight the specific role of the CDN 2',3'-cyclic GMP–AMP (cGAMP) in spreading the inflammatory response to neighbouring cells. We then focus on the regulatory mechanisms that prevent activation by self DNA under steady-state conditions, and why and how cGAS–STING becomes activated in disease states. Finally, we consider therapeutic approaches designed to either attenuate cGAS–STING activation in inflammatory diseases or to stimulate inflammation in immune-suppressed states such as cancer.

## Immune sensing and inflammation

In the field of medicine, inflammation is generally considered a pathological process, yet inflammation is a necessary process for survival. Inflammatory responses initiated by innate immune cells are not only triggered by viruses, bacteria and fungi as an essential response

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## Key points

- Cyclic GMP–AMP synthase (cGAS) is an evolutionarily conserved cytosolic nucleic acid sensor that synthesizes the cyclic dinucleotide second messenger 2',3'-cyclic GMP–AMP (cGAMP), which engages stimulator of interferon genes (STING) to trigger the production of inflammatory cytokines, including type I interferons.
- Extracellular cGAMP is hydrolysed by transmembrane and soluble ectonucleotide pyrophosphatase/phosphodiesterase 1, and enters neighbouring cells via cGAMP importers. Transfer to adjoining and distant cells enables cGAMP to act as an immunotransmitter and modulate antiviral responses, antitumour immunity and tumour metastasis.
- Activation of cGAS–STING by genomic or mitochondrial self DNA has been implicated in numerous autoinflammatory, autoimmune and neurodegenerative diseases, cell senescence and ageing, as well as in acute and chronic kidney injury.
- The microbiome can stimulate or inhibit cGAS–STING via cGAS sensing of commensal DNA, microorganism-induced endogenous retroviruses, or self DNA released owing to infection-induced cell damage. These interactions can affect gut and skin homeostasis.
- Modulating the proteins involved in the activation of the cGAS–STING pathway and its regulators provides therapeutic opportunities to attenuate or enhance cGAS–STING-driven inflammatory responses.

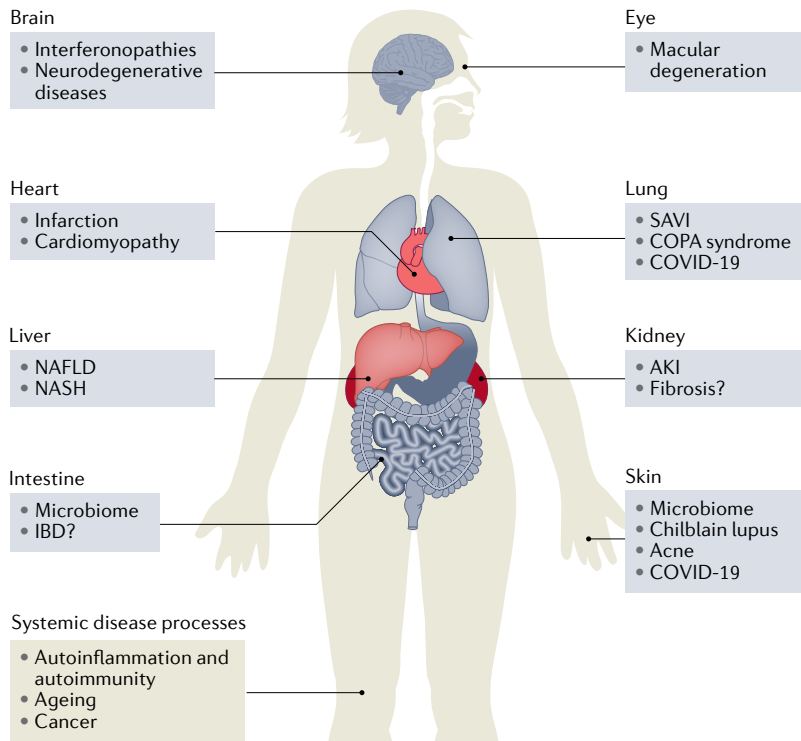
to eliminating infection, but inflammation is also necessary to repair tissue damage. TLRs are the best known innate sensors responsible for host immune defence. These receptors were first discovered in the fruit fly, *Drosophila*, and homologs were subsequently identified in all multicellular organisms (reviewed in REF.<sup>10</sup>). Although discovered more recently in mammals than TLRs<sup>2,11</sup>, ancient homologs of cGAS and STING have been detected in bacteria and in unicellular choanoflagellates, where they might have similar functions to those of the vertebrate proteins<sup>12–14</sup>. For example, in bacteria, cGAS activates a phospholipase to destroy the bacterial cell membrane and cause cell death. This mechanism probably functions as a bacterial defence against virus (that is, bacteriophage) reproduction<sup>14</sup>.

Although host sensors have evolved to detect and respond to microbial proteins, carbohydrates and lipids, these ligands can be modified by the microorganism to escape detection. By contrast, nucleic acids cannot be readily modified by the pathogens without affecting function. Unsurprisingly, host cells are equipped with multiple sensors for nucleic acid detection. In mammals, cytoplasmic nucleic acid sensors have a key role in the detection of pathogens that have breached membrane barriers<sup>15</sup>. Binding of double-stranded DNA to cGAS triggers a protein conformational change that results in the formation of cGAS dimers, activation of its catalytic site and synthesis of cGAMP (reviewed in REF.<sup>16</sup>; FIG. 2). cGAMP then functions as an endogenous second messenger and binds to STING, which resides in the endoplasmic reticulum (ER). After activation by cGAMP, STING is transferred from the ER to the Golgi via the ER–Golgi intermediate compartment<sup>17</sup>. Here, STING oligomerizes and acts as a platform to recruit and phosphorylate the serine/threonine-protein kinase TBK1. This kinase, in turn, phosphorylates the transcription factor interferon regulatory factor 3 (IRF3), which dimerizes and translocates to the nucleus to stimulate the expression of IFN $\beta$ . In addition to the

canonical pathway of cGAS-mediated activation of STING, other DNA sensors (for example, the inflammasome receptor AIM2 and IFN $\gamma$ -inducible protein 16, (IFI-16)), as well as RNA sensors (for example, retinoic acid-inducible gene-I, (RIG-I), and interferon-induced helicase C domain-containing protein 1 (MDA5)) signal through or cooperate with STING to drive IFN-I production. For example, etoposide-induced nuclear DNA damage in keratinocytes involves TNF receptor-associated factor 6 (TRAF6)-mediated assembly of K63-linked ubiquitin chains on STING and downstream activation of NF- $\kappa$ B, rather than IRF3 (REF.<sup>18</sup>). These non-canonical pathways of STING activation are independent of cGAS.

The IFN-I family comprises a large number of related proteins<sup>19</sup> — 13 IFN $\alpha$  proteins, 1 IFN $\beta$  and several less-studied members, including IFN $\epsilon$ , IFN $\kappa$ , IFN $\tau$  and IFN $\omega$ , all of which act on the IFN-I receptor (IFNAR). IFN $\beta$  can be produced by almost any cell type following stimulation by nucleic acids. Release of this cytokine serves to prime or amplify IFN-I production by other cells, including plasmacytoid dendritic cells, which are the main producers of IFN $\alpha$ <sup>20</sup>. IFN-I exerts its effector functions through the activation of >200 interferon-stimulated genes (ISGs), which in turn regulate translational control, and viral RNA stability and editing<sup>21</sup>. Of high relevance to autoimmunity, IFN-I is highly immunostimulatory and can induce the activation and maturation of dendritic cells, upregulation of MHC and co-stimulatory molecule expression, activation of natural killer (NK) cells, T cells and B cells, and suppression of regulatory T cells<sup>20</sup>. STING also forms a scaffold for activation of IKK $\epsilon$ , leading to the nuclear translocation of NF- $\kappa$ B and the production of other inflammatory cytokines such as tumour necrosis factor (TNF), IL-6 and IL-1 $\beta$ , which have very broad activating potential both within and outside of the immune system (FIG. 2).

cGAS–STING and endosomal TLRs (E-TLRs; comprising TLRs 3, 7, 8 and 9) signal through overlapping downstream pathways to activate IFN-I and IFN-III, respectively, as well as inflammatory cytokines (FIG. 2). Only ligands that enter endosomes through phagocytosis or endocytosis can engage E-TLRs and their common downstream pathways require one adaptor, either MyD88 or TRIF<sup>10</sup>. By contrast, the cGAS–STING pathway has several unique features: its DNA-sensing proteins are located in the cytoplasm, cGAS serves both as a DNA receptor and as a nucleotidyl transferase enzyme, and cGAS communicates with its effector platform STING at a distance by generating a CDN messenger (FIG. 2). Of note, although the E-TLR and cGAS–STING pathways are generally regarded as distinct, under certain conditions, membrane rupture might allow the escape of endosomal or lysosomal contents into the cytoplasm where they might be recognized by the cGAS–STING pathway<sup>22–24</sup> (FIG. 2). The proteins RIG-I and MDA5 use the adaptor protein mitochondrial antiviral-signalling protein (MAVS) and act as cytoplasmic RNA sensors that can also stimulate the production of IFN-I<sup>25</sup>; however, they are not considered further here.



**Fig. 1 | Involvement of cGAS and/or STING in disease.** The cyclic GMP–AMP synthase (cGAS)–stimulator of interferon genes (STING) pathway has been implicated in systemic disease processes, including autoimmunity, autoinflammation, ageing and cancer, as well as organ-specific diseases that target the eyes, brain, lungs, heart, kidneys, skin and intestines. cGAS–STING is also involved in the response to many viral infections, which can be systemic or local. AKI, acute kidney injury; COPA, coatomer protein subunit- $\alpha$ ; IBD, inflammatory bowel disease; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; SAVI, STING-associated vasculopathy with onset in infancy.

**cGAS–STING biochemistry and cell biology**

To understand how cGAS–STING functions in response to activating stimuli, it is useful to consider the subcellular localization of these proteins and their relationship to different organelles as well as the regulation of cGAMP as a mediator of cGAS activation.

**Regulation and transport of cGAS**

Subtle biochemical features unique to microbial DNA or RNA might influence the avidity of binding to intracellular sensors<sup>26</sup> but both E-TLRs and cGAS can be activated by host and microbial nucleic acids. Consequently, self versus non-self discrimination is not particularly relevant to the regulation of these receptors. Rather, several strategies, such as rapid phagocytosis and efficient processing of host apoptotic cells, absence of nucleic acid sensors on most cell surface membranes and an abundance of extracellular and intracellular nucleases, prevent these pathways from promoting the development of autoimmune and autoinflammatory diseases (reviewed in REF.<sup>27</sup>).

When cGAS was initially characterized and identified as a cytoplasmic DNA sensor, overexpression of Flag-tagged cGAS indicated that the protein localized to the cytoplasm; a minor fraction could be detected in the nuclear or perinuclear regions<sup>2</sup>. However, subsequent

studies showed that, under steady-state conditions, cGAS was predominantly detected in the nucleus, where it was tightly sequestered to and inhibited by chromatin in a high-avidity salt-resistant interaction<sup>28</sup>. This nuclear tethering prevents cGAS dimerization and activation, maintaining its resting state. Cryo-EM structures of the human cGAS–nucleosome core particle complex revealed that cGAS anchors to the nucleosome acidic patch on the histone H2A–H2B dimer through two conserved arginines. In this configuration, the three cGAS–DNA binding sites that are required for cGAS activation become inaccessible and cGAS dimerization is inhibited<sup>29–34</sup>. In addition, in the nucleus, the barrier to autointegration factor (BAF) protein competes with DNA for binding to cGAS<sup>35</sup> and, in the cytoplasm, low levels of DNA fail to trigger cGAS activation owing to a threshold requirement for ligand-induced phase separation<sup>36</sup>. Notably, during mitosis, the N terminus of cGAS is hyperphosphorylated by kinases, including Aurora kinase B, thereby preventing its interaction with nuclear chromatin<sup>37</sup>. Other post-translational modifications of cGAS and their functional consequences are discussed elsewhere<sup>38,39</sup>. Of note, following DNA damage or under conditions of DNA instability, genomic DNA might form micronuclei that mis-segregate, rupture and interact with cGAS during mitosis<sup>40</sup>. Damaged DNA might also generate chromatin bridges through chromosome breakage–fusion–bridge cycles that are associated with weakened nuclear membranes and DNA release<sup>41</sup>. These findings suggest that cGAS might participate in the immunosurveillance of mitotic errors and/or the generation of micronuclei. Despite its nuclear location, cGAS activation and downstream signal transduction occur in the cytoplasm. Although the signals required to release cGAS from nuclear tethering remain unclear, a functional nuclear export signal (NES; 169LEKLLK174) has been identified on cGAS, which allows it to traffic from the nucleus to the cytoplasm<sup>42</sup>. cGAS can encounter genomic DNA in the cytoplasm under several circumstances (FIG. 3).

Nucleases such as DNase1, DNase1L3 and RNase1 provide an additional layer of protection against chronic responses to nucleic acids released from cells into the extracellular environment. For example, DNase1L3 contains a C-terminal tail that interacts with and degrades chromatin on microparticles<sup>43</sup>. Nucleases are also abundant within the cell; some are present in the cytoplasm, such as DNase III (also known as TREX1), and others are compartmentalized within endosomes (for example, DNase II). TREX1 is an abundant 3′–5′ exonuclease with a preference for single-stranded DNA and is thought to degrade DNA arising from retroelements and from mitochondria<sup>44</sup>. Furthermore, DNA–RNA hybrids that are produced by an infection with retroviruses or that occur in the life cycle of endogenous retroelements are degraded by RNaseH2 enzymes<sup>45</sup>. The action of these enzymes might be attenuated during inflammation because digestion is less efficient if the DNA is oxidized (that is, contains 8-OH-deoxyguanine), which can occur following the generation of reactive oxygen intermediates<sup>46</sup>.

**Micronuclei**  
Small membrane-bound compartments that contain genomic DNA and form during mitosis in certain pathological states.

**Retroelements**  
Genetic elements that can be incorporated into a DNA sequence after reverse transcription of an RNA molecule.

**cGAMP metabolism and transport**

cGAMP is a small charged CDN that mediates cGAS activation and must also be regulated to avoid chronic activation of STING. CDNs were initially discovered as bacterial second messengers that have crucial roles in bacterial virulence, motility, metabolism and survival. Various bacteria produce CDNs, including cGMP–GMP, cAMP–AMP and 3',3'-cGAMP<sup>47</sup>. These CDNs comprise two nucleotide monophosphates interlinked by phosphodiester bonds to form a cyclic structure. Unlike bacterial CDNs, which have two 3'–5' bonds, mammalian cells produce a 2',3'-cGAMP, which has 2'–5' as well as 3'–5' phosphodiester bonds. Although bacterial CDNs can also activate STING, 2',3'-cGAMP binds to STING with a much higher affinity<sup>48</sup>, leading to cellular production of IFN-I and inflammatory cytokines (FIG. 2). Interestingly, *Drosophila* cells contain a STING orthologue that responds to exogenously administered 2',3'-cGAMP by activating the transcription factor Relish, which is an orthologue of NF-κB<sup>49,50</sup>. The discovery of two cGAS-like receptors (cGLRs) in *Drosophila* helps to explain cGLR–STING antiviral immunity in the absence of IFN. cGLR1 is activated by double-stranded RNA to produce 3',2'-cGAMP, whereas cGLR2 produces a combination of 2',3'-cGAMP and 3',2'-cGAMP, which shows the remarkable diversity of responses triggered by the cGAS–cGAMP–STING triad<sup>49,50</sup>.

The mechanisms that prevent accumulation of cGAMP and continuous stimulation of STING are incompletely defined. Whereas intracellular phosphodiesterases that hydrolyse cGAMP have not yet been identified<sup>51,52</sup>, evidence suggests that cells continuously export cGAMP into the extracellular space, where it might either be imported into neighbouring cells or hydrolysed by the multifunctional single-pass transmembrane protein ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1)<sup>51,52</sup> (FIG. 4). The catalytic domain of ENPP1 faces the extracellular space

enabling this enzyme to hydrolyse the extracellular pool of cGAMP into AMP and GMP<sup>52</sup>. ENPP1 is expressed on the surface of many cells, including skin cells, plasma cells and tumour cells, and can also be cleaved to form a highly bioactive serum protein<sup>53,54</sup>.

cGAMP transport is highly relevant in inflammation, tumour immunity and autoimmunity. The intercellular transport of cGAMP was first recognized in virus-infected cells, where cGAMP trafficked between cells through gap junctions (that is, intercellular channels) formed by clusters of connexin proteins<sup>55</sup>. Connexins 43 and 45 were required for cGAMP transfer to adjoining cells but other connexins in this family of 20 proteins might also be involved. Of note, cGAMP transport via connexin 43 from virus-infected endothelial cells to macrophages amplified the antiviral response, which suggested that this mechanism promotes antiviral immunity<sup>56</sup>. Connexin-mediated transfer of cGAMP has also been reported to modulate inflammatory responses in cancer<sup>57</sup>, alcohol-induced hepatocyte injury<sup>58</sup> and in inflamed joints<sup>56</sup>. Studies of tumour models and immunotherapy revealed that extracellular cGAMP acts as a soluble immune mediator in the tumour microenvironment (TME) to propagate the production of inflammatory cytokines, including IFN-I, and promote immune cell activation<sup>52</sup>. By acting as an immunotransmitter, cGAMP can modulate local antitumour immunity and affect tumour metastasis<sup>57,59</sup>.

In addition to transmission through channels that link two adjoining cells, cGAMP can enter the cytoplasm of surrounding cells via fusion of extracellular vesicles or through specialized cell-surface importers that can handle its negative charge (FIG. 4). Initial genome-wide CRISPR genetic screens identified two cGAMP importers — SLC19A1 and LRRC8. These large channel complexes enable cGAMP-mediated communication in non-adjacent or migrating cells. SLC19A1 is a carrier that transports folates and a variety

Table 1 | Systemic diseases associated with cGAS–STING activation in humans or mice

| Disease names         | Mouse models  | Human diseases   |
|-----------------------|---|--|
| AGS                   | Activation of cGAS–STING by loss of function mutations of DNases or nucleic acid metabolizing enzymes <sup>43,65,86,87</sup>  | Mutations in <i>TREX1</i> , <i>RNASEH</i> and other nucleic acid processing enzymes lead to the activation of cGAS and the production of IFNβ <sup>83,84</sup>   |
| SAVI                  | Gain-of-function mutations in <i>Sting1</i> lead to SAVI-like diseases <sup>90–92</sup>   | Gain-of-function mutations in <i>STING1</i> result in a systemic inflammatory disease with vasculitis and pulmonary fibrosis <sup>88,89</sup>  |
| SLE                   | cGAS–STING is activated in the lupus-prone <i>TREX1</i> D18N mutant <sup>95</sup> and <i>Fcgr2b</i> -knockout mice <sup>96</sup>  | <i>TREX1</i> heterozygous mutations associated with familial chilblain lupus <sup>100</sup><br><br>cGAMP expression in PBMCs of 15% of patients with SLE and in microvesicles from SLE blood cells <sup>102,103</sup><br><br>Mitochondria-rich red blood cells activate IFN-I through cGAS–STING in lupus monocytes <sup>108</sup> |
| Ageing and senescence | Activation of cGAS–STING-triggered immune senescence <sup>123,126,127</sup><br><br>cGAS drives non-canonical inflammasome activation in age-related macular degeneration <sup>180</sup> | Activation of cGAS–STING triggered immune senescence <sup>123,126,127</sup><br><br>cGAS drives non-canonical inflammasome activation in age-related macular degeneration <sup>180</sup>  |

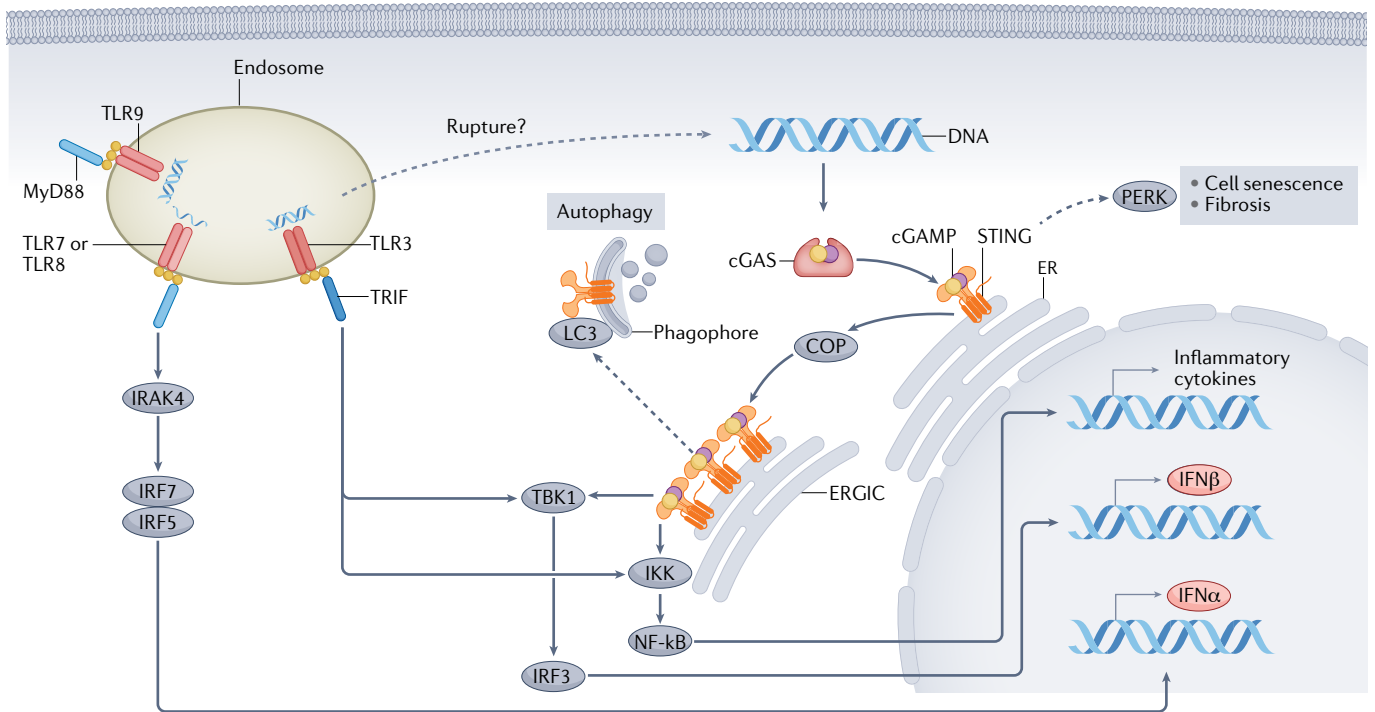
AGS, Aicardi–Goutières syndrome; cGAS, cyclic GMP–AMP synthase; IFN-I, type I interferon; PBMCs, peripheral blood mononuclear cells; SAVI, STING-associated vasculopathy with onset in infancy; SLE, systemic lupus erythematosus; STING, stimulator of interferon genes.



of organic phosphates, such as thiamine derivatives and nucleotides, including cGAMP<sup>60,61</sup>. The requirement for SLC19A1 was demonstrated in primary human peripheral mononuclear cells and in lineage-specific cell lines (for example, in haematological but not epithelial or endothelial cell lines)<sup>60,62</sup>. LRRC8A is a crucial component of the ubiquitous volume-regulated anion channel (VRAC) and enables intercellular communication by transporting Cl<sup>-</sup> and osmolytes across the plasma membrane<sup>63</sup>. Unlike SLC19A1, which was dispensable for cGAMP transport in endothelial cells, the LRRC8A:C heteromer was required for cGAMP import into both immortalized human microvascular endothelial cells and primary human endothelial cells<sup>64</sup>. Depending on the concentration gradient of cGAMP, LRRC8A can act bi-directionally and export cGAMP out of endothelial cells that overexpress cGAS<sup>63</sup>. Although LRRC8A is ubiquitously expressed across cell types, other components of the heterodimeric VRAC complex (such as LRRC8C or LRRC8E) differentially contribute to cGAMP transport in a cell- and species-specific manner; LRRC8D inhibits cGAMP transport<sup>63,64</sup>. The discovery of LRRC8 as a cGAMP transporter enabled *in vivo*

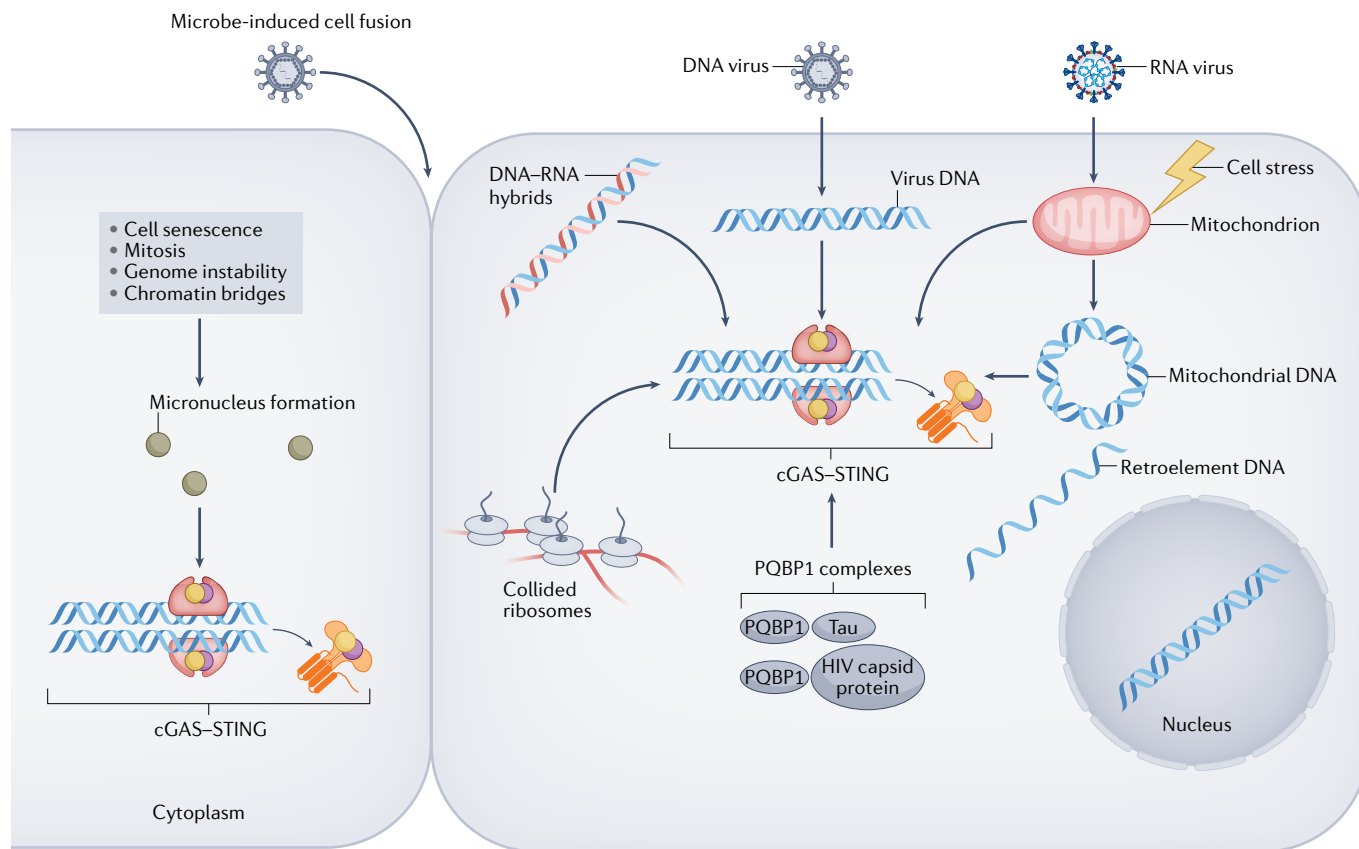
studies that revealed that cGAMP transport is required for clearance of herpes simplex virus 1 (HSV1)<sup>63</sup>. In 2021, SLC46A2 was identified as a third cGAMP importer that is specifically expressed in human CD14<sup>+</sup> monocytes and in monocyte-derived macrophages<sup>62</sup>.

Further studies are required to elucidate the roles of different cGAMP transporters in disease states associated with cGAS–STING activation. Although cGAMP export and import by adjacent cells may be advantageous to protect against viral infection, persistent cGAMP spreading and STING activation might lead to excessive IFN-I production and pathogenic autoinflammatory or autoimmune responses in genetically predisposed individuals. The findings that cGAMP functions as an immunotransmitter and can be imported into naïve cells also have important therapeutic implications. Of note, although cGAS–STING activation can be assessed by quantifying cGAMP, detection of this CDN is technically difficult because the gold standard method is mass spectrometry and concentrations in tissues are low<sup>65</sup> (nanomolar range); the increasing availability of cGAMP ELISAs might facilitate the detection of cGAS activation in the future.



**Fig. 2 | Signalling pathways downstream of cGAS and of endosomal TLRs.** Binding to double-stranded DNA induces dimerization of cyclic GMP–AMP synthase (cGAS), which, in turn, leads to the synthesis of the second messenger 2',3'-cyclic GMP–AMP (cGAMP). cGAMP binds with high affinity to stimulator of interferon genes (STING) leading to STING oligomerization and exit from the endoplasmic reticulum (ER); coatomer protein (COP) complexes facilitate the transport of STING. In the ER–Golgi intermediate compartment (ERGIC), STING recruits serine/threonine-protein kinase TBK1. The transcription factor interferon regulatory factor 3 (IRF3), which is a key TBK1 substrate, enters the nucleus following phosphorylation and promotes the transcription of IFN $\beta$ . Oligomerization of STING also enables activation of the inhibitor of nuclear factor- $\kappa$ B (NF- $\kappa$ B) kinase (IKK) complex, which promotes activation of the NF- $\kappa$ B transcription factor. NF- $\kappa$ B translocates to the nucleus where it

promotes transcription of inflammatory cytokines tumour necrosis factor (TNF), IL-6 and IL-1 $\beta$ . By contrast, endosomal Toll-like receptors (TLRs) use the MyD88 or TRIF to generate platforms for downstream signal transduction. The DNA sensor TLR9 and the single-stranded RNA sensors, TLRs 7 and 8, activate IRF5 and IRF7 via Myd88 and IL-1 receptor-associated kinase 4 (IRAK4), which results in the synthesis of IFN $\alpha$ . The double-stranded RNA sensor TLR3, engages the TRIF platform, which leads to the activation of TBK1 and IKK, leading to IFN $\beta$  transcription. In non-classical pathway activation (dashed arrows), activated STING molecules on the ERGIC can bind to microtubule-associated protein 1 light chain 3 (MAP1LC3; also known as LC3) on the phagophore, which leads to STING degradation through autophagy. Moreover, STING activation in the ER might lead to PKR-like ER kinase (PERK) activation and subsequent cell senescence or fibrosis.



**Fig. 3 | Mechanisms of activation of the cGAS–STING pathway.** In the cytoplasm, cyclic GMP–AMP synthase (cGAS) can be activated by microbial or host DNA. The best-studied sources of cGAS-activating nucleic acids are DNA viruses and host mitochondria. However, several other DNA sources and cGAS-activating stimuli, such as endogenous retroelement DNA, micronuclei generated in senescent cells or owing to genomic instability, and the formation of chromatin bridges, have been reported<sup>40,41,44,195</sup>. Microbe-induced cell fusion also enables the release of chromatin into the cytosol and RNA viruses can promote cGAS activation through ribosomal collision<sup>79,81</sup>. Finally, certain DNA-binding proteins such as polyglutamine-binding protein 1 (PQB1) might co-activate cGAS in combination with the HIV capsid protein<sup>77</sup> or Tau<sup>138</sup>.

**Autophagy and cell death**

Autophagy is a primordial function of cGAS–STING observed in invertebrates that do not synthesize IFN-I, such as the sea anemone<sup>12</sup>. Nonetheless, studies in this organism suggest that cGAS activation stimulates antiviral and antibacterial responses through ISG homologs. In mammalian cells, STING orchestrates several functions within the cell<sup>66</sup>. Activated STING molecules on the ER–Golgi intermediate compartment bind to microtubule-associated protein 1 light chain 3 (MAP1LC3; also known as LC3) on the autophagy membrane (that is, the phagophore), which leads to degradation of STING and termination of the activation signal (FIG. 2). This degradation process also helps to remove cytoplasmic DNA of host or microbial origin through enzymatic destruction in the autolysosome<sup>57</sup>. In clinical studies, cGAS–STING-induced autophagy was observed after radiotherapy or infection with *Mycobacterium tuberculosis*<sup>68,69</sup>; the bacterial DNA–STING interaction stimulated resistance to infection by eliciting a T helper 17 cell immune response<sup>70</sup>.

In certain cell types, intense STING activation drives apoptosis. In mice, this pro-apoptotic programme was driven by activation of mitochondrial B cell lymphoma

2-homology domain 3 only (BH3-only) proteins and was observed in T cells but not in macrophages nor dendritic cells<sup>71</sup>. The BH3-only protein PUMA also caused an increase in necroptosis, which required activation of STING following the release of mtDNA<sup>72</sup>. Certain viruses, such as murine  $\gamma$ -herpesvirus 68, induce necroptosis in a TNF- and STING-dependent manner<sup>73</sup>.

**cGAS–STING in disease**

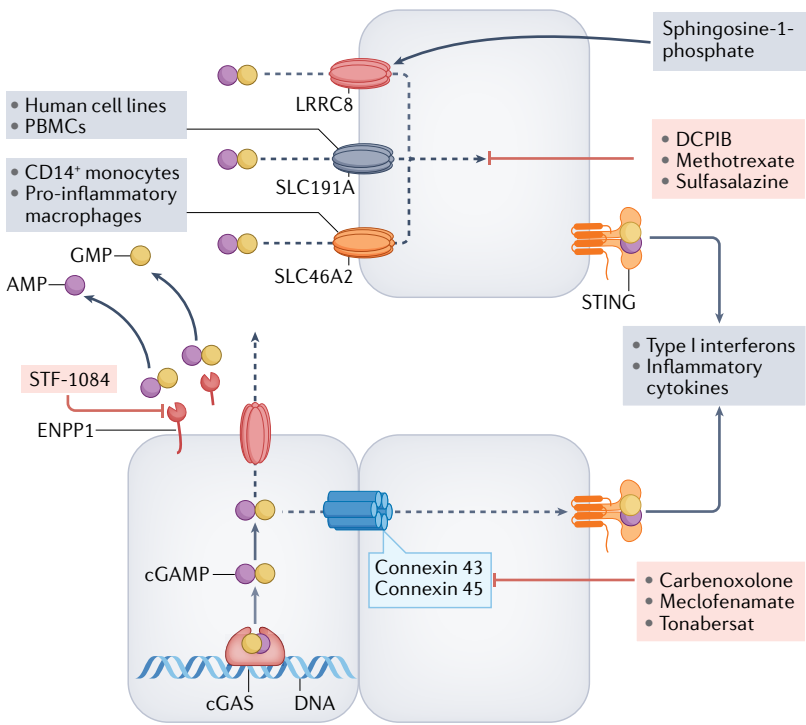
The cGAS–STING pathway can be activated by a number of microbes that infect humans. In addition, substantial evidence implicates the cGAS–STING pathway in mouse models of inflammatory and neurodegenerative diseases, suggesting that this pathway is activated in several human diseases (FIG. 1; TABLES 1 and 2).

**Viruses and other infections**

cGAS can be activated by a considerable number of DNA-containing viruses (for example, vaccinia, herpes simplex, hepatitis B and cytomegalovirus) given its broad DNA ligand specificity<sup>74</sup>. Surprisingly, RNA viruses such as HIV and SARS-CoV-2 can also activate cGAS, although this activation occurs through mechanisms distinct from those involving DNA viruses. For

**Necroptosis**  
A form of regulated cell death involving the activation of receptor-interacting protein kinases-1 (RIPK1) and RIPK3, and cell rupture (that is, necrosis).

example, during HIV infection, a Y-form DNA intermediate with an overhanging stretch of guanines activates cGAS<sup>75</sup>, possibly through a protein intermediate, polyglutamine-binding protein 1 (PQB1)<sup>76</sup> (FIG. 3). In addition, the DNA-binding HIV capsid protein is recognized by the nuclear non-POU domain-containing octamer-binding protein (NONO), which results in cGAS activation<sup>77</sup>; cGAS was engaged in the nucleus but the location of the downstream signal transduction remains to be determined. By contrast, the positive-sense RNA virus SARS-CoV-2 activated cGAS by inducing mitochondrial damage<sup>78</sup>, micronuclei generation and cell fusion, which led to nuclear export of chromatin DNA into the cytoplasm, which can also be induced by bacteria with a type VI secretion system 5 (REF.<sup>79</sup>) (FIG. 3). Accordingly, in COVID-19, cGAS–STING activation has been implicated in lung inflammation and in skin manifestations of the disease through the induction of endothelial cell death and IFN-I production<sup>78</sup>.



**Fig. 4 | Transport and metabolism of cGAMP.** Following activation, cyclic GMP–AMP synthase (cGAS) produces 2',3'-cyclic GMP–AMP (cGAMP), which binds to stimulator of interferon genes (STING) within cells. The fate of intracellular cGAMP depends on its rate of synthesis, the cell type in which it is produced and the biological context. Excess cGAMP is transported out of cells and is hydrolysed by membrane-bound or soluble ectonucleotide pyrophosphatase phosphodiesterase 1 (ENPP1) to yield AMP and GMP in the extracellular space<sup>51,52</sup>. When production and export of cGAMP exceeds the catabolic capacity of ENPP1, cGAMP is imported into adjacent or distal cells through specific transporters, some of which can mediate bidirectional transport (LRRc8). The best-studied transporter in vascular endothelial cells is the volume-regulated anion (VRAC) channel LRRc8A:C heteromer, whereas the solute carrier SLC19A1 is functional in human cell lines and peripheral blood mononuclear cells, and SLC46A2 is active in CD14<sup>+</sup> monocytes and pro-inflammatory macrophages<sup>60,62–64</sup>. In tumours, cGAMP can be transported between adjoining cells through gap junctions (for example, connexins 43 and 45)<sup>55,57,59</sup>, which can be blocked by the compounds shown. Several chemical compounds can block ENPP1 and cGAMP import as indicated. Sphingosine 1-phosphate can activate LRRc8 to potentiate cGAMP transport<sup>64</sup>.

Single-stranded RNA viruses such as dengue virus can also activate cGAS by causing mitochondrial damage and release of mtDNA<sup>80</sup>. Notably, the translational stress induced by DNA or RNA viruses in infected cells frequently results in slowing of ribosomal movement (stalling) and can lead to collisions. Collided ribosomes also bind to cGAS and reduce its threshold of activation by DNA 15-fold, so viruses that induce ribosome collisions act as cGAS co-activators<sup>79,81</sup> (FIG. 3). These studies indicate that, although DNA is the prototypic cGAS ligand, several factors can influence the threshold for cGAS activation, including infection with an RNA virus. Intracellular bacteria such as *M. tuberculosis*, *Listeria* and *Chlamydia*, as well as protozoan parasites such as *Plasmodium*, activate cGAS with variable effects on the host immune response (reviewed in REF.<sup>82</sup>).

**Autoinflammatory and autoimmune diseases**

The disease-causing potential of nucleic acid ligands, cGAS–STING and its downstream pathways is underscored by monogenic interferonopathies, which are disorders associated with the aberrant production of IFN-I. Over more than a decade, several studies have revealed that interferonopathies are caused by a variety of mutations in genes that are involved in nucleic acid metabolism. For example, mutations in *TREX1*, *RNASEH2B* and *SAMHD1* lead to activation of cGAS and production of IFNβ<sup>83,84</sup>. Many patients with interferonopathies produce autoantibodies<sup>85</sup>, which supports the hypothesis that elevated levels of IFN-I contribute to lupus-like features owing to the aforementioned adjuvant effects of IFN-I on the immune system. Moreover, observations from studies of loss-of-function mutations in genes encoding DNases or certain nucleic acid-metabolizing enzymes substantiate the requirement for chronic activation of cGAS–STING to maintain the high IFN state typical of interferonopathies and of some systemic autoimmune diseases<sup>43,65,86,87</sup>. *STING1* gain-of-function mutations cause STING-associated vasculopathy with onset in infancy (SAVI), which is a systemic inflammatory disease characterized by vasculitis and pulmonary fibrosis<sup>88,89</sup>. In mouse models, SAVI-like disease is largely, but not entirely, IFN-I dependent, and innate activation of monocytes and neutrophils has a key pathogenic role<sup>90–92</sup>. Coatmer protein subunit-α (COPA) syndrome, which is a disease with overlapping lung, joint and kidney involvement similar to SAVI, is also caused by chronic stimulation of STING owing to retention of STING at the Golgi<sup>89,93,94</sup>. Moreover, cGAS–STING activation has been demonstrated in certain lupus-prone mouse strains, including the *TREX1* D18N mutant<sup>95</sup> and *Fcgr2b*-knockout mice<sup>96</sup>.

Chronically elevated levels of IFN-I are observed in the blood of patients with many systemic autoimmune disorders, including systemic lupus erythematosus (SLE), Sjögren syndrome, scleroderma and dermatomyositis<sup>97</sup>. Although TLRs almost certainly have a role in perpetuating the IFN response following entry of immune complexes containing nucleic acid antigens into plasmacytoid dendritic cells<sup>98</sup>, emerging evidence indicates that the cGAS–STING pathway is also engaged, at least in some patients; 1–2% of patients

Table 2 | Organ-specific diseases associated with cGAS–STING activation in humans or mice

| Disease names and/or affected tissues                                 | Mouse models  | Human diseases  |
|---|---|---|
| ALS   | Loss or blockade of STING rescued ALS phenotype in Prp-TDP-43 <sup>Tg/+</sup> mice <sup>133</sup>   | cGAMP detected in iPSC-derived motor neurons from patients with <i>TARDBP</i> mutations; increased cGAMP concentrations in spinal cord extracts from patients with ALS <sup>133</sup> |
| PD  | Lack of PINK1 or parkin led to oxidation of mtDNA and cGAS–STING activation <sup>135</sup>  | <i>PINK1</i> or <i>PRKN</i> mutations associate with early-onset PD <sup>134</sup>  |
| AD  | NAD <sup>+</sup> supplementation reduced neuroinflammation by suppressing cGAS–STING <sup>137</sup>   | NA  |
| Kidney disease  | Cisplatin-induced tubular injury was attenuated by STING deficiency and inhibition <sup>5</sup> . Blocking STING trafficking from the ER improved glomerular pathology scores <sup>122</sup>  | cGAS and STING expression correlated with kidney fibrosis in patients with CKD <sup>7</sup> . cGAS–STING pathway implicated in diabetic kidney disease <sup>116</sup>                 |
| Intestinal inflammation and microbiome                                | STING deficiency or antagonism exacerbates gut inflammation <sup>142,144,146</sup><br>STING contributes to gut microbial homeostasis <sup>143</sup><br>Constitutive STING activation leads to dysbiosis and intestinal inflammation <sup>145</sup>  | Increased STING levels in the colon of patients with ulcerative colitis <sup>145</sup>  |
| Familial chilblain lupus, UV skin responses, skin microbiome and acne | Skin colonization with <i>S. epidermidis</i> led to cGAS–STING activation in keratinocytes <sup>140</sup><br>UV light induces a IFN-I signature dependent on cGAS–STING <sup>106,107</sup>  | <i>Cutibacterium acnes</i> associated with acne vulgaris stimulates IFN-I production in macrophages via cGAS–STING <sup>149</sup>   |
| Myocardial infarction, heart failure                                  | cGAS- or STING-deficient mice are protected against myocardial infarction <sup>181,182</sup> . cGAS has an essential pathogenic role in pressure overload-induced heart failure <sup>182</sup>  | NA  |
| Viral hepatitis   | HBV evades immune sensing by inhibiting cGAS–STING expression and signalling <sup>183</sup>   | Decreased STING expression in peripheral blood of patients with HBV infection <sup>184</sup>  |
| NAFLD and NASH  | Reduced liver injury in mice with impaired STING and high fat diet-induced NAFLD <sup>185</sup> . Macrophage activation via STING stimulates hepatocyte fat deposition and activates hepatic stellate cells <sup>186</sup> , possibly through sensing of mtDNA <sup>187</sup>   | Increased STING levels in livers of patients with NAFLD <sup>186,188</sup>  |
| ALD   | Ethanol-triggered ER stress causes activation of cGAS–STING <sup>58</sup> , STING-mediated apoptosis of hepatocytes <sup>189</sup> , and liver injury and fibrosis <sup>190</sup><br>cGAMP transport through hepatic gap junctions amplifies tissue injury <sup>58</sup>  | Increased cGAS–STING activation in the liver of patients with ALD <sup>58</sup>   |
| Obesity   | In DsbA-L KO, cGAS–STING activation suppresses thermogenesis and stimulates metabolic disorder <sup>117,191</sup><br>High-fat diet causes metabolic stress and STING activation via mtDNA; STING deficiency partially prevented inflammation of adipose tissue, insulin resistance and obesity <sup>192</sup>   | NA  |
| Arthritis   | <i>Sting1</i> loss protects <i>Dnase2<sup>-/-</sup>Ifnar<sup>-/-</sup></i> mice from arthritis <sup>193</sup><br>cGAS activation by self DNA causes arthritis in <i>Dnase2<sup>-/-</sup>Ifnar1<sup>-/-</sup></i> mice <sup>65</sup><br>TNF induces release of mtDNA, which activates cGAS; <i>Cgas</i> KO mice are protected from arthritis in the K/BxN arthritis model <sup>194</sup> | NA  |

AD, Alzheimer disease; ALD, alcoholic liver disease; ALS, amyotrophic lateral sclerosis; cGAMP, 2',3'-cyclic GMP–AMP; cGAS, cyclic GMP–AMP synthase; CKD, chronic kidney disease; ER, endoplasmic reticulum; HBV, hepatitis B virus; IFN-I, type I interferon; iPSCs, induced pluripotent stem cells; KO, knockout; mtDNA, mitochondrial DNA; NA, not available; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; PD, Parkinson disease; STING, stimulator of interferon genes; TNF, tumour necrosis factor; UV, ultraviolet. cGAS involvement in microbial infections is discussed in the main text.

with SLE have *TREX1* mutations<sup>99,100</sup>, which, in mice, result in IFN-I production exclusively through the cGAS–STING pathway<sup>87,101</sup>. Moreover, in SLE, cGAMP could be detected in the peripheral blood cells of 15% of patients<sup>102,103</sup> and in microvesicles released from blood cells<sup>102,103</sup>. Oxidized mtDNA released in the context of neutrophil extracellular traps formation in SLE was also linked to cGAS activation in humans<sup>104,105</sup>. Of note, ultraviolet light, which is known to precipitate cutaneous lupus and exacerbations of systemic lupus, induces

a IFN-I signature that, in mice, was cGAS–STING dependent<sup>106,107</sup>. Mitochondria-rich red blood cells can also activate IFN-I through cGAS–STING in lupus monocytes<sup>108</sup>. In dermatomyositis, detection of IFN $\beta$  and phosphorylated IRF3 in skin biopsy samples suggested cGAS–STING activation, but this hypothesis has not been tested<sup>109</sup>.

In addition to the key role of IFN-I in host defence and tumour regulation, clear examples exist of IFN-I-independent STING activities that contribute to



an inflammatory immune response. For example, several groups showed that mice with the STING S365A mutation, in which IRF3 cannot be recruited and activated to transcribe IFN $\beta$ , are still able to respond to a virus infection<sup>110–112</sup>. DNase II-deficient mice developed auto-inflammatory arthritis and retained the ability to restrict HSV-1 infection and control tumour immune evasion. Although *Dnase2*<sup>-/-</sup> STING-S365A mice had reduced ability to control viral infection and tumour growth, they still exhibited severe polyarthritis.

### Kidney disease

IFN-I might cause collapsing glomerulopathy, proliferative nephritis and vasculitis in cases of SAVI and coatomer protein subunit- $\alpha$  syndrome<sup>113</sup>. The cGAS–STING pathway might also contribute to acute kidney injury (AKI) and CKD characterized by kidney fibrosis<sup>114</sup>. In mice, the nephrotoxic agent cisplatin stimulated the expression of cGAS and STING in the damaged tubules, which mimicked high STING levels observed in the kidneys of patients with AKI<sup>6</sup>. Cisplatin-induced tubular injury, including apoptosis and inflammation, as well as kidney failure, were attenuated in STING-deficient mice and by pharmacological inhibition of STING with C-176 or H151 (REF.<sup>5</sup>). Researchers hypothesized that mtDNA leaked into the cytoplasm through the pores of damaged mitochondria and activated the cGAS–STING pathway in these AKI models. Mitochondrial damage is a well-recognized trigger of AKI (reviewed in REF.<sup>4</sup>) and targeting STING might therefore help to prevent AKI in patients receiving platinum-based chemotherapy who have increased plasma levels of mtDNA<sup>5</sup>. The cGAS–STING pathway was similarly activated in the ischaemia–reperfusion injury model of AKI<sup>6</sup>.

Given that mitochondrial damage leads to tubular cell injury, the mitochondrial transcription factor A (TFAM), which is downregulated in patients with CKD, was deleted in kidney tubule cells specifically to create a mouse model of CKD<sup>7</sup>. Akin to the AKI models, absence or inhibition of STING attenuated tubular structural damage, and reduced inflammatory cytokine levels and tubular apoptosis. Moreover, in 433 kidney tissue samples from patients with CKD, *CGAS* and *STING1* expression correlated positively with the degree of kidney fibrosis<sup>7</sup>. Although these studies did not interrogate the downstream signalling cascade, cGAS–STING activation was shown to mediate kidney fibrosis through a non-canonical pathway (that is, independently of the cGAS–STING–TBK1–IRF3 pathway)<sup>115</sup>. In the unilateral ureteral obstruction model of kidney fibrosis, upon binding to cGAMP, STING was shown to interact directly with PKR-like ER kinase (PERK), which triggered phosphorylation of eukaryotic initiation factor 2 $\alpha$  (eIF2 $\alpha$ ) and led to increased collagen expression and kidney fibrosis by modulating damage-initiated mRNA translation<sup>115</sup>. The cGAS–STING pathway has also been implicated in diabetic kidney disease<sup>116</sup>, where mitochondrial stress and release of mtDNA have important roles in disease pathogenesis<sup>117</sup>. cGAS–STING stimulation with mtDNA might also participate in metabolic diseases of the liver and adipose tissue (TABLE 2), which, in humans, are associated with CKD<sup>118</sup>, and can lead to

tubular injury and kidney fibrosis in murine models of obesity<sup>119</sup>.

In contrast to AKI and the aforementioned CKD models, the role of cGAS–STING in lupus nephritis has been controversial. Initial studies in lupus mouse models indicated that STING suppresses systemic autoimmunity, including kidney disease<sup>120</sup>. Accordingly, loss of *Cgas* on the lupus-prone MRL/Fas.lpr background resulted in increased proteinuria and cellular infiltration in the kidney<sup>121</sup>. By contrast, a pro-inflammatory effect of STING activation was reported to drive lupus pathology in *Fcgr2b*<sup>-/-</sup> mice<sup>96,122</sup>. Administration of the ISD017 peptide, which blocks STING trafficking from the ER, improved glomerular pathology scores and reduced IgG deposition in the kidney, which suggested that STING-targeted therapeutics might be useful in lupus nephritis<sup>122</sup>. The opposing findings in these studies suggest that the roles of cGAS and STING in autoimmune kidney diseases are complex and likely model dependent. Moreover, phenotypes might differ between models in which the cGAS–STING pathway is defective from birth, in which case compensatory mechanisms might be triggered, and those in which the pathway is inhibited pharmacologically in animals with established disease.

### Immune senescence and ageing

Cells are constantly exposed to stress. When stress-induced damage is irreparable, cells undergo apoptosis but milder forms of injury can drive aged or stressed cells to exit the cell cycle and become senescent<sup>123</sup>. Senescent cells are characterized by elevated expression of the cell cycle inhibitor p16<sup>Ink4a</sup> and by major changes in transcriptional profiles, metabolism and chromatin organization<sup>124</sup>. These cells have a distinctive secretory phenotype known as the senescence-associated secretory phenotype, which is defined by changes in the expression of metalloproteinases, chemokines and cytokines (especially TNF, IL-6 and IL-1 $\beta$ )<sup>125</sup>. The senescence-associated secretory phenotype is thought to contribute to many chronic diseases associated with ageing, including atherosclerosis, osteoarthritis and possibly neurodegeneration. In the last 5 years, several studies have shown that immune senescence is often triggered by cGAS–STING activation following the release of chromatin fragments into the cytoplasm, possibly owing to defects in nuclear lamins<sup>123,126,127</sup>. Activating DNA ligands are likely to originate from multiple sources, including mitochondria and aberrant cytoplasmic chromatin fragments that emerge following oxidative stress, exposure to certain drugs and irradiation.

### Neurodegenerative diseases

Accumulation of aggregated misfolded proteins in neurons and the surrounding glia is a pathological hallmark of many neurodegenerative diseases, including Alzheimer disease (AD), Parkinson disease (PD) and amyotrophic lateral sclerosis (ALS). Each of these diseases has a variable inflammatory component, which might depend on genetic susceptibility factors and stage of disease. The presence of aggregated or misfolded proteins triggers a stress response leading to cell death or

activation of inflammatory cytokines, including IFN-I, which has long been known to exert neurocytotoxic effects. These effects were demonstrated clearly in transgenic mice that overexpress IFN $\alpha$ <sup>128</sup>, in studies of neurotropic viruses<sup>129</sup> and in studies of interferonopathies. Moreover, selected mouse models of AD, PD and ALS are associated with a high IFN-I signature. In patients with ALS, several reports demonstrate that inflammatory cytokine levels, including IFN-I levels, as well as the frequency of autoimmune disorders, are high<sup>130–132</sup>. Moreover, cGAMP was detected in iPSC-derived motor neurons from patients with ALS associated with *TARDBP* mutations and cGAMP concentrations were elevated in spinal cord extracts from patients with spontaneous ALS<sup>133</sup>. Mutations that prevent the normal expression of the mitochondrial proteins PINK1 or parkin, are linked to early-onset PD in humans<sup>134</sup>. In mice that lack these mitochondrial-protective proteins, cell stress induces mitochondrial damage and mtDNA oxidation, which, in turn, activates the cGAS–STING pathway<sup>135</sup>. In patients with AD, ISG-expressing microglia surrounded amyloid plaques and IFN-I expression correlated with disease severity<sup>136</sup>. Of note, NAD<sup>+</sup> supplementation reduced neuroinflammation in a mouse model of AD at least partly by suppressing cGAS–STING<sup>137</sup>. In mice, PQBP1 binds to aggregated tau proteins and activates cGAS–STING in microglia, resulting in neuroinflammation in the brain<sup>138</sup>. These findings from mouse models might have important implications for PQBP1-associated neurodegenerative diseases such as Huntington's disease and spinocerebellar ataxia type 1 in humans.

#### Relationship with the microbiome

Commensal bacteria can alter IFN-I production via cGAS–STING signalling at multiple epithelial sites<sup>139–141</sup>. Conversely, STING expression levels affect commensal bacteria in the intestine, skin, lung and the TME.

**Intestine.** The IFN-I response triggered by cGAS sensing of DNA derived from commensal bacteria influences intraepithelial lymphocyte numbers, the distribution of different types of innate lymphoid cells and the function of regulatory T cells<sup>142</sup>. The effects of STING deficiency and overactivation in the intestine are controversial. Loss of *Sting* in mice led to altered development and function of regulatory T cells and was accompanied by dysbiosis, rendering the mice more prone to gut inflammation and enteric infection than controls<sup>142</sup>. Similarly, another study reported that *Sting*<sup>−/−</sup> mice were more susceptible than controls to inflammatory colitis owing to loss of IL-10 that was indirectly stimulated by STING<sup>143</sup>. By contrast, STING deficiency was reported to prevent the induction of colitis, which was exacerbated by a STING agonist<sup>144</sup>. Others observed that constitutive activation of STING (mouse model of SAVI) led to intestinal dysbiosis and spontaneous colitis; the altered microbiome propagated intestinal inflammation through STING ubiquitination and stabilization in myeloid cells<sup>145</sup>. The same study found elevated STING levels in the colon of patients with ulcerative colitis<sup>145</sup>. These findings demonstrate a reciprocal relationship between STING and the

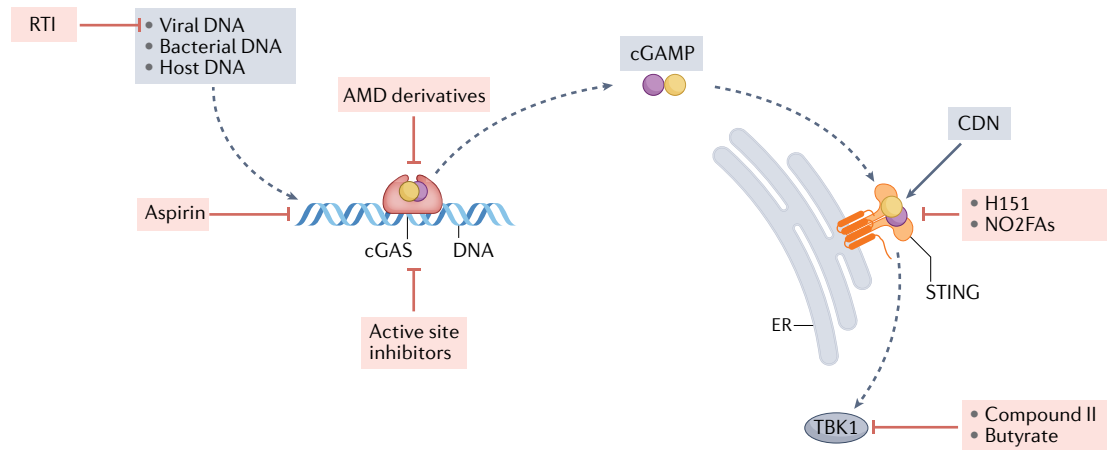
microbiome in shaping intestinal health and response to disease. Whether differences between the observations in mouse models can be explained by different approaches to genetic disruption of *Sting1* (*Sting*<sup>−/−</sup> versus *Sting1*<sup>g/g</sup>), environmental factors or other experimental approaches, remains to be determined.

Additional studies revealed that microbial insults resulting from pathogenic bacteria triggered the release of mtDNA from dying intestinal endothelial cells, which resulted in STING activation and intestinal barrier dysfunction<sup>146</sup>. Furthermore, STING antagonists prevented sepsis-induced intestinal injury and protected mice from lipopolysaccharide-induced endotoxaemia<sup>146,147</sup>. Therefore, commensal and pathogenic bacteria can both lead to STING-mediated intestinal inflammation, although potentially through different mechanisms.

**Lung.** In addition to the intestinal inflammation discussed above, SAVI mice (carrying a *Sting1* N153S gain-of-function mutation) develop lung disease<sup>141</sup>. Depleting gut commensals with oral antibiotics eliminated lung disease<sup>141</sup> and prevented intestinal inflammation<sup>145</sup>. In this model, STING-mediated lung inflammation was attenuated in the presence of *Bacteroides thetaiotaomicron*, suggesting that specific commensal species might have inhibitory effects on the cGAS–STING pathway. However, in the intestine, bacterial products (for example, c-GMP–GMP), rather than a specific bacterial species, promoted STING-mediated inflammation<sup>145</sup>. These seminal studies introduced the potential therapeutic role of commensals and their products in regulating the cGAS–STING pathway and IFN-I production.

**Skin.** Skin colonization with *Staphylococcus epidermidis* led to cGAS–STING activation in keratinocytes, namely the sensing of microbe-induced endogenous retroviruses<sup>140</sup>. This cGAS-mediated IFN-I response was required for the induction of homeostatic T cell responses to this skin microorganism. Microbe-induced IFN-I responses have also been reported to promote tissue repair in the skin<sup>148</sup>, although the role of the cGAS–STING pathway was not investigated. By contrast, this pathway might contribute to inflammation in certain skin diseases. For example, the Gram-positive bacterium *Cutibacterium acnes*, which is associated with the inflammatory skin disease acne vulgaris, stimulates IFN-I production in macrophages via cGAS–STING<sup>149</sup>. Whether the cGAS–STING pathway links dysbiosis and high IFN-I in autoimmune diseases with skin involvement such as lupus, psoriasis, dermatomyositis and scleroderma warrants further investigation.

**Tumour microenvironment.** Therapeutic approaches to modulate the cGAS–STING pathway have been of particular interest in tumour therapy as IFN-I signalling can enhance antitumour immunity. A 2021 study<sup>150</sup> showed that CDNs from commensal bacteria in the TME activate STING in monocytes, thereby potentiating the IFN-I response and anti-tumorigenic properties of NK cells and DCs. Faecal transplants from patients with



**Fig. 5 | Targeting the cGAS–STING pathway.** The cyclic GMP–AMP synthase (cGAS)–stimulator of interferon genes (STING) pathway can be inhibited at many stages. Reverse transcriptase inhibitors (RTI) block the synthesis of cGAS-activating complementary DNA from endogenous retroelements. Aminoquinoline-type antimalarial drugs (AMDs) block DNA binding to cGAS<sup>166</sup>, whereas aspirin acetylates the cGAS residues Lys414, Lys384 and Lys394, which leads to its inactivation<sup>167</sup>. Active site inhibitors block the synthesis of the second messenger 2',3'-cyclic GMP–AMP (cGAMP). For STING inhibition, both naturally occurring nitro-fatty acids (NO<sub>2</sub>-FAs) and the nitrofuranyl derivative, H-151, block palmitoylation of crucial cysteine residues on STING, preventing oligomerization of the protein<sup>171</sup>. A number of different TBK1 inhibitors, mostly belonging to the aminopyrimidine class, such as compound II, have been generated and used to treat autoimmune disorders in mice. In contrast to attenuation of the cGAS–STING pathway, STING can be activated by synthetic cyclic dinucleotides (CDNs), which might be useful in enhancing antitumour immunity.

melanoma who responded to the checkpoint inhibitor anti-PD-1 induced an intra-tumoural IFN-I signature in non-responders<sup>150</sup>, which supports the therapeutic use of commensal bacteria to modulate cGAS–STING function. Indeed, in murine models of melanoma and colon cancer, oral administration of *Lactobacillus rhamnosus* GG improved tumour responses to anti-PD-1 therapy via cGAS–STING-mediated IFN $\beta$  production in DCs<sup>151</sup>. This treatment altered the composition of the gut microbiome, and increased DC infiltration in the tumours and activation of cytotoxic CD8<sup>+</sup> T cells in the TME. Of note, commensal bacteria (*Lachnospiraceae* species) suppressed radiation therapy-induced IFN-I production in murine colon and metastatic melanoma cancers by secreting butyrate, which inhibited the activation of TBK1 and IRF3 downstream of cGAS–STING<sup>152</sup> (FIG. 5). Modulation of gut microbiota by vancomycin restored the antitumour effects of radiation therapy, which further supports the notion that altering the gut microbiome might have a therapeutic effect in cancer<sup>152</sup>.

**Modulation of cGAS–STING in disease**

As discussed above, cGAS, cGAMP, STING, as well as downstream kinases, are implicated in many diseases and are therefore therapeutic targets. Importantly, compounds that enhance or inhibit cGAS–STING have theoretical risks associated with them, and whether unwanted effects will be observed in humans treated with cGAS–STING-modifying compounds remains to be determined. The risk–benefit ratio of cGAS–STING modulators will also need to be compared with other approaches that target upstream or downstream molecules. These alternative targets for therapy would include oxidized mtDNA<sup>104</sup> (upstream) or IFN receptor (IFNAR) blocking antibodies<sup>153</sup> (downstream) of cGAS–STING.

Here, we discuss the use of small molecules to activate or inhibit cGAS–STING in preclinical studies.

**Pathway activation**

In diseases such as cancer and in certain viral infections, enhancing the IFN-I response might have beneficial adjuvant effects on immune cells, especially CD8 cytotoxic T cells and antigen-presenting cells<sup>154</sup>.

**Direct STING activation.** Administration of IFN $\alpha$  has been used as adjunctive therapy in patients with certain types of cancer or chronic viral infections such as hepatitis C infection<sup>154</sup>. 5,6-Dimethylxanthenone-4-acetic acid (also known as vadimezan or ASA-404), is a potent selective agonist of the STING pathway and exerted significant antitumour and antiviral effects in mice owing to its stimulation of IFN-I and other cytokines<sup>155,156</sup>. However, 5,6-dimethylxanthenone-4-acetic acid does not activate human STING. Many different CDNs with agonist activity on human STING have been developed and are being evaluated as primary or adjunctive therapy in human cancers (reviewed in REFS<sup>8,9</sup>). Unsurprisingly, CDNs have also been used to enhance inflammatory responses and reduce SARS-CoV-2 infection of human bronchial epithelial cells in vitro and mouse cells in vivo<sup>157</sup>. Of note, STING activation can induce apoptosis in T cells; therefore, careful evaluation of the differential effects on immune cell populations is necessary when assessing the therapeutic potential of STING agonists.

**Targeting ENPP1 or cGAMP transport.** Tumour cells upregulate ENPP1 on their surface, potentially as an immune evasion mechanism<sup>59</sup>. High ENPP1 expression has been associated with worse prognosis and response to therapy in different murine models of breast and

colorectal cancer, probably owing to low extracellular cGAMP levels coupled with high levels of adenosine, which is a breakdown product of cGAMP hydrolysis and suppresses immune activation<sup>59,158</sup>. Given that secreted cGAMP activates and recruits both innate and adaptive immune cells that can reduce tumour growth and spreading<sup>59,159–161</sup>, extending the lifespan of extracellular cGAMP by targeting ENPP1 (FIG. 4) is of therapeutic interest in cancer. This approach is supported by evidence that inhibiting ENPP1 enhances the efficacy of current standard-of-care cancer therapies. ENPP1 inhibition with the phosphonate analogue STF-1084 not only enhanced IFN $\beta$  production and improved the response to radiation therapy in breast and pancreatic cancer<sup>54</sup> but also potentiated the effects of immune checkpoint blockade on antitumour immunity<sup>59,159</sup>. Although an ENPP1 inhibitor was not effective when used alone in human cancers, clinical trials are evaluating its potential as a combination therapy.

Other cGAMP targeting approaches might involve the use of compounds that block gap junctions, such as carbenoxolone<sup>55,56</sup>, meclofenamate<sup>56</sup> and tonabersat<sup>57</sup>. Pharmacological inhibition of cGAMP transport through gap junctions with these drugs controlled viral dissemination<sup>55</sup>, infection-induced toxic inflammation<sup>56</sup> and tumour growth in a model of established brain metastasis<sup>57</sup>. Another therapeutic approach involves the targeting of cGAMP transporters. Two FDA-approved drugs, methotrexate and sulfasalazine, blocked cGAMP import through SLC19A1 (REFS<sup>60,61</sup>) and new chemical tools to modulate LRRC8 channels on vascular endothelial cells have been developed<sup>64</sup>: moreover, sphingosine 1-phosphate (S1P) potentiates cGAMP import by endothelial cells, whereas the small molecule VRAC inhibitor DCPIB blocks LRRC8A-dependent uptake of extracellular cGAMP (FIG. 5).

Of note, selective delivery to the tumour site while avoiding the unwanted effects of systemic IFN-I induction, including the risk of autoimmunity, remains a considerable challenge for the implementation of CDN therapy. However, the cell type-specific expression of cGAMP importers and differential responsiveness to extracellular cGAMP provide opportunities for targeted therapy. For example, sulfasalazine inhibited cGAMP transport in macrophages and T cell-specific loss of LRRC8A:C channels in mice boosted T cell-driven immune responses<sup>162</sup>.

### Pathway inhibition

In diseases where chronic IFN-I production is thought to contribute to autoinflammatory and autoimmune pathogenesis, attenuation of IFN-I activity might improve outcomes. Many small compounds inhibit cGAS, STING or downstream kinases such as TBK1 (FIG. 5). When targeting cGAS, small molecules that bind to and inhibit the nucleotidyl transferase active site of cGAS with nanomolar affinity have, so far, only been useful in vitro<sup>163–165</sup>. By contrast, the antimalarial aminoquinoline compound X6 inhibited DNA binding to cGAS in vitro and in *Trex1*-knockout mice<sup>166</sup>. Treatment with X6 attenuated the production of cGAMP and IFN-I, and reduced inflammation in the mouse heart<sup>166</sup>. Notably, sodium

salicylate (also known as aspirin) inhibited cGAS activation by acetylating cGAS residues (specifically, Lys414, Lys384 and Lys394) that mediate DNA–cGAS interactions<sup>167</sup>. cGAS inhibition was also demonstrated in the *Trex1*-knockout mouse model but only at high concentrations of aspirin (equivalent to 3.5 g in a 70-kg adult, which would result in a blood concentration of ~1.5 mM), thus precluding its therapeutic use<sup>168</sup>.

Palmitoylation of Cys88 and Cys91 is necessary for the multimerization of STING, which, in turn, enables the binding of TBK1 (REF.<sup>169</sup>) (FIG. 2). Both naturally occurring nitro-fatty acids (NO<sub>2</sub>-FAs) and the nitro-furan derivative H-151 interact with one or more of these cysteines and attenuate STING-mediated inflammatory pathways<sup>170,171</sup> (FIG. 5). The inhibitory effect of H-151 was demonstrated in *Trex1*<sup>-/-</sup> mice and in mouse models of ALS. The small molecule SN-011, which interacts with the cGAMP binding pocket was also an efficient STING inhibitor and reduced disease severity in *Trex1*<sup>-/-</sup> mice<sup>172</sup>. Reverse transcriptase inhibitors that prevent the accumulation of ligands such as retroelements or DNA–RNA hybrids have had some therapeutic success in murine lupus<sup>173</sup>, but whether they can prevent cGAS–STING activation remains to be determined.

Importantly, long-term inhibition of the cGAS–STING pathway could potentially increase susceptibility to infection and cancer. To date, spontaneous infections or increased cancer incidence in mice deficient for cGAS or STING has not been reported. However, specific pathogen-free environment and differences in mouse and human predisposition to cancers should be kept in mind.

The majority of compounds that target TBK1, which acts downstream of STING, TLR3, TLR-4 and TRIF pathways, have low nanomolar affinity (for example, aminopyrimidines) and also target other serine/threonine kinases<sup>174</sup>. More selective inhibitors have been subsequently developed and they exert inhibitory effects in *Trex1*<sup>-/-</sup> mice<sup>175</sup> and mice with arthritis<sup>176,177</sup>.

Hydroxychloroquine (HCQ) is widely used to treat SLE and other autoimmune diseases. In vitro and animal studies suggest that this drug can attenuate both E-TLR and cGAS pathways<sup>166,178</sup>. In patients with SLE, HCQ is effective in the treatment of skin and joint disease but does not reduce life-threatening complications such as kidney disease<sup>179</sup>. Whether this limitation is due to inadequate dosing (HCQ at doses >400 mg/day might be associated with retinal and heart complications) or the presence of additional mechanisms of tissue injury is unclear. Nonetheless, more potent inhibitors of E-TLR and cGAS–STING are needed and are already in preclinical development. Given that cGAS activation seems to only occur in a subset of patients with SLE, such compounds might require a precision medicine approach guided by potential biomarkers such as cGAMP<sup>102</sup>.

### Conclusions

Despite only being discovered in 2013, progress in delineating the mode of action of the DNA sensor cGAS, its crucial partnership with STING and its roles in host defence and inflammation have been remarkably rapid. At present, substantial evidence implicates



the cGAS–STING pathway in mouse models of disease and accumulating data suggest that this pathway is also active in several human diseases. Over the next few years, improvements in cGAMP detection methods and in the evaluation of response to therapies designed to enhance or attenuate the cGAS–STING pathway will clarify its role in human diseases. Whether cGAS, cGAMP or STING will be easiest to target and have the fewest side-effects remains to be determined. Given that all of these molecules are upstream of IFN-I, targeting the cGAS–STING pathway is likely to be less disabling to host defences than blocking IFN-I, for example, through the common IFNAR.

Key questions remain to be addressed, for example, with regard to the cellular localization of cGAS and its regulation. The nature of the DNA ligands that trigger cGAS in autoinflammatory, autoimmune and degenerative disease states must also be elucidated. Finally, although mtDNA and retroelement DNA are frequently implicated in activation of the cGAS–STING pathway, precisely how and why these types of DNA activate cGAS remains to be clarified. Understanding these mechanisms might allow the pathway to be therapeutically targeted upstream of cGAS.

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K.B.E. and J.A. are co-founders of Amdax Therapeutics, LLC. S.S.-G. declares no competing interests.

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