Translating nucleic acid-sensing pathways into therapies

Tobias Junt[1] *and Winfried Barchet*[2]

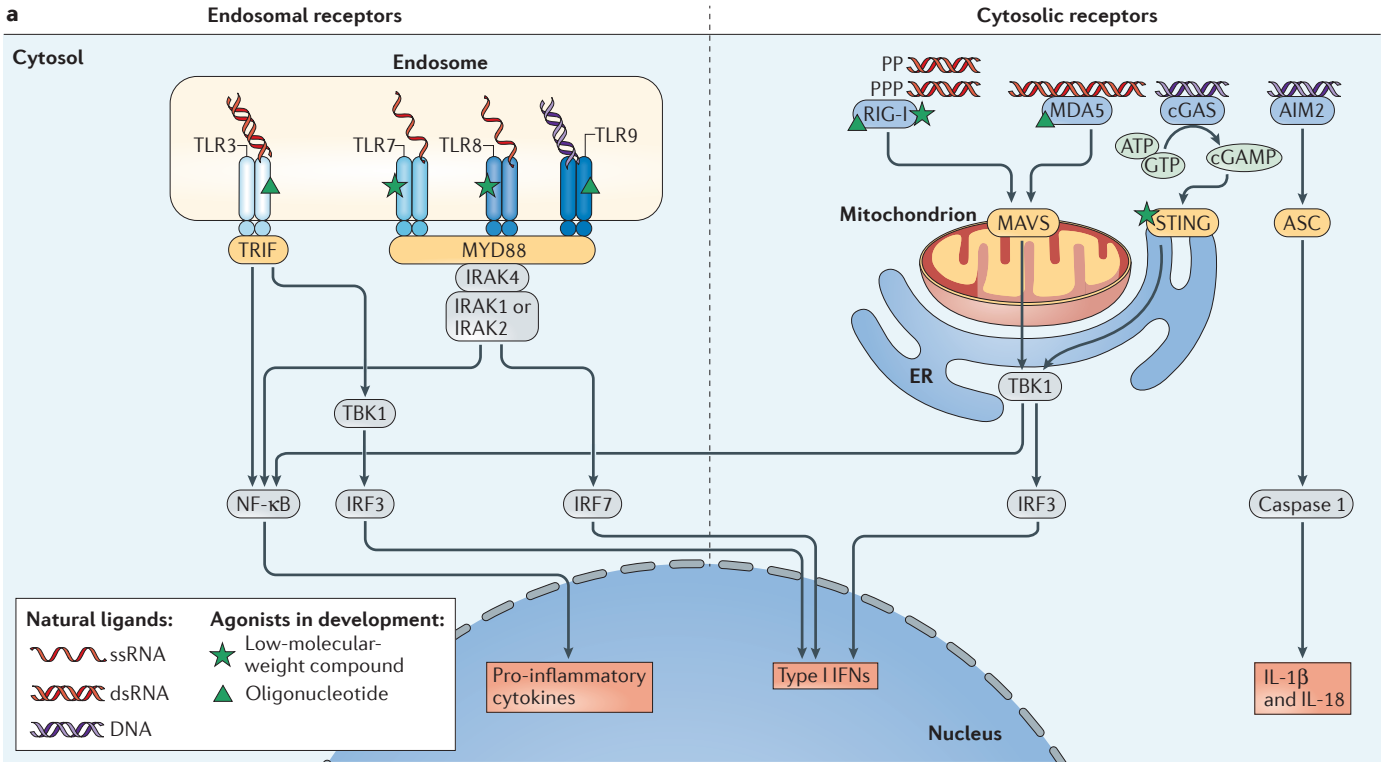
Abstract | Nucleic acid sensing by innate receptors initiates immune defences against viruses and other pathogens. A hallmark of this response is the release of interferons (IFNs), which promote protective immunity by inducing IFN-stimulated genes (ISGs). A similar ISG signature is found in autoinflammatory and autoimmune conditions, indicating that chronic activation of nucleic acid-sensing pathways may contribute to these diseases. Here, we review how nucleic acid-sensing pathways are currently being targeted pharmacologically with both agonists and antagonists. We discuss how an improved understanding of the biology of these pathways is leading to novel therapies for infections, cancer, and autoimmune and autoinflammatory disorders, and how new therapeutics will, in turn, generate a deeper understanding of these complex diseases.

The immune system has evolved to protect against infections, as swift and adequate immune responses to pathogens are critical for host survival. Prompt pathogen detection relies on a limited set of germline-encoded innate immune sensors termed pattern recognition receptors (PRRs). These receptors are triggered by molecular structures — so-called pathogen-associated molecular patterns (PAMPs) — that are conserved across a wide range of microorganisms. Bacterial and fungal PAMPs are not expressed by the mammalian host and thus are truly foreign molecules. Viruses, by contrast, instruct mammalian cells to synthesize all of their components and thus do not contain molecules that are foreign to the host. Over the past decade, the concept has emerged that virus recognition by the innate immune system hinges on the detection of viral RNA or DNA structures by relatively few PRRs (FIG. 1).

A hallmark consequence of innate nucleic acid sensing is the secretion of the key antiviral cytokines: type I interferons (IFNs; comprising IFNα1–IFNα13 and IFNβ) and type III IFNs (IFNλ1–IFNλ4)[1,2]. Although conventional dendritic cells (DCs), plasmacytoid DCs (pDCs) and macrophages are particularly prone to producing large amounts of type I IFNs, many somatic cell types selectively secrete type III IFNs[1,2]. Type I and type III IFNs activate type I IFN receptor (IFNAR) and interleukin-28 receptor (IL-28R) signalling, respectively, and promote a state of cell-autonomous antiviral defence by inducing the expression of dozens of IFN-stimulated genes (ISGs)[3]. In this Review, both IFN families are collectively referred to as IFNs.

The necessity to rely on nucleic acid sensing for the prompt response to infections with diverse viruses bears the inherent risk of misdirected immune activation by autologous DNA or RNA. Three critical safeguards are in place to prevent chronic IFN-associated autoinflammation. First, nucleic acid receptors are confined to the cellular compartments of the endosome and cytosol that are accessed during the course of virus replication yet contain physiologically low concentrations of potentially activating host nucleic acids[4]. Second, agonistic features of autologous nucleic acids are masked by elaborate modifications: for example, there are rich methylation patterns in mammalian DNA and RNA, which most rapidly replicating pathogens cannot readily obtain[5]. Third, nucleases are constitutively expressed within cells and counteract the endosomal or cytosolic accumulation of potentially stimulatory nucleic acids (FIG. 2). The importance of these nucleases is highlighted in monogenic autoinflammatory diseases such as Aicardi–Goutières syndrome (AGS), in which missense mutations in the cytosolic enzyme 3′ repair exonuclease 1 (TREX1; also known as DNase III) lead to intracellular accumulation of DNA, chronic expression of an ISG signature and debilitating autoinflammatory disease. In mice, genetic deficiency of *Trex1* leads to myocarditis[6], and *Dnase2* deficiency leads to embryonic death due to the activation of IFN-dependent pathways[7]. This is consistent with data showing that nucleic acid sensors continuously monitor normal developmental processes that involve relocation of nucleic acids between subcellular compartments: for example, cell division[8], cell

[1]*Autoimmunity, Transplantation and Inflammation, Novartis Institute of Biomedical Research, Novartis Campus Forum 1, CH-4056 Basel, Switzerland.*
[2]*German Center for Infection Research Cologne–Bonn, Institute of Clinical Chemistry and Clinical Pharmacology, University of Bonn, Sigmund-Freud Strasse 25, 53127 Bonn, Germany.*
Correspondence to T.J.
e-mail:
tobias.junt@novartis.com
doi:10.1038/nri3875
Published online
21 August 2015



b

	Human				Mouse			
	TLR3	TLR7	TLR8	TLR9	TLR3	TLR7	TLR8*	TLR9
pDCs		+		+		+		+
B cells		+		+		+		+
Monocytes	+		+		+	+		+
XCR1⁻ DCs			+			+		+
XCR1⁺ DCs	+				+			
Neutrophils			+			+		

*Not functional.

Figure 1 | **Endosomal and cytosolic nucleic acid-sensing pathways that provide pharmacological targets.** **a** | The figure indicates some of the major nucleic acid-sensing pathways that are being targeted. Sensors for nucleic acids are shown in blue, adaptor molecules are shown in yellow and downstream signalling molecules are shown in grey. Activation of these pathways may result in effector mechanisms beyond the production of interferon (IFN) and other cytokines (red boxes): for example, the expression of IFN-stimulated genes or B cell proliferation (see the main text). Current drug discovery efforts for these pathways are summarized in TABLE 2. **b** | The expression of endosomal Toll-like receptors (TLRs) across different immune cell subsets, with notable differences between humans and mice. AIM2, absent in melanoma 2; cGAMP, cyclic GMP–AMP; cGAS, cGAMP synthase; DC, dendritic cell; dsRNA, double-stranded RNA; ER, endoplasmic reticulum; IL, interleukin; IRAK, IL-1 receptor-associated kinase; IRF, IFN-regulatory factor; MAVS, mitochondrial antiviral signalling protein; MDA5, melanoma differentiation-associated protein 5; MYD88, myeloid differentiation primary response protein 88; NF-κB, nuclear factor-κB; pDC, plasmacytoid DC; RIG-I, retinoic acid-inducible gene I; ssRNA, single-stranded RNA; STING, stimulator of IFN genes; TBK1, TANK-binding kinase 1; TRIF, TIR domain-containing adaptor protein inducing IFNβ; XCR1, XC-chemokine receptor 1.

death[9] and autophagy[10]. A currently emerging complementary function of intracellular nucleases is their ability to trim certain nucleic acids to defined structures or lengths, thereby improving recognition by nucleic acid receptors[11,12]. Altogether, it seems that nucleic acid-sensing pathways are continuously active at a basal level, in equilibrium with intracellular nucleases, and ready for prompt immune activation once this balance is offset.

Aside from pathogens, sterile cell stress can also offset this equilibrium (FIG. 2). This can be beneficial for immune surveillance in situations such as cancer, where the physiological turnover of cells is disturbed[13]. However, a pathologically enhanced influx of autologous nucleic acids into cells can also contribute to autoimmune diseases. As an example, autoimmune inflammation leads to increased expression of molecular shuttles that stabilize

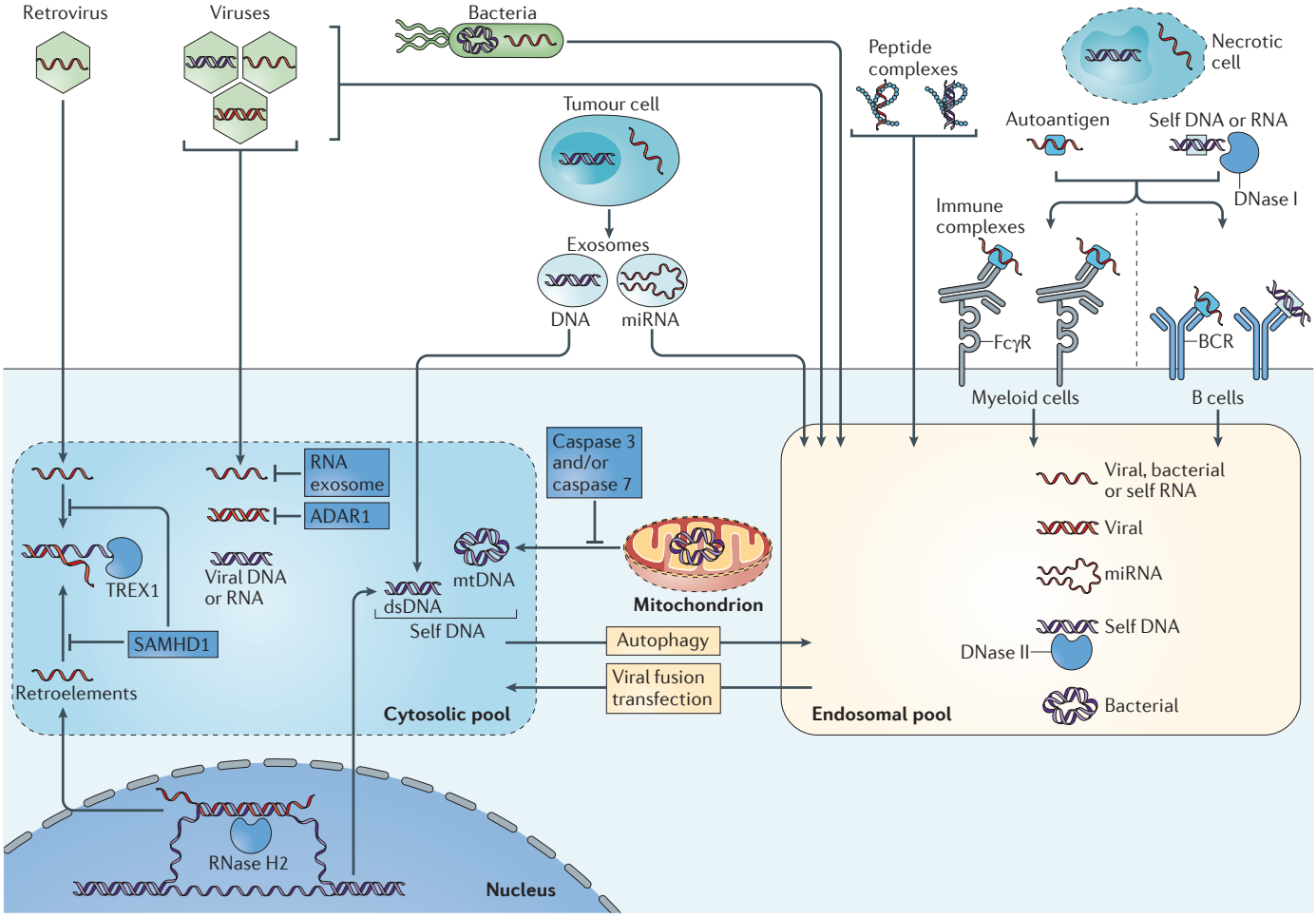


Figure 2 | **Access routes of self or foreign DNA and RNA to endosomes and to the cytosol.** Self DNA or RNA may enter cells after complexation with peptides[14,96], or within immune complexes[15,63]. DNA may enter the cytoplasm after disintegration of mitochondria[9,127], from the nucleus[10] or as tumour-derived particles[13]. Passage of nucleic acids between the cytosolic and the endosomal pools may occur. Nucleases that counteract the accumulation of DNA and RNA (and thus avoid inappropriate stimulation of nucleic acid sensors) are shown in blue boxes. ADAR1, double-stranded RNA-specific adenosine deaminase; BCR, B cell receptor; dsDNA, double-stranded DNA; miRNA, microRNA; mtDNA, mitochondrial DNA; TREX1, 3′ repair exonuclease 1.

extracellular DNA and RNA and deliver them into cells. The simplest of these shuttle molecules are cationic amphiphilic peptides that are highly expressed in psoriatic skin[14]. In systemic lupus erythematosus (SLE) and Sjögren syndrome, antibodies against double-stranded DNA (dsDNA) or RNA-associated antigens function as delivery vehicles for DNA or RNA molecules[15,16].

The involvement of nucleic acid-sensing mechanisms in the anti-pathogen response and in various autoinflammatory and autoimmune diseases makes these pathways attractive drug targets. A key challenge for drug discovery is to identify the nucleic acid-sensing pathways that match the pathophenotype of each disease. In this Review, we discuss how agonists and antagonists of nucleic acid-sensing pathways are being developed for clinical application based on our current knowledge of their biology. We provide a brief overview of the various nucleic acid sensors before describing the drugs that have been designed to target them and the indications for which they are being developed.

Pathophenotype
A disease subtype within a complex disease that is distinguished by certain clinical symptoms. Complex diseases such as systemic lupus erythematosus contain multiple pathophenotypes. The key challenge of molecular pathology is to match pathophenotypes to the activation of specific pathogenic pathways.

Nucleic acid sensors

Endosomal nucleic acid sensors. Nucleic acid sensors of the Toll-like receptor (TLR) family are confined to endosomes[17] and are selectively expressed by a few cell types, mainly those in the innate immune system (FIG. 1). TLR3 is activated by dsRNA, TLR7 detects single-stranded RNA (ssRNA) and short dsRNA, and recent evidence suggests that TLR8 binds short ssRNA and ssRNA breakdown products[18]. Short synthetic GU-rich oligonucleotides showed an enhanced agonistic effect on both TLR7 and TLR8, indicating a preference for certain nucleotides. TLR9 instead senses DNA with a distinct preference for DNA that contains unmethylated CpG motifs[19]. TLRs are transmembrane proteins consisting of amino-terminal leucine-rich repeats (LRRs) on the luminal side and a cytosolic Toll/IL-1R (TIR) domain. In TLR7, TLR8 and TLR9, the TIR domain initiates signalling by aggregating the adaptor protein myeloid differentiation primary response protein 88 (MYD88). The MYD88-dependent signalling cascade ultimately

Table 1 | **Agonists of nucleic acid sensors in preclinical and clinical development***

Compound (Company)	Chemical composition	Clinical Phase	Comments	Refs and ClinicalTrials.gov identifiers
TLR3 agonists				
Poly-ICLC/ Hiltonol (Oncovir)	Nucleotide-based agonist	I–II	Enhancement of multi-peptide vaccine efficacy in glioblastoma	NCT01920191
		II	Solid tumours	NCT01984892
Poly(I:C_{12}U)/ Rintatolimod. Trade name: Ampligen (HemispheRx Biopharma)	Nucleotide-based agonist	III	Chronic fatigue syndrome	NCT00215813
		I–II	Adjuvant for influenza vaccine	NCT01591473
TLR7 agonists				
Imiquimod (originator 3M Pharmaceuticals; owners: Valeant Pharmaceuticals International and MEDA; licensee for Japan: iNova Pharmaceuticals; sub-licensee for Japan: Mochida Pharmaceutical; generic versions: Taro Pharmaceutical Industries, Glenmark Generic). Trade names: Aldara, Zyclara (both MEDA, Valeant Pharmaceuticals), Vyloma (Valeant Pharmaceuticals), Beselna (Mochida Pharmaceuticals).	Low-molecular-weight compound	Approved	• Approved for genital warts and skin cancers (including actinic keratosis and basal cell carcinoma) • Additional clinical trials are ongoing	33
AZD8848 (AstraZeneca and Dainippon Sumitomo)	Low-molecular-weight compound	II	Intranasal administration for allergic rhinitis	NCT01788813
GSK-2245035 (GlaxoSmithKline)	Low-molecular-weight compound	II	Respiratory allergies including asthma	NCT01607372
GS-9620 (Gilead)	Low-molecular-weight compound	II	HBV infection	NCT02166047
			HCV infection	NCT01591668
TMX-101/ Vesimune (Telormedix)	Low-molecular-weight compound	II	Liquid formulation of imiquimod (intravesical administration) for the treatment of non-invasive bladder cancer	NCT01731652
TLR7 and TLR8 agonists				
Resiquimod (MEDA)	Low-molecular-weight compound	II	Adjuvant in protein or peptide vaccines for tumours	NCT00960752, NCT01204684 and others
Resiquimod topical (Spirig)	Low-molecular-weight compound	II	Actinic keratosis	NCT01583816
TLR8 agonists				
VTX-2337 (VentiRx)	Low-molecular-weight compound	II	VTX2337 used alongside chemotherapy and cetuximab (EGFR-specific antibody) for the treatment of squamous cell carcinoma of the head and neck	NCT01836029
TLR9 agonists				
Kappaproct (InDex Pharmaceuticals)	Nucleotide-based agonist	III	Used to increase steroid sensitivity in patients with ulcerative colitis	NCT01493960
MGN-1703/dSLIM (Mologen)	Nucleotide-based agonist	II–III	Colorectal cancer	NCT01208194
			SCLC	NCT02200081
MGN-1601 (Mologen)	Nucleotide-based agonist	PoC study	MGN-1601 is a combination of MGN-1703 plus allogeneic cancer cells for the treatment of renal cell cancer	NCT01265368
SD101 (Dynavax)	Nucleotide-based agonist	I–II	Used in combination with low-dose radiation for lymphomas	NCT02266147 and NCT01745354
Heplisav-B (Dynavax)	Nucleotide-based agonist	III	Hepatitis B virus (HBV) vaccine comprising a TLR9 agonist (1018 ISS) and HBV surface antigen	NCT02117934
AV7909 (Emergent Biosolutions)	Nucleotide-based agonist	II	New anthrax vaccine comprising a TLR9 agonist (CpG7909) and the existing anthrax vaccine BioThrax	NCT01263691

Table 1 (cont.) | **Agonists of nucleic acid sensors in preclinical and clinical development***

Compound (Company)	Chemical composition	Clinical Phase	Comments	Refs and ClinicalTrials.gov identifiers
Cytosolic nucleic acid sensor agonists (in preclinical Phases)				
ADU-S100 (Aduro and Novartis)	Nucleotide-based agonist	STING	Cyclical dinucleotides for the treatment of cancer	NA
ImOl-100 (Rigontec)	Nucleotide-based agonist	RIG-I	Minimal RNA mimic of PPP-RNA for the treatment of cancer and infection	NA
MCT-465 (Multicell Technologies)	Nucleotide-based agonist	RIG-I, MDA5 and TLR3	High-molecular-weight dsRNA for the treatment of cancer, and HBV and HCV infections	NA
Isoflavones e.g. KIN-100 (Kineta)	Low-molecular-weight compound	IRF3 or an upstream target	Low-molecular-weight compounds for the treatment of viral infections	NA
SB-9200 (Spring Bank Pharmaceuticals)	Low-molecular-weight compound	RIG-I and NOD2	Small-molecule nucleic acid hybrid for the treatment of viral Infections	NA

*Data correct as of March 2015. dsRNA, double-stranded RNA; EGFR, epidermal growth factor receptor; HBV, hepatitis B virus; HCV, hepatitis C virus; MDA5, melanoma differentiation-associated protein 5; NA, not applicable; NOD2, nucleotide-binding oligomerization domain 2; PoC study, proof-of-concept study; RIG-I, retinoic acid-inducible gene I; SCLC, small-cell lung cancer; STING, stimulator of interferon genes; TLR, Toll-like receptor.

leads to the activation of nuclear factor-κB (NF-κB) and the transcription of genes encoding pro-inflammatory cytokines. Exclusively in pDCs, the MYD88 signalling complex is able to induce ample transcription of genes encoding IFNα subtypes via a direct activation of IFN-regulatory factor 7 (IRF7). By contrast, TLR3 signals via TIR domain-containing adaptor protein inducing IFNβ (TRIF), which activates NF-κB, mitogen activated protein kinase (MAPK) and IRF3 signalling and results in the transcription of *IFNB* and pro-inflammatory cytokines[20].

Cytosolic nucleic acid sensors. Another set of RNA and DNA sensors is broadly expressed in the cytosol of immune and non-immune cells. The DExD/H-box helicases retinoic acid-inducible gene I (RIG-I; also known as DDX58) and melanoma differentiation-associated protein 5 (MDA5) detect complementary dsRNA structures. RIG-I is activated by 5′ triphosphorylated or 5′ diphosphorylated ends of short dsRNA[21,22]. The RIG-I dependent detection of endogenous mRNAs is prevented by 2′ -O-methylation at the first base pair[154]. The MDA5 ligand is less well defined but was reported to be present in long dsRNA and in branched high-molecular RNA forms[23,24]. It was recently shown that MDA5-activating structures are also present in endogenously synthesized RNAs, but these are removed by adenosine deaminase 1 (ADAR1)-mediated editing[155,156]. Both RIG-I and MDA5 consist of a carboxy-terminal ligand-binding domain, a central DEAxD/H-box helicase domain and an N-terminal caspase activation and recruitment domain (CARD). These CARDs engage similar CARDs of the signalling adaptor mitochondrial antiviral signalling protein (MAVS) on the outer mitochondrial membrane, which leads to multimerization of MAVS and activation of the MAVS signalling complex[25]. Another cytosolic sensor, cyclic GMP–AMP (cGAMP) synthase (cGAS)[26], is a receptor for dsDNA. Upon ligand binding, cGAS produces the non-canonically linked cyclic dinucleotide (CDN) [G(2′,5′)pA(3′,5′)p] (2′3′-cGAMP), which functions as a second messenger to activate the stimulator of IFN genes (STING) on the endoplasmic reticulum (ER)[27,28]. Mouse STING is also activated directly by bacterial CDNs, including c-di-AMP and c-di-GMP[29,30].

Both endosomal and cytosolic nucleic acid sensors are themselves ISGs: that is, their expression is inducible by innate immune activation and IFN receptor signalling[3]. These feedback amplification cycles enhance the acute responsiveness to infection in many tissues. The functional expression of cytosolic and endosomal RNA and DNA receptors is cell-type specific and sometimes differs between humans and mice (FIG. 1b). This type of information provides useful guidance for drug discovery. For example, it suggests that antagonists of endosomal TLRs will mainly target haematopoietic cells and may have limited direct effects on non-haematopoietic cells. By contrast, non-haematopoietic cells are direct target cells of agonists or antagonists of cytosolic nucleic acid receptors.

A common feature of the signalling adaptors MAVS, STING and TRIF is their ability to activate TANK-binding kinase 1 (TBK1) and IRF3 upon phosphorylation[31]. Similarly to endosomal TLRs, these cytosolic sensor pathways eventually converge on activation of the transcription factors NF-κB, MAPK, and IRF3 and IRF7 homodimers and heterodimers, and the production of pro-inflammatory cytokines and IFNs. By contrast, absent in melanoma 2 (AIM2), which is another cytosolic receptor for dsDNA, triggers the release not of IFNs but of IL-1β and IL-18. AIM2 has recently been reviewed in the context of ASC inflammasome activators[32].

Of note, in addition to those mentioned above, several other nucleic acid sensors have been described. We focus here on the sensors that are currently being targeted in the clinic. In the sections below, we discuss the agonists and antagonists that are being used to target these receptors in different target indications.

Agonists of nucleic acid-sensing pathways

The engagement of nucleic acid receptors activates the innate immune system in multiple ways. Aside from triggering cell-intrinsic and IFN-mediated antiviral effector mechanisms, nucleic acid sensor agonists activate DCs, promoting cytokine secretion, maturation and antigen presentation. This, in turn, enhances and shapes the quality of adaptive immune responses. Owing to their antiviral and immune-enhancing properties, oligonucleotide or small-molecule agonists of nucleic acid sensors are being used in clinical trials to boost the immune response against poorly immunogenic cancers, and as adjuvants in therapeutic immunizations against cancer or in prophylactic vaccines against infections (TABLE 1). The most notable adverse effects of nucleic acid sensor agonists are attributed to systemic inflammatory responses and include flu-like symptoms or arthralgia. Therefore, agonists of nucleic acid sensors are often used topically[33], or targeted to antigen-presenting cells by covalent linkage to protein antigens or by packaging into nanoparticles[34–37].

The recent wealth of structural and functional knowledge provides exciting opportunities for rational agonist design. However, as we discuss below, most agonists that are currently in late stages of clinical development were discovered as immune stimulators long before their mechanism of action was uncovered.

Empirical discovery of agonists for nucleic acid sensors. Polynucleotide products of the enzyme polynucleotide phosphorylase (PNPase 1) were first intensively studied as synthetic inducers of IFN activity[38]. The most consistently active variant, the dsRNA mimetic polyinosinic:polycytidylic acid (poly(I:C)), was much later shown to function as an agonist for both TLR3 and MDA5 (REFS 39,40). Ongoing clinical trials are using the analogue poly-ICLC that is formulated with poly-L-lysine in order to increase RNase resistance. Poly-ICLC provides potent virus-like adjuvant activity[41] and is currently being evaluated as a promising cancer vaccine adjuvant in diverse clinical settings (reviewed in REF. 42). Another variant, poly(I:C_{12}U) (rintatolimod) has been developed for its favourable toxicity profile, which is probably due to an inability to activate MDA5. It is being clinically evaluated for the treatment of chronic fatigue syndrome[43].

Similarly, the small molecule imiquimod, an imidazoquinoline derivative, had already reached approval as an antiviral for topical treatment of papillomavirus-induced genital warts before it was shown to act via TLR7 (REF. 44). Imiquimod is still the only agonist of nucleic acid sensors that is approved by the US Food and Drug Administration (FDA). It has revolutionized the treatment of dermatological neoplasias, such as basal cell carcinoma and actinic keratosis, obviating the need for surgery. Local induction of pro-inflammatory cytokines, local recruitment of immune cells, and improved antigen presentation for the induction of T helper 1 (T_H1) and $CD8^+$ T cell responses are considered to be crucial for treatment success. High response rates and an excellent safety profile after topical treatment have led to the use of imiquimod in the treatment of other dermatological malignancies such as lentigo maligna[33]. The imidazoquinoline resiquimod is a dual agonist of TLR7 and TLR8. In humans, resiquimod therefore potently activates additional cell types and elicits a broader range of cytokines. This probably contributes to the more frequent occurrence of systemic adverse effects observed in clinical trials[33]. Resiquimod and structurally unrelated TLR7-selective compounds are currently being evaluated in clinical trials of allergy, viral infections and non-dermatological localized cancers (TABLE 1). Of note, the lack of functional TLR8 in the mouse (FIG. 1b) and other rodent models has made the evaluation and preclinical development of TLR8-specific agonists more demanding. Moreover, compensating for their lack of TLR8 expression, mice show more widespread expression of TLR7 than is found in humans. This also restricts the predictive value of mouse models for the development of human TLR7 agonists.

Originally identified as a small-molecule inducer of IFN, 5,6-dimethylxanthenone-4-acetic acid (DMXAA; also known as vadimezan) was clinically developed for the therapy of non-small-cell lung cancer due to its effectiveness in disrupting the tumour vascularization in mouse xenotransplantation models[45]. Despite its promise, it ultimately failed in clinical trials[46]. A mechanistic explanation for this discrepancy was recently offered by the finding that DMXAA activated mouse STING but had no effect on human variants of STING[47]. New structure–function studies of DMXAA using human STING now enable the design of cell-permeable DMXAA variants that are active in humans[48]. The ability of bacterial CDNs to activate STING[29], and thus function as potent immune stimulants in mice[49], prompted their development as human vaccine adjuvants[50]. However, similarly to DMXAA, bacterial CDNs are much weaker agonists of human variants of STING than of mouse STING[51]. This indicates that agonists for human STING should instead be modelled on the physiological agonist 2′3′-cGAMP, which has recently been shown to have vaccine adjuvant properties as well[52].

Rational design of agonists for nucleic acid sensors. In contrast to the empirically identified agonists described above, TLR9-stimulatory synthetic CpG oligodeoxynucleotides (CpG-ODNs) were designed rationally, based on the immune-stimulatory properties of bacterial DNA that, in contrast to human DNA, is rich in unmethylated CpG motifs[53]. Optimization of sequence features and backbone modifications led to CpG-ODN subtypes that preferentially activate either B cells or pDCs. Dozens of clinical trials have focused on the antitumour activity of CpG-ODNs, in combination with chemotherapy or in therapeutic vaccines. However, despite a favourable safety profile and substantial *in vivo* activity in mice, none of these cancer trials has so far demonstrated sufficient clinical benefit. One reason could be that in humans, TLR9 is selectively expressed in B cells and pDCs, but in mice it is also expressed by some DC subsets (FIG. 1b). After a transient waning of interest, CpG-ODNs are currently making a comeback as adjuvants for use in anti-infection vaccines. The most promising results were obtained in vaccine trials against anthrax and hepatitis B virus (HBV), even in patients whose immune function was compromised due to chronic HIV infection (reviewed

in REF. 54). Therefore, CpG-ODNs may eventually reach the clinic as adjuvants for prophylactic vaccines, rather than in the far more demanding context of therapeutic immunizations against tumours.

As exemplified above for DMXAA and CpG-ODNs, differences in nucleic acid sensor expression patterns between humans and preclinical test species, or differing sensitivity to experimental agonist compounds, can hamper the translation of preclinical results into humans. By contrast, RIG-I is broadly expressed in somatic and immune cells of both mice and humans. Both species show comparable reactivity to different viral, synthetic oligonucleotide and small-molecule RIG-I ligands. However, in comparison to other stimuli of cytosolic nucleic acid receptors, RIG-I agonists induce a much more pronounced IFN response in humans[21,22,55]. Structural analysis of RIG-I co-crystallized with its RNA ligands, in combination with extensive functional studies, enabled the design of optimized RNA-based agonists for RIG-I[56,57]. Given that RIG-I agonists have not yet advanced to clinical testing, it is too early to gauge their full therapeutic potential.

Nucleic acid sensor agonists as vaccine adjuvants. The vaccines that have most successfully reduced the world's burden of infectious diseases rely on live attenuated vaccine strains that are inherently immunogenic, presumably due to autonomous replication and concerted activation of multiple PRRs[58]. Modern subunit vaccines consist of defined antigens — for example, recombinant proteins — and are preferred because of their safety profile and because they can be manufactured at a consistent quality and in consistent amounts. However, they are poorly immunogenic by themselves and therefore require the co-administration of adjuvants that activate antigen-presenting cells. Currently, only a few adjuvants — including aluminium salts (alum), squalene emulsions and saponins — are approved for clinical use, and they mainly induce antibody responses. However, the induction of T_H cells is equally important for sustaining antibody responses, and the activation of $CD8^+$ T cells is key for protection against intracellular pathogens such as *Plasmodium* spp. and *Mycobacterium tuberculosis*. Agonists of nucleic acid recognition pathways are promising novel candidates that license DCs for the induction of protective immune responses mediated by both T_H1 cells and cytotoxic $CD8^+$ T cells[59]. The combined use of different agonists may prove even more powerful, as recent data have indicated that co-engagement of multiple nucleic acid receptors elicited the most potent immune responses in preclinical trials of vaccine adjuvants[60,61].

Antagonists of nucleic acid-sensing pathways

Rationale for blocking nucleic acid-sensing pathways in IFN-associated inflammatory diseases. Nucleic acid-sensing pathways and ISGs offer numerous potential drug targets that are currently being evaluated preclinically for the treatment of IFN-associated inflammatory diseases. These diseases are characterized by elevated levels of type I IFN or ISGs, and they are frequently associated with antinuclear antibodies (ANAs) against dsDNA or RNA-associated antigens. The prototypical and consequently most well-studied member of this group is SLE. Other examples are autoimmune diseases such as Sjögren syndrome, dermatomyositis, polymyositis and systemic sclerosis, as well as an increasing number of monogenic IFN-associated autoinflammatory diseases.

Endosomal DNA- and RNA-sensing pathways were first implicated in the pathogenesis of these disorders by studies in mice[62] and by experiments showing that sera from patients with SLE elicit type I IFN production from pDCs via nucleic acid-sensing TLRs[63]. These data led to the hypothesis that antagonists of these pathways could be effective treatments. Clinical data further strengthen this hypothesis: hydroxychloroquine, which is a standard medication for SLE, was shown to interfere with the function of endosomal TLRs[64].

The drugs that are currently in development to antagonize nucleic acid-sensing pathways rely on one of three principal mechanisms of action (TABLE 2). First, they may neutralize type I IFN or block signalling through IFNAR. The IFNα-neutralizing antibody sifalimumab showed clinical efficacy in moderate-to-severe SLE[128], and the IFNAR-blocking antibody anifrolumab led to a downregulation of pathogenically elevated ISGs[129]. Second, these drugs may target extracellular DNA- and RNA-containing autoantigens: for example, recombinant DNases or RNases can be used to degrade extracellular nucleic acids. The feasibility of this principle has been demonstrated in a mouse model of lupus-like disease[65]. The third group of antagonists that are in development directly target DNA- and RNA-sensing receptors or inhibit their downstream signalling components. FIGURE 3 summarizes the hypothetical clinical outcomes of these interventions in specific indications.

Challenges for the development of antagonists of nucleic acid sensors. Despite the high unmet medical need in IFN-associated inflammatory diseases, few antagonists of nucleic acid sensors have entered clinical development. This may be due to several factors. First, the development of agonists has been aided by compounds such as poly(I:C), imidazoquinolines and DMXAA, which were available long before the discovery of the corresponding nucleic acid-sensing pathways, whereas such historical knowledge is not available for antagonists of nucleic acid-sensing pathways. Second, the preclinical safety assessments for antagonists of nucleic acid sensors must be extensive, as their target indications are mostly systemic and chronic and call for long-term treatment. Third, and most importantly, the complexity of IFN-associated target indications, such as SLE, makes the design and interpretation of clinical trials for antagonists of nucleic acid-sensing pathways challenging. Patient selection or stratification criteria are required that reflect pathogenic activity of specific nucleic acid-sensing pathways. For example, an ISG signature has been used to stratify patients with SLE in a clinical trial of the IFNα-neutralizing antibody rontalizumab[66]. However, as nucleic acid sensors have effects beyond the modulation of the IFN response, further pathway-specific biomarkers are required to support patient stratification in trials with novel antagonists.

Antinuclear antibodies (ANAs). Autoantibodies against double-stranded DNA or RNA-containing antigens (for example, Sjögren syndrome-related antigen A (SS-A; also known as Ro), SS-B (also known as La), Sm (spliceosomal) and small nuclear ribonucleoprotein 70 kDa (snRNP70)). Their presence in patient sera is a diagnostic hallmark of autoimmune diseases such as Sjögren syndrome and systemic lupus erythematosus. One diagnostic test for ANAs relies on specific staining pattern of cell nuclei with patient sera by immunofluorescence, hence the name.

Biomarkers
Measurable parameters that are reflective of specific biological processes in living organisms. Diagnostic biomarkers point to disease type or severity and may support patient stratification or selection. Pharmacodynamic biomarkers are measured in clinical trials to indicate pharmacological responses to compounds.

Table 2 | **Inhibitors of nucleic acid-sensing pathways in clinical Phase II and Phase III trials***

Compound (company)	Clinical Phase	Indication	Mode of action	Comments	Refs and ClinicalTrials.gov identifiers
Neutralization of IFN, IFNAR or IFN signalling					
Sifalimumab‡ (AstraZeneca and MedImmune)	Phase II	SLE	IFNα-specific monoclonal antibody	After 1 year, there was more patient-reported improvement for sifalimumab-treated patients than for those treated with placebo plus standard of care, although the former group showed an increased risk of infections	128
Anifrolumab (AstraZeneca and MedImmune)	Phase II	SLE	IFNAR1-specific monoclonal antibody	Anifrolumab treatment led to increased and more sustained ISG suppression compared with sifalimumab, with patients showing an SRI response at 6 months	129
IFNα kinoid (Neovacs)	Phase II	SLE	Therapeutic vaccine to raise endogenous IFN-specific antibodies	Reduction of ISG expression	130
GSK-2586184 (GlaxoSmithKline and Galapagos)	Stopped during Phase II	SLE	Small-molecule antagonist of JAK1	Primary endpoints not met	NCT01777256
Rontalizumab‡ (Roche and Genentech)	Stopped after Phase II	SLE	IFNα-neutralizing antibody	SRI response only in a biomarker-defined pre-specified subgroup	66
Targeting nucleic-acid containing autoantigens					
Lupuzor (ImmuPharma)	Phase III	SLE	snRNP70-derived peptide	Phase IIb trial results: SRI response at week 12 improved relative to placebo	131
RSLV-132 (Resolve Therapeutics)	Phase II	SLE	RNase–Fc	Causes degradation of RNA in autoantigens	NCT02194400
Targeting signalling pathways of nucleic acid sensors					
Amlexanox; trade name Aphthasol (Uluru)	Approved and discontinued	Aphthous ulcers and asthma	TBK1 and IKKε antagonist	None.	132
LMW antagonists (Domainex)	Preclinical	COPD, cancers and inflammation	TBK1 and IKKε antagonist	None.	Domainex
ND-2110 and ND-2158 (Nimbus)	Preclinical	Front-runner indication: *MYD88*^{L265P}-mutant B cell lymphomas	IRAK4 antagonists	None.	133
LMW antagonists (Aurigene and Curis)	Preclinical	Front-runner indication: *MYD88*^{L265P}-mutant B cell lymphomas	IRAK4 antagonists	None.	134
LG0224912 and LG0250276 (TG Therapeutics)	Preclinical	Front-runner indication: *MYD88*^{L265P}-mutant B cell lymphomas	IRAK4 antagonists	None.	135
Nucleic acid sensor antagonists					
Hydroxychloroquine; trade name Plaquenil (Sanofi-Aventis)	Approved	SLE and rheumatoid arthritis	TLR7, TLR9 and cGAS antagonist activity, probably by binding to nucleic acid ligands	• Slow onset of action • Key safety risk is toxicity in the eye	117
IMO-3100 ((Idera Pharmaceuticals)	Phase II	Psoriasis	TLR7 and TLR9 oligonucleotide antagonist	• Clinical response • Reduction of T_H17-type gene signature	NCT01622348
IMO-8400 (Idera Pharmaceuticals)	Phase I–II	Waldenström macroglobulinaemia and *MYD88*^{L265P} – mutant DLBCL	TLR7, TLR8 and TLR9 oligonucleotide antagonist	Ongoing	NCT02092909 and NCT02252146
	Phase II	Psoriasis	TLR7, TLR8 and TLR9 oligonucleotide antagonist	• Clinical response • No sign of dose-dependency	NCT01899729
DV-1179‡ (Dynavax)	Stopped after Phase II	SLE	TLR7 and TLR9 oligo-nucleotide antagonist	No modification of ISG expression	Dynavax

*Data correct as of March 2015. ‡Clinical development has been stopped for this indication. cGAS, cyclic GMP–AMP synthase; COPD, chronic obstructive pulmonary disease; DLBCL, diffuse large B cell lymphoma; IFN, interferon; IFNAR, type I IFN receptor; IFNAR1, IFNAR subunit 1; IKKε, IκB kinase-ε; IRAK4, IL-1 receptor-associated kinase 4; ISG, IFN-stimulated gene; JAK1, Janus kinase 1; LMW, low molecular weight; *MYD88*, myeloid differentiation primary response protein 88; SLE, systemic lupus erythematosus; SRI, SLE responder index; snRNP70, small nuclear ribonucleoprotein 70 kDa; TBK1, TANK-binding kinase 1; T_H17, T helper 17; TLR, Toll-like receptor.

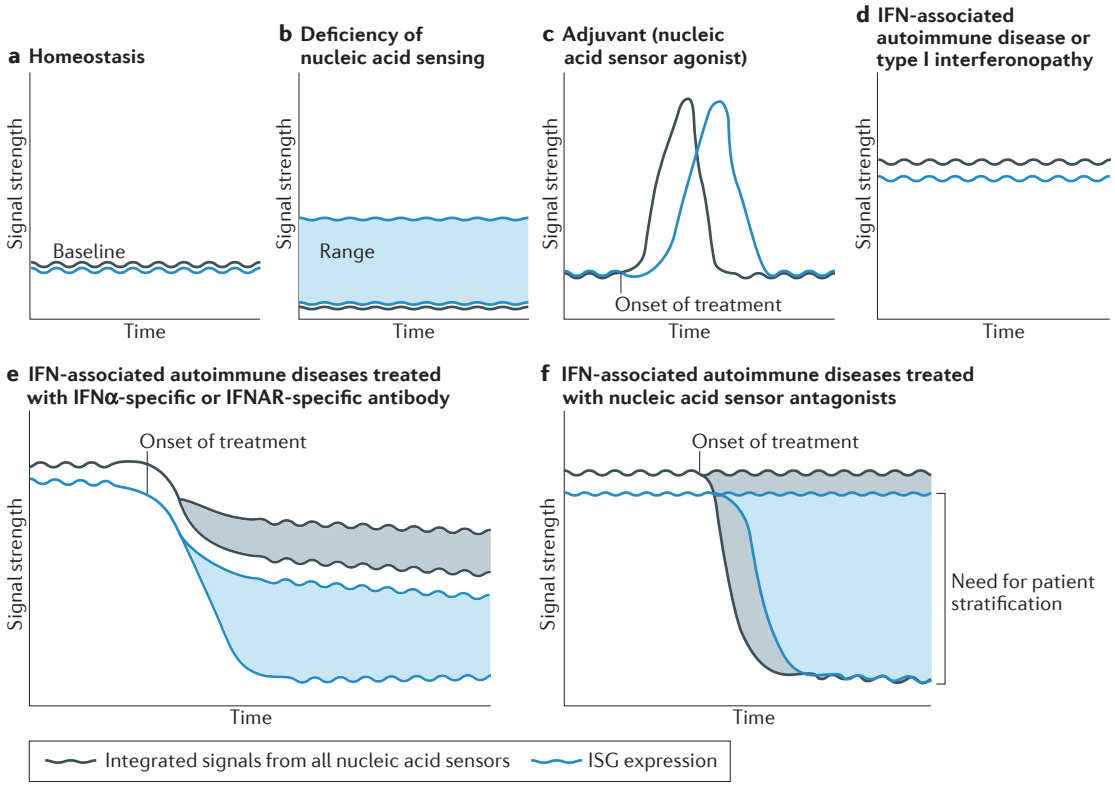


Figure 3 | **Hypotheses about nucleic acid sensor activity and expression of the ISG signature over time in different physiological states.** The blue lines indicate the expression level of interferon (IFN)-stimulated genes (ISGs) in the body, and the black lines indicate the total strength of signals from all nucleic acid sensors in each state. **a** | Homeostasis is characterized by low levels of homeostatic nucleic acid sensing and low expression of ISGs. The signals probably fluctuate over time. **b** | Homeostatic deficiency of nucleic acid sensing can occur, for example, in gene-deficient mice. Note that this signalling deficiency may paradoxically increase ISG expression in homeostasis (within the range indicated by the blue shaded area), for example, in cases where tonic nucleic acid sensing may provide protection against retroelements[120]. **c** | Nucleic acid sensor activation by agonistic adjuvant (black line) leads to temporary ISG expression. **d** | Untreated IFN-associated autoimmune diseases or interferonopathies are characterized by chronically elevated activity of nucleic acid-sensing pathways and ISGs. **e** | Treatment of autoimmune diseases or type I interferonopathies with antibodies against IFNα or the type I IFN receptor (IFNAR) leads to a reduction of nucleic acid sensor signalling (grey shaded area) because some nucleic acid sensors are ISGs. Patients show different responsiveness (blue shaded area) depending on their ISG expression[66]. **f** | If the sensor targeted by a nucleic acid sensor antagonist is a key driver for disease, the integrated nucleic acid-sensing signal will be reduced to low levels, and if it is not involved, it will stay unaltered (grey shaded area); ISG expression (blue shaded area) will correlate with treatment success. This highlights the need for patient stratification in complex inflammatory diseases.

Strategy for the development of antagonists of nucleic acid sensors. Preclinical drug development is an iterative compound optimization process that includes genetic, functional and translational studies and aims to generate safe and efficacious candidate compounds for clinical trials (BOX 1). Clinical development of antagonists of nucleic acid sensors is based on mechanistic insight into the role of nucleic acid-sensing pathways in IFN-associated inflammatory diseases. Conversely, results from recent clinical trials with antagonists have started to provide deeper insight into the pathogenic mechanisms of target indications. Therefore, rather than focusing on individual nucleic acid-sensing pathways, we discuss in the following sections how the understanding of the molecular pathophysiology and the emerging clinical experience of antagonizing nucleic acid sensors inform each other in specific target indications.

SLE as an indication for antagonists of endosomal TLRs. The rationale for developing antagonists to endosomal TLRs in order to treat lupus first emerged from the discovery that IFN-producing pDCs infiltrate the skin of patients with cutaneous lupus[67,68] and that IgG from the sera of patients with ANA-positive SLE elicited IFN secretion from human pDCs in a TLR7- and TLR9-dependent fashion[15,63]. This was further corroborated by data describing a strong genetic linkage of *TLR7* to SLE (TABLE 3). Moreover, TLR7-dependent IFNα production is significantly higher in females than in males[69], in line with the significantly higher incidence of SLE in females. This is considered to be a consequence of both oestrogen signalling and *TLR7* gene dosage, as *TLR7* is on the X chromosome[70].

Beyond pDCs, the mechanistic involvement of TLR7 and TLR9 in patients with SLE has been addressed for neutrophils and B cells. Ribonucleoprotein (RNP)-specific IgG from the sera of patients with SLE

Box 1 | **Basic principles of drug discovery**

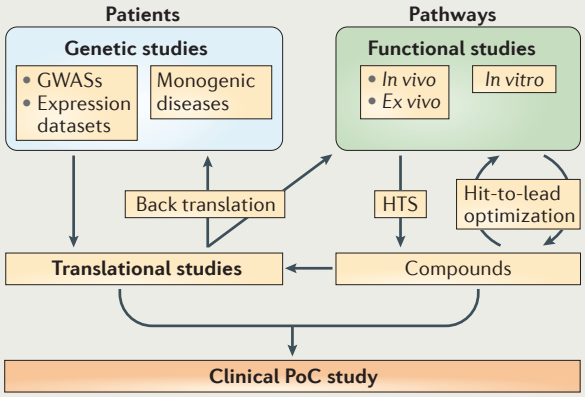
Preclinical validation of innovative drug targets relies on a combination of genetic, functional and translational studies (see the figure).

Genetic studies include both genome-wide association studies (GWASs) and correlations of gene expression levels with disease phenotype. Strong genetic data supporting a role for a disease-relevant target pathway may come from rare monogenic diseases, in which a single genetic defect drives a specific disease phenotype. In polygenic diseases, several signalling pathways may be deregulated. Therefore, functional studies are needed to identify key intervention points within these pathways, which then become molecular drug targets. Functional studies include:

- First, *in vivo* or *ex vivo* studies using mouse strains or cells in which the target molecule or pathway is genetically modified.
- Second, *in vitro* studies using the recombinant drug target or transfectants overexpressing the drug target. These studies may lead to biochemical or cellular assays that can be adapted for high-throughput screening (HTS) campaigns, either directly on the target or on a specific phenotypic outcome (phenotypic screen). Tool compounds from the screening campaign may further support functional target validation experiments *in vitro* or *in vivo*, as compounds are optimized to become safe and efficacious clinical candidates.

Translational studies are *in vitro* experiments that use compounds to study the modification of disease-relevant parameters on patient material (for example, serum or tissue biopsies). These studies help to establish biomarkers that are reflective of compound's mode of action, to support patient stratification based on pathway activity and to refine the target indication for the clinical proof-of-concept (PoC) study. At the same time, they generate an improved understanding of pathway and disease ('back translation').

Small PoC studies are the first clinical trials of novel compounds in patients. They are aimed at clinical validation of a target in indications that are defined at the molecular level. PoC studies enable rapid, science-based decisions about larger Phase II studies because they link a compound's mode of action with patient outcome. As an example, a positive PoC study for the interleukin-1β (IL-1β)-neutralizing antibody canakinumab in cryopyrin-associated periodic syndrome (CAPS) — a monogenic disease in which hyperactivation of NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasomes results in pathological overproduction of IL-1β — allowed for the expansion of clinical studies of canakinumab to other disease indications, such as juvenile idiopathic arthritis[125,126].



triggers neutrophil extracellular trap (NET) release in a TLR7-dependent manner[71]. NETs, in turn, induce IFN secretion from pDCs via TLR9 (REF. 72), particularly after complexation with autoantibodies from the sera of patients with SLE. Together, this suggests a pathogenic neutrophil–pDC axis in SLE. Mechanistic investigations in B cells suggested that DNA- or RNA-associated autoantigens co-activate B cells via the B cell receptor (BCR) and TLR9 (REF. 73) or TLR7 (REF. 74). Of note, DNA found in the plasma of patients with SLE is biased towards short, hypomethylated DNA stretches[75], which are sequences known to favour TLR9 recognition. In addition, the RNA that associates with SLE autoantigens (such as small nuclear ribonucleoprotein 70 kDa (snRNP70)) has been shown to contain uridine-rich TLR7-recognition sequences[76]. So far, a clear role for TLR3 or TLR8 (REF. 77) in patients with SLE has not yet been identified by *in vitro* or *in vivo* studies.

The above data raise the important question of which TLR selectivity profile of antagonists is optimal for therapy of IFN-associated autoimmune diseases. Data, mostly from mouse models, about the functional hierarchy of endosomal TLRs have provided some answers.

Elevated expression of *Tlr7* in lupus-prone mice led to increased levels of autoantibodies against RNA-associated antigens and accelerated autoimmunity[78]. Moreover, *Tlr7* overexpression by itself led to an autoimmune syndrome in which disease severity correlated with the gene dose of *Tlr7* (REF. 78). Conversely, TLR7 deficiency protected mice from developing lupus-like disease in several mouse models of lupus[79,80]. By contrast, knock out of *Tlr8* in mice led to spontaneous inflammation[81], and TLR9 deficiency exacerbated disease in several mouse models of lupus[62]. There are two possible explanations for these findings. First, expression of TLR8 and TLR9 may limit TLR7 expression[82,83] and pathogenic function[81,84,85]. Second, TLR9 signalling may have an anti-inflammatory role. TLR9 activation by CpG injection is protective in mouse models of arthritis[86] and diabetes[87]. In the latter system this protection was linked to CpG-dependent induction of indoleamine 2,3-dioxygenase 1 (IDO1), an enzyme that favours the induction of regulatory T (T_{Reg}) cells by tryptophan depletion[88]. Similarly, activation of human pDCs via TLR9 was shown to promote T_{Reg} cell generation[89], and activation of B cells via TLR9 promoted IL-10 secretion[90].

Together, the above data build the case for TLR7 antagonists as an effective approach for SLE therapy. In further support of TLR7 as a dominant driver of lupus, oligonucleotides that are antagonistic for TLR7, TLR8 and TLR9, or for TLR7 and TLR9 or for TLR7 all reduced lupus-like disease in animal models[91–93].

Table 3 | **Disease associations of gene mutations or autoantibodies targeting nucleic acid sensors**

Disease	Sensor	Finding	Refs
SLE	TLR3	Association of rs3775291 with SLE and T1D	136,137
	TLR7	• Elevated *TLR7* copy number is a risk factor for juvenile SLE • Oestrogen and X chromosome dosage drive TLR7 activity • Disease-associated SNPs: rs3853839 (in the 3′ UTR; correlated with increased TLR7 expression) and rs179010 (in intron 2)	70,138,139
	TLR9	rs352140 associated with renal disease:	140
	MDA5	rs1990760 associated with disease	141
Dermatomyositis and polymyositis	MDA5	MDA5-specific antibodies in a group of patients with milder disease	142
T1D	MDA5	• Disease-associated of SNPs: rs1990760 • Defective MDA5 variants associated with diabetes risk	141,143
	RIG-I	Defective RIG-I variants associated with diabetes risk	144

*Only SNPs that were reported more than once are shown. MDA5, MDA5, melanoma differentiation-associated protein 5; RIG-I, retinoic acid-inducible gene I; SLE, systemic lupus erythematosus; SNP, single-nucleotide polymorphism; T1D, type 1 diabetes; TLR, Toll-like receptor; UTR, untranslated region.

In spite of this compelling rationale for targeting endosomal TLRs in SLE, no nucleic acid sensor antagonist has so far been successfully tested in the clinic in patients with SLE. DV1179, a oligonucleotide antagonist of TLR7 and TLR9, failed to inhibit an ISG signature in clinical trials of SLE for undisclosed reasons (TABLE 2), although a related compound, IRS954, showed efficacy *in vitro*[63] and in animal models[92], and sensitized pDCs to glucocorticoid-mediated death[94]. This confirms the need for novel biomarkers for patient selection and stratification, for example, based on specific gene signatures of specific nucleic acid-sensing pathways.

TLR antagonists in autoimmune diseases and lymphoma. In a complementary approach to clinical studies in SLE, it is appealing to establish clinical efficacy and safety of novel antagonists of nucleic acid-sensing pathways in proof-of-concept trials in indications with a more uniform clinical picture (BOX 1).

Psoriasis has been proposed as a clinical entry point for antagonists of nucleic acid sensing based on the expression of an ISG signature in psoriatic lesions[95] and based on the ability of LL37 (also known as CAMP) — a polycationic peptide that is strongly upregulated in psoriasis lesions — to deliver RNA and DNA into pDCs for TLR7 and TLR9 activation, and into monocytes for TLR8 activation[14,96]. This therapeutic hypothesis was tested in clinical studies of involving the use of IMO-3100, an oligonucleotide antagonist of TLR7 and TLR9, in patients with psoriasis. This compound is a derivative of a CpG-containing TLR9 oligonucleotide that was chemically engineered to result in TLR7- and TLR9-antagonistic properties[97]. A clinical response in psoriasis was observed for IMO-3100, and the compound interfered with an IL-17 signature[98]. As recent clinical data establish that psoriasis is a strongly IL-17-dependent disease[99], this result indicates that dual antagonists of TLR7 and TLR9 modify a disease-relevant pathway. However, it has been proposed that additional TLR8 antagonism on top of TLR7 and TLR9 inhibition may enhance therapeutic efficacy[100], potentially because TLR8 signalling induces the production of IL-1β and IL-6, two cytokines that support T_H17 cell development[101]. In support of this hypothesis, a Phase II study in psoriasis using IMO-8400, an oligonucleotide that is antagonistic for TLR7, TLR8 and TLR9 (ClinicalTrials.gov identifier NCT01899729), has shown signs of a clinical response[102]. As a follow-up, clinical trials of IMO-8400 in other dermatological indications have been announced. However, it is of note that small-molecule antagonists of endosomal TLRs have not yet advanced to clinical trials.

B cell tumours such as ABC-DLBCL (diffuse large B cell lymphoma of the activated B cell type) often harbour the activating $MYD88^{L265P}$ mutation[103]. This mutation may sensitize tumour cells to tonic signalling via TLR9 (REF. 104) or TLR7. Idera has started a Phase I–II clinical trial of IMO-8400 in patients with Waldenström macroglobulinaemia who are $MYD88^{L265P}$ positive (NCT02092909). These trials are expected to provide evidence as to whether inhibition of endosomal TLRs is indeed critical to halt tumour cell proliferation and to identify which patients are most responsive to this therapy, because *in vitro* data suggest that the $MYD88^{L265P}$ mutation alone is not sufficient to sustain B cell proliferation[104].

Antagonizing cytosolic nucleic acid sensors in type I interferonopathies. The functional role of cytosolic nucleic acid-sensing pathways in IFN-associated inflammatory diseases has been addressed much more recently, and this has led to first hypotheses about the clinical use of antagonists. Type I interferonopathies[105] have recently been described as rare monogenic autoinflammatory diseases that are characterized by high expression of ISG signatures (TABLE 4). Phenotypic overlap with SLE and genetic association of some causative genes with SLE[106] suggest that type I interferonopathies probably belong to the same group of IFN-associated inflammatory diseases. They could provide a novel opportunity for proof-of-concept trials of antagonists of cytosolic nucleic acid sensors before entering complex SLE trials.

Table 4 | **Type I interferonopathies***

Disease	Mutated gene (locus name)	Proposed molecular mechanism	Symptoms	Refs
AGS	*TREX1* (*AGS1*)	Deficiency of 3′–5′ exonuclease with DNA preference leads to the accumulation of DNA in the cytosol, which activates STING, leading to increased expression of IFN and ISGs	• Inflammatory encephalopathy • Calcification of basal ganglia • Spasticity • Presence of ANAs in some patients • Chilblains • Glaucoma • Hypothyroidism • Cardiomyopathy • Increased levels of IFNs in cerebrospinal fluid • May develop SLE • (For AGS5: cerebral vasculopathy and arthropathy)	145
	RNASEH2A (*AGS4*)	Defects in the RNAseH2 complex lead to the accumulation of ribonucleotides in genomic DNA, which results in a DNA damage response and the expression of IFN and ISGs		
	RNASEH2B (*AGS2*)			
	RNASEH2C (*AGS3*)			
	SAMHD1 (*AGS5*)	Defective SAMHD1 function results in the accumulation of dNTPs, which leads to a DNA damage response and the expression of IFN and ISGs		
	ADAR1 (*AGS6*)	Defective ADAR1 function has effects on the integrity of cytosolic dsRNAs, which as a consequence become activators of MDA5		
	IFIH1	MDA5 gain of function leads to higher affinity for RNA and the expression of IFN and ISGs		
Familial chilblain lupus	*TREX1*	Similar to *AGS1*?	• Cutaneous chilblain lesions • ANAs in some patients	107
	SAMHD1	Similar to *AGS5*?		146
RVCL	*TREX1*	• *TREX1* frameshift mutation • Aberrant intracellular localization of TREX1	• Retinal vasculopathy, infarcts and calcification of the white matter • Renal or hepatic involvement possible • ISG signature debatable	147
SMS	*IFIH1*	MDA5 gain of function leads to increased expression of IFN and ISGs	• Arterial calcification • Dental inflammation • Bone resorption	148
	DDX58	RIG-I gain of function leads to increased expression of IFN and ISGs	Atypical SMS: aortic calcification, glaucoma and skeletal but no dental abnormalities	149
SAVI	*TMEM173*	Stabilized STING dimerization leads to constitutive activation and constitutive expression of IFN and ISGs	• Cutaneous vasculopathy • Pulmonary fibrosis and immune cell infiltration into the lung parenchyma • High levels of serum IFN	110
ISG15 deficiency	*ISG15*	Absence of a negative regulator of IFN signalling leads to increased expression of IFN and ISGs	• Calcification of basal ganglia • High levels of serum IFN may occur • Presence of ANAs	150
THES	*SKIV2L*	• Deficient *SKIV2L* function leads to excessive unfolded protein response • Hyperactive RIG-I activity • Increased expression of ISGs	• Facial abnormalities and hair growth defects • Severe diarrhoea • Immunodeficiency	151
CECR1 deficiency ‡	*CECR1*	Neutrophils driving endothelial damage?	• Polyarteritis nodosa • Neutrophil signature	152
SPENCD‡	*ACP5*	• *ACP5* deficiency • Downstream effects of ACP5 deficiency unknown	• Calcification of basal ganglia • Spasticity • Diverse autoimmune symptoms	153

*All of the above diseases except for RVCL show an elevated ISG signature, but not all show elevated levels of type I IFN or ANAs. ‡Primary genetic defects lie entirely outside nucleic acid-sensing or metabolism pathways. *ACP5*, tartrate-resistant acid phosphatase 5; ADAR1, dsRNA-specific adenosine deaminase; AGS, Aicardi–Goutières syndrome; ANAs, antinuclear antibodies; *DDX58*, gene encoding RIG-I; DSH, dyschromatosis symmetrica hereditaria; dsRNA, double-stranded RNA; *IFIH1*, gene encoding MDA5; IFN, interferon; ISG, IFN-stimulated gene; MDA5, melanoma differentiation-associated protein 5; RIG-I, retinoic acid-inducible gene I; RVCL, retinal vasculopathy with cerebral leukodystrophy; SAVI, STING-associated vasculitis of infancy; *SKIV2L*, superkiller viralicidic activity 2-like; SLE, systemic lupus erythematosus; SMS, Singleton–Merten syndrome; SPENCD, spondyloenchondrodysplasia; STING, stimulator of IFN genes; THES, trichohepatoenteric syndrome; TREX1, 3′ repair exonuclease 1; *TMEM173*, gene encoding STING.

For example, a subgroup of patients with AGS shows expression of a gain-of-function mutant of *IFIH1*, which encodes MDA5 (REF. 107). Similarly, constitutively active MDA5 causes an IFN-dependent inflammatory pathology in mice[108], and an increased gene dosage of *Mda5* accelerated autoimmunity in lupus-prone animals[109]. Expression of a non-functional MDA5 variant is associated with protection from dermatomyositis and type 1 diabetes (T1D; TABLE 3). In aggregate, the above results suggest that antagonists of MDA5 may be suitable anti-inflammatory agents and that patients with AGS associated with MDA5 hyperactivity could provide a clinical entry point in a molecularly defined patient cohort when such compounds become available.

Antagonists of the cGAS–STING pathway are also being considered as anti-inflammatory therapies based on observations showing that the expression of a constitutively active form of STING leads to STING-associated vasculitis of infants (SAVI), another type I interferonopathy[110] (TABLE 4). In further support of this hypothesis, STING deficiency protected mice from inflammation in models of defective DNA metabolism, such as the *Trex1*$^{-/-}$ model of myocarditis[8]. However, contrasting results were recently reported in the MRL.Fas^{lpr} mouse model of lupus, in which STING deficiency was found to exacerbate disease[111]. This was attributed to a role of STING activation in constraining TLR7 and TLR9 signalling and in enhancing *Ido1* expression and T_{Reg} cell development[111]. Another study confirmed the anti-inflammatory effect of STING activation through an IDO1-dependent mechanism[112], similar to what was observed for TLR9 activation[88]. Therefore, it will be necessary to carefully dissect which patients may benefit from antagonists of the cGAS–STING pathway at the preclinical stage. This will support the design of meaningful clinical studies when cGAS or STING antagonists are available for clinical testing.

Although patients with hyperactive MDA5 or STING may benefit from antagonists of these proteins in clinical proof-of-concept trials, it is possible that they express these proteins in an alternatively folded form and thus do not respond to antagonists that target the native proteins. This point will have to be addressed preclinically in translational studies.

Novel approaches and new challenges for the development of nucleic acid sensor antagonists. Continuing technical advances are expected to further enhance drug discovery efforts for antagonists of nucleic acid sensors. For example, the co-crystal structures of recombinant cGAS, STING, RIG-I, TLR8 and TLR9 with their ligands have recently been solved[18,19,28,51,56,113–116] and enable virtual library screens for compounds that act on the respective target. Recombinant expression of nucleic acid sensor proteins may support co-crystallization efforts with candidate compounds for potential *in silico* optimization. In addition, recombinant DNA and RNA sensor proteins allow for direct binding assays to differentiate receptor-binding compounds from compounds that affect DNA- and RNA-sensing pathways by a receptor-independent mode of action. In a recent example of TLR9 antagonists, suppressive activity was shown to depend on interaction of TLR9 antagonists with DNA and accumulation in endosomes[117].

The crosstalk of sensing pathways has recently been identified as a potential key challenge for the clinical development of antagonists of nucleic acid sensors in complex diseases. For example, it has been observed in mouse models of lupus that STING deficiency leads to enhanced responsiveness of endosomal TLRs in macrophages[111], and it will be important to evaluate whether STING inhibitors induce a similar effect in man. Recent data from animal models also suggest that different symptoms of a complex disease may be driven by distinct pathways. This is highlighted by a recent study showing that in mice that are double deficient for *Dnase2* and IFNAR1, the development of arthritis was prevented by the ablation of either STING or AIM2, whereas the concurrent production of ANAs was shown to be dependent on endosomal TLRs[118]. Another example is the observed potentiation of the cGAS–STING pathway by ultraviolet light-induced oxidation of cellular DNA[119]. As many patients with SLE are prone to developing skin lesions following sun exposure, it is possible that signalling through the STING pathway becomes particularly important during cutaneous lupus flares. Together, these data underscore the importance of dissecting complex diseases to identify the pathways that drive individual symptoms before approaching clinical studies with antagonists of nucleic acid-sensing pathways.

New knowledge on the physiology and pathophysiology of nucleic acid-sensing pathways is also guiding safety considerations for antagonists. Antagonists of endosomal TLRs can theoretically increase the risk of infections: for example, reactivation of endogenous retroviruses has been observed in mice incapable of TLR7-mediated RNA sensing[120], and human IRF7 deficiency can lead to severe influenza virus infections[121]. By contrast, patients with broad defects in TLR signalling due to MYD88 deficiency- or IL-1 receptor-associated kinase 4 (IRAK4) deficiency show only mild impairments in immunity to infections[122]. Safety studies for antagonists of cytosolic nucleic acid sensors will need to take into account the widespread expression of these sensors in somatic cells and their diverse homeostatic functions. For example, data from animal models suggest that tumour immunosurveillance relies on STING signalling[13]. Moreover, sensing of mitochondrial DNA through STING can elicit IFN-dependent inflammation following cell death in the absence of apoptotic caspases[9,123], and efficient B cell responses to T cell-independent type 2 antigens seem to rely on BCR-dependent reactivation of endogenous retroviruses and subsequent MDA5 and STING signalling[124]. These data raise the possibility that cytosolic DNA and RNA sensors regulate a mutualistic relationship of cells with their retrovirome. Although this is an exciting prospect for basic research, it could pose a challenge for the development of safe antagonists.

The field of nucleic acid sensing is currently witnessing a convergence of knowledge from molecular immunology and molecular pathology. However, the ultimate proof for our understanding of nucleic acid-sensing pathways and of related diseases will be the successful clinical development of antagonists of nucleic acid sensors.

Conclusion

As we have outlined in this Review, novel agonists and antagonists of nucleic acid-sensing pathways are anticipated to improve treatment options for a wide range of diseases, ranging from chronic infections and cancer to autoimmune disorders. However, a prerequisite for clinical success will be a better understanding of the complex molecular pathophysiology of these indications. For some cancers, molecular profiling and identification of the corresponding pathophenotypes has become a reality, and as a consequence, personalized cancer therapies are emerging. Autoimmune diseases still lack this level of molecular insight, which could be why targeted treatments for

T cell-independent type 2 antigens
Polyvalent antigens that activate B cells by efficient crosslinking of the B cell receptor (BCR), without the need of T help. They differ from T cell-independent type 1 antigens, which are polyclonal B cell stimulants that activate B cells independently of BCR ligation.

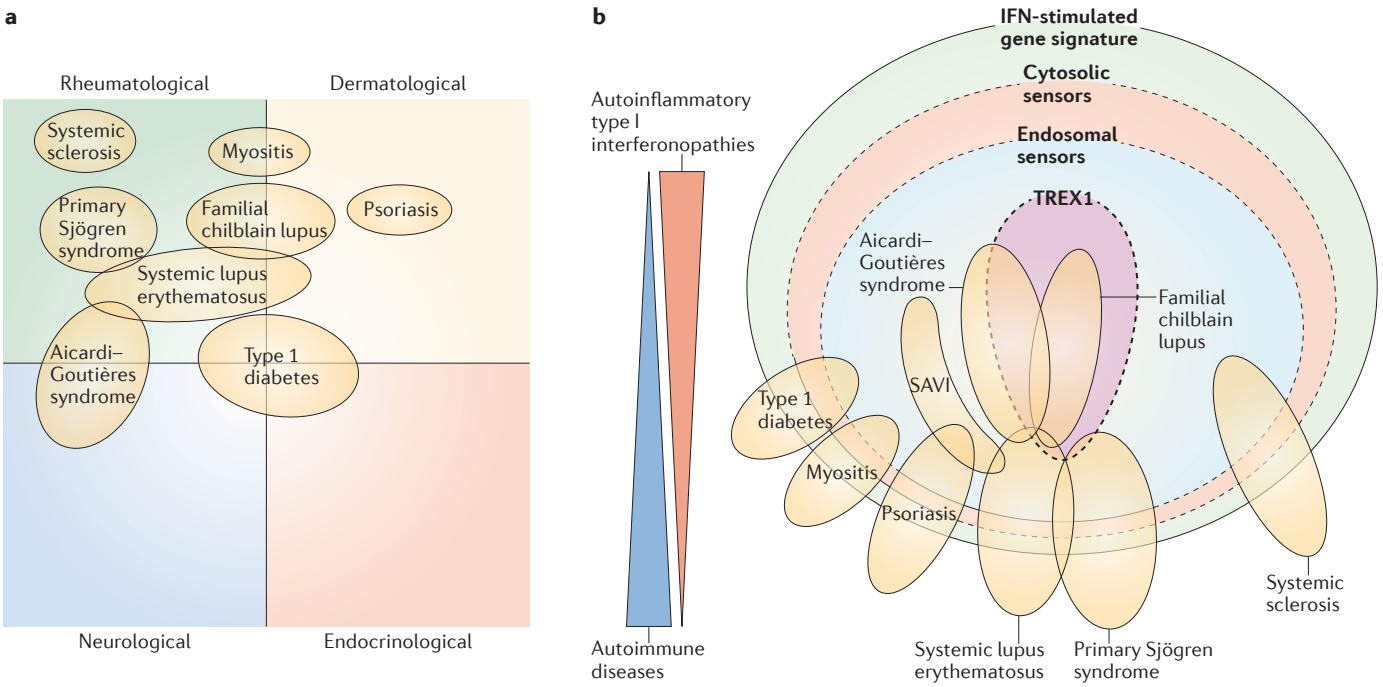


Figure 4 | **Classification of interferon-associated inflammatory diseases.** **a** | Classical disease categories according to the involved organ system. Complex indications affect different organ systems, as shown by the overlap of the yellow ovals with different quadrants. **b** | Proposed classification of indications according to pathway activity. Red, blue and purple ovals denote the activation of specific molecular pathways within the range of interferon-associated diseases (green oval). The position of the yellow ovals on this scheme denotes confirmed or proposed pathway involvement in the respective indications. Dashed lines indicate that pathway activity still awaits confirmation in the respective diseases. Overlapping pathophenotypes are shown by the overlap of yellow ovals. ISG, interferon-stimulated gene; SAVI, stimulator of interferon genes (STING)-associated vasculitis of infants; TREX1, 3′ repair exonuclease 1.

chronic inflammatory conditions are scarce. The recent definition of type I interferonopathies based on monogenic defects and ISGs has provided insight into the molecular analysis of less heterogeneous inflammatory disease phenotypes. A next step will be to understand which nucleic acid-sensing pathways are active in distinct pathophenotypes and how an ISG signature can be connected to such diverse symptoms. Will the connection between the activity of nucleic acid-sensing pathways and specific symptoms be the same in monogenic type I interferonopathies as in the more complex autoimmune diseases? Specific compounds that target nucleic acid-sensing pathways will help in this deconvolution challenge, as they provide tools to functionally probe pathway activity in patient samples. This knowledge about pathogenic pathways will support patient stratification for more targeted interventions and may eventually lead to a new functional definition of inflammatory diseases (FIG. 4). Therefore, drug discovery for nucleic acid sensors is an endeavour that promotes our understanding of complex pathway biology and targets the as-of-yet unmet medical needs of patients suffering from complex diseases.

1. Theofilopoulos, A. N., Baccala, R., Beutler, B. & Kono, D. H. Type I interferons (α/β) in immunity and autoimmunity. *Annu. Rev. Immunol.* **23**, 307–336 (2005).
2. Egli, A., Santer, D. M., O'Shea, D., Tyrrell, D. L. & Houghton, M. The impact of the interferon-λ family on the innate and adaptive immune response to viral infections. *Emerg. Microbes Infect.* **3**, e51 (2014).
3. Schneider, W. M., Chevillotte, M. D. & Rice, C. M. Interferon-stimulated genes: a complex web of host defenses. *Annu. Rev. Immunol.* **32**, 513–545 (2014).
4. Pichlmair, A. & Reis, E. Sousa, C. Innate recognition of viruses. *Immunity* **27**, 370–383 (2007).
5. Garcīa-Sastre, A. 2 methylate or not 2 methylate: viral evasion of the type I interferon response. *Nat. Immunol.* **12**, 114–115 (2011).
6. Morita, M. *et al.* Gene-targeted mice lacking the trex1 (DNase III) 3′–5′ DNA exonuclease develop inflammatory myocarditis. *Mol. Cell. Biol.* **24**, 6719–6727 (2004).
7. Yoshida, H., Okabe, Y., Kawane, K., Fukuyama, H. & Nagata, S. Lethal anemia caused by interferon-β produced in mouse embryos carrying undigested DNA. *Nat. Immunol.* **6**, 49–56 (2005).
8. Ahn, J., Ruiz, P. & Barber, G. N. Intrinsic self-DNA triggers inflammatory disease dependent on STING. *J. Immunol.* **193**, 4634–4642 (2014).
9. Rongvaux, A. *et al.* Apoptotic caspases prevent the induction of type I interferons by mitochondrial DNA. *Cell* **159**, 1563–1577 (2014).
10. Lan, Y. Y., Londono, D., Bouley, R., Rooney, M. S. & Hacohen, N. Dnase2a deficiency uncovers lysosomal clearance of damaged nuclear DNA via autophagy. *Cell Rep.* **9**, 180–192 (2014).
11. Chan, M. P. *et al.* DNase II-dependent DNA digestion is required for DNA sensing by TLR9. *Nat. Commun.* **6**, 5853 (2015).
12. Pawaria, S. *et al.* Cutting edge: DNase II deficiency prevents activation of autoreactive B cells by double-stranded DNA endogenous ligands. *J. Immunol.* **194**, 1403–1407 (2015).
13. Woo, S.-R. *et al.* STING-dependent cytosolic DNA sensing mediates innate immune recognition of immunogenic tumors. *Immunity* **41**, 830–842 (2014).
This study was the first to indicate a crucial role for cytosolic sensing of self DNA in tumour surveillance.
14. Lande, R. *et al.* Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. *Nature* **449**, 564–569 (2007).
This was the first paper to demonstrate that an antimicrobial peptide from psoriatic skin shuttles DNA into cells and induces type I IFN release via TLR9.
15. Lovgren, T., Eloranta, M. L., Bave, U., Alm, G. V. & Ronnblom, L. Induction of interferon-α production in plasmacytoid dendritic cells by immune complexes containing nucleic acid released by necrotic or late apoptotic cells and lupus IgG. *Arthritis Rheum.* **50**, 1861–1872 (2004).
This paper demonstrates that sera from patients with SLE can form immune complexes with necrotic material and induce type I IFN release from pDCs.
16. Bave, U. *et al.* Activation of the type I interferon system in primary Sjogren's syndrome: a possible etiopathogenic mechanism. *Arthritis Rheum.* **52**, 1185–1195 (2005).

17. Blasius, A. L. & Beutler, B. Intracellular toll-like receptors. *Immunity* **32**, 305–315 (2010).
18. Tanji, H. *et al.* Toll-like receptor 8 senses degradation products of single-stranded RNA. *Nat. Struct. Mol. Biol.* **22**, 109–115 (2015).
19. Ohto, U. *et al.* Structural basis of CpG and inhibitory DNA recognition by Toll-like receptor 9. *Nature* **520**, 702–705 (2015).
References 18 and 19 provided the first structural insight into unexpected aspects of single-stranded nucleic acid ligand binding by TLR8 and TLR9, respectively. TLRs have proven notoriously difficult to express and crystallize.
20. Hornung, V. SnapShot: nucleic acid immune sensors, part 1. *Immunity* **41**, 868–868.e1 (2014).
21. Hornung, V. *et al.* 5′-triphosphate RNA is the ligand for RIG-I. *Science* **314**, 994–997 (2006).
22. Goubau, D. *et al.* Antiviral immunity via RIG-I-mediated recognition of RNA bearing 5′-diphosphates. *Nature* **514**, 372–375 (2014).
23. Kato, H. *et al.* Length-dependent recognition of double-stranded ribonucleic acids by retinoic acid-inducible gene-I and melanoma differentiation-associated gene 5. *J. Exp. Med.* **205**, 1601–1610 (2008).
24. Pichlmair, A. *et al.* Activation of MDA5 requires higher-order RNA structures generated during virus infection. *J. Virol.* **83**, 10761–10769 (2009).
25. Hou, F. *et al.* MAVS forms functional prion-like aggregates to activate and propagate antiviral innate immune response. *Cell* **146**, 448–461 (2011).
26. Sun, L., Wu, J., Du, F., Chen, X. & Chen, Z. J. Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. *Science* **339**, 786–791 (2013).
27. Wu, J. *et al.* Cyclic GMP–AMP is an endogenous second messenger in innate immune signaling by cytosolic DNA. *Science* **339**, 826–830 (2013).
References 26 and 27 report the seminal discovery of the cytosolic DNA receptor, as well as the small-molecule second messenger, that links to STING activation.
28. Gao, P. *et al.* Cyclic [G(2′,5′)pA(3′,5′)p] is the metazoan second messenger produced by DNA-activated cyclic GMP-AMP synthase. *Cell* **153**, 1094–1107 (2013).
29. Burdette, D. L. *et al.* STING is a direct innate immune sensor of cyclic di-GMP. *Nature* **478**, 515–518 (2011).
30. Jin, L. *et al.* MPYS is required for IFN response factor 3 activation and type I IFN production in the response of cultured phagocytes to bacterial second messengers cyclic-di-AMP and cyclic-di-GMP. *J. Immunol.* **187**, 2595–2601 (2011).
31. Liu, S. *et al.* Phosphorylation of innate immune adaptor proteins MAVS, STING, and TRIF induces IRF3 activation. *Science* **347**, aaa2630 (2015).
This study highlights a previously unappreciated role for adaptor phosphorylation as an integral step in the activation of nucleic acid signalling pathways.
32. Latz, E., Xiao, T. S. & Stutz, A. Activation and regulation of the inflammasomes. *Nat. Rev. Immunol.* **13**, 397–411 (2013).
33. Huen, A. O. & Rook, A. H. Toll receptor agonist therapy of skin cancer and cutaneous T-cell lymphoma. *Curr. Opin. Oncol.* **26**, 237–244 (2014).
34. Kasturi, S. P. *et al.* Programming the magnitude and persistence of antibody responses with innate immunity. *Nature* **470**, 543–547 (2011).
35. Kastenmüller, K. *et al.* Protective T cell immunity in mice following protein–TLR7/8 agonist-conjugate immunization requires aggregation, type I IFN, and multiple DC subsets. *J. Clin. Invest.* **121**, 1782–1796 (2011).
36. Ilyinskii, P. O. *et al.* Adjuvant-carrying synthetic vaccine particles augment the immune response to encapsulated antigen and exhibit strong local immune activation without inducing systemic cytokine release. *Vaccine* **32**, 2882–2895 (2014).
37. Kastenmuller, W., Kastenmüller, K., Kurts, C. & Seder, R. A. Dendritic cell-targeted vaccines — hope or hype? *Nat. Rev. Immunol.* **14**, 705–711 (2014).
38. Field, A. K., Tytell, A. A., Lampson, G. P. & Hilleman, M. R. Inducers of interferon and host resistance. II. Multistranded synthetic polynucleotide complexes. *Proc. Natl Acad. Sci. USA* **58**, 1004–1010 (1967).
39. Alexopoulou, L., Holt, A. C., Medzhitov, R. & Flavell, R. A. Recognition of double-stranded RNA and activation of NF-κB by Toll-like receptor 3. *Nature* **413**, 732–738 (2001).
40. Gitlin, L. *et al.* Essential role of mda-5 in type I IFN responses to polyriboinosinic:polyribocytidylic acid and encephalomyocarditis picornavirus. *Proc. Natl Acad. Sci. USA* **103**, 8459–8464 (2006).
41. Caskey, M. *et al.* Synthetic double-stranded RNA induces innate immune responses similar to a live viral vaccine in humans. *J. Exp. Med.* **208**, 2357–2366 (2011).
42. Ammi, R. *et al.* Poly(I:C) as cancer vaccine adjuvant: knocking on the door of medical breakthroughs. *Pharmacol. Ther.* **140**, 120–131 (2014).
43. Strayer, D. R. *et al.* A double-blind, placebo-controlled, randomized, clinical trial of the TLR-3 agonist rintatolimod in severe cases of chronic fatigue syndrome. *PLoS ONE* **7**, e31334 (2012).
44. Hemmi, H. *et al.* Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. *Nat. Immunol.* **3**, 196–200 (2002).
45. Baguley, B. C. & Ching, L. M. DMXAA: an antivascular agent with multiple host responses. *Int. J. Radiat. Oncol. Biol. Phys.* **54**, 1503–1511 (2002).
46. Lara, P. N. *et al.* Randomized Phase III placebo-controlled trial of carboplatin and paclitaxel with or without the vascular disrupting agent vadimezan (ASA404) in advanced non-small-cell lung cancer. *J. Clin. Oncol.* **29**, 2965–2971 (2011).
47. Conlon, J. *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.* **190**, 5216–5225 (2013).
48. Gao, P. *et al.* Binding-pocket and lid-region substitutions render human STING sensitive to the species-specific drug DMXAA. *Cell Rep.* **8**, 1668–1676 (2014).
49. Karaolis, D. K. R. *et al.* Bacterial c-di-GMP is an immunostimulatory molecule. *J. Immunol.* **178**, 2171–2181 (2007).
50. Dubensky, T. W., Kanne, D. B. & Leong, M. L. Rationale, progress and development of vaccines utilizing STING-activating cyclic dinucleotide adjuvants. *Ther. Adv. Vaccines* **1**, 131–143 (2013).
51. Gao, P. *et al.* Structure–function analysis of STING activation by c[G(2′,5′)pA(3′,5′)p] and targeting by antiviral DMXAA. *Cell* **154**, 748–762 (2013).
52. Li, X.-D. *et al.* Pivotal roles of cGAS–cGAMP signaling in antiviral defense and immune adjuvant effects. *Science* **341**, 1390–1394 (2013).
53. Krieg, A. M. *et al.* CpG motifs in bacterial DNA trigger direct B-cell activation. *Nature* **374**, 546–549 (1995).
54. Scheiermann, J. & Klinman, D. M. Clinical evaluation of CpG oligonucleotides as adjuvants for vaccines targeting infectious diseases and cancer. *Vaccine* **32**, 6377–6389 (2014).
55. Bedard, K. M. *et al.* Isoflavone agonists of IRF-3 dependent signaling have antiviral activity against RNA viruses. *J. Virol.* **86**, 7334–7344 (2012).
56. Wang, Y. *et al.* Structural and functional insights into 5′-ppp RNA pattern recognition by the innate immune receptor RIG-I. *Nat. Struct. Mol. Biol.* **17**, 781–787 (2010).
57. Schlee, M. *et al.* Recognition of 5′ triphosphate by RIG-I helicase requires short blunt double-stranded RNA as contained in panhandle of negative-strand virus. *Immunity* **31**, 25–34 (2009).
58. Querec, T. *et al.* Yellow fever vaccine YF-17D activates multiple dendritic cell subsets via TLR2, 7, 8, and 9 to stimulate polyvalent immunity. *J. Exp. Med.* **203**, 413–424 (2006).
59. Coffman, R. L., Sher, A. & Seder, R. A. Vaccine adjuvants: putting innate immunity to work. *Immunity* **33**, 492–503 (2010).
60. Goff, P. H. *et al.* Synthetic TLR4 and TLR7 ligands as influenza virus vaccine adjuvants induce rapid, sustained and broadly protective responses. *J. Virol.* **89**, 3221–3335 (2015).
61. Temizoz, B. *et al.* TLR9 and STING agonists synergistically induce innate and adaptive type II IFN. *Eur. J. Immunol.* **45**, 1159–1169 (2015).
62. Marshak-Rothstein, A. & Rifkin, I. R. Immunologically active autoantigens: the role of toll-like receptors in the development of chronic inflammatory disease. *Annu. Rev. Immunol.* **25**, 419–441 (2007).
A comprehensive review of the role of endosomal TLRs in SLE, with a particular focus on B cell biology.
63. Barrat, F. J. *et al.* Nucleic acids of mammalian origin can act as endogenous ligands for Toll-like receptors and may promote systemic lupus erythematosus. *J. Exp. Med.* **202**, 1131–1139 (2005).
64. Kuznik, A. *et al.* Mechanism of endosomal TLR inhibition by antimalarial drugs and imidazoquinolines. *J. Immunol.* **186**, 4794–4804 (2011).
65. Sun, X. *et al.* Increased ribonuclease expression reduces inflammation and prolongs survival in TLR7 transgenic mice. *J. Immunol.* **190**, 2536–2543 (2013).
66. Kalunian, K. *et al.* Efficacy and safety of rontalizumab (anti-interferon α) in SLE subjects with restricted immunosuppressant use: results of a randomized, double-blind, placebo-controlled Phase 2 study. *Arthritis Rheumatol. Abstr. Suppl.* **64**, S1111 (2012).
A conference abstract showing that ISGs can be used for patient stratification in clinical studies.
67. Blomberg, S. *et al.* Presence of cutaneous interferon-α producing cells in patients with systemic lupus erythematosus. *Lupus* **10**, 484–490 (2001).
68. Farkas, L., Beiske, K., Lund-Johansen, F., Brandtzaeg, P. & Jahnsen, F. L. Plasmacytoid dendritic cells (natural interferon- α/β-producing cells) accumulate in cutaneous lupus erythematosus lesions. *Am. J. Pathol.* **159**, 237–243 (2001).
69. Berghofer, B. *et al.* TLR7 ligands induce higher IFN-α production in females. *J. Immunol.* **177**, 2088–2096 (2006).
70. Laffont, S. *et al.* X-chromosome complement and estrogen receptor signaling independently contribute to the enhanced TLR7-mediated IFN-α production of plasmacytoid dendritic cells from women. *J. Immunol.* **193**, 5444–5452 (2014).
71. Garcia-Romo, G. S. *et al.* Netting neutrophils are major inducers of type I IFN production in pediatric systemic lupus erythematosus. *Sci. Transl Med.* **3**, 73ra20 (2011).
72. Lande, R. *et al.* Neutrophils activate plasmacytoid dendritic cells by releasing self-DNA-peptide complexes in systemic lupus erythematosus. *Sci. Transl Med.* **3**, 73ra19 (2011).
73. Leadbetter, E. A. *et al.* Chromatin–IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. *Nature* **416**, 603–607 (2002).
74. Lau, C. *et al.* RNA-associated autoantigens activate B cells by combined B cell antigen receptor/Toll-like receptor 7 engagement. *J. Exp. Med.* **202**, 1171–1177 (2005).
75. Chan, R. W. *et al.* Plasma DNA aberrations in systemic lupus erythematosus revealed by genomic and methylomic sequencing. *Proc. Natl Acad. Sci. USA* **111**, E5302–E5311 (2014).
76. Vollmer, J. *et al.* Immune stimulation mediated by autoantigen binding sites within small nuclear RNAs involves Toll-like receptors 7 and 8. *J. Exp. Med.* **202**, 1575–1585 (2005).
77. Annable, T. *et al.* Using poly I:C as an adjuvant does not induce or exacerbate models of systemic lupus erythematosus. *Autoimmunity* **48**, 29–39 (2015).
78. Deane, J. A. *et al.* Control of TLR7 expression is essential to restrict autoimmunity and dendritic cell proliferation. *Immunity* **27**, 801–810 (2007).
79. Christensen, S. R. *et al.* Toll-like receptor 7 and TLR9 dictate autoantibody specificity and have opposing inflammatory and regulatory roles in a murine model of lupus. *Immunity* **25**, 417–428 (2006).
80. Savarese, E. *et al.* Requirement of Toll-like receptor 7 for pristane-induced production of autoantibodies and development of murine lupus nephritis. *Arthritis Rheum.* **58**, 1107–1115 (2008).
81. Demaria, O. *et al.* TLR8 deficiency leads to autoimmunity in mice. *J. Clin. Invest.* **120**, 3651–3662 (2010).
82. Wang, J. *et al.* The functional effects of physical interactions among Toll-like receptors 7, 8, and 9. *J. Biol. Chem.* **281**, 37427–37434 (2006).
83. Fukui, R. *et al.* Unc93B1 restricts systemic lethal inflammation by orchestrating Toll-like receptor 7 and 9 trafficking. *Immunity* **35**, 69–81 (2011).
84. Nickerson, K. M. *et al.* TLR9 regulates TLR7- and MyD88-dependent autoantibody production and disease in a murine model of lupus. *J. Immunol.* **184**, 1840–1848 (2010).
85. Desnues, B. *et al.* TLR8 on dendritic cells and TLR9 on B cells restrain TLR7-mediated spontaneous autoimmunity in C57BL/6 mice. *Proc. Natl Acad. Sci. USA* **111**, 1497–1502 (2014).
86. Wu, H. J. *et al.* Inflammatory arthritis can be reined in by CpG-induced DC–NK cell cross talk. *J. Exp. Med.* **204**, 1911–1922 (2007).
87. Fallarino, F. *et al.* IDO mediates TLR9-driven protection from experimental autoimmune diabetes. *J. Immunol.* **183**, 6303–6312 (2009).
88. Wingender, G. *et al.* Systemic application of CpG-rich DNA suppresses adaptive T cell immunity via induction of IDO. *Eur. J. Immunol.* **36**, 12–20 (2006).
89. Moseman, E. A. *et al.* Human plasmacytoid dendritic cells activated by CpG oligodeoxynucleotides induce the generation of $CD4^+CD25^+$ regulatory T cells. *J. Immunol.* **173**, 4433–4442 (2004).

90. Miles, K. *et al.* A tolerogenic role for Toll-like receptor 9 is revealed by B-cell interaction with DNA complexes expressed on apoptotic cells. *Proc. Natl Acad. Sci. USA* **109**, 887–892 (2012).
91. Pawar, R. D. *et al.* Inhibition of Toll-like receptor-7 (TLR-7) or TLR-7 plus TLR-9 attenuates glomerulonephritis and lung injury in experimental lupus. *J. Am. Soc. Nephrol.* **18**, 1721–1731 (2007).
92. Barrat, F. J., Meeker, T., Chan, J. H., Guiducci, C. & Coffman, R. L. Treatment of lupus-prone mice with a dual inhibitor of TLR7 and TLR9 leads to reduction of autoantibody production and amelioration of disease symptoms. *Eur. J. Immunol.* **37**, 3582–3586 (2007).
93. Zhu, F. G. *et al.* A novel antagonist of Toll-like receptors 7, 8 and 9 suppresses lupus disease-associated parameters in NZBW/F1 mice. *Autoimmunity* **46**, 419–428 (2013).
94. Guiducci, C. *et al.* TLR recognition of self nucleic acids hampers glucocorticoid activity in lupus. *Nature* **465**, 937–941 (2010).
95. van der Fits, L., van der Wel, L. I., Laman, J. D., Prens, E. P. & Verschuren, M. C. In psoriasis lesional skin the type I interferon signaling pathway is activated, whereas interferon-α sensitivity is unaltered. *J. Investig. Dermatol.* **122**, 51–60 (2004).
96. Ganguly, D. *et al.* Self-RNA-antimicrobial peptide complexes activate human dendritic cells through TLR7 and TLR8. *J. Exp. Med.* **206**, 1983–1994 (2009).
97. Yu, D. *et al.* Modifications incorporated in CpG motifs of oligodeoxynucleotides lead to antagonist activity of Toll-like receptors 7 and 9. *J. Med. Chem.* **52**, 5108–5114 (2009).
98. Suarez-Farinas, M. *et al.* Treatment of psoriasis patients with IMO-3100 shows improvement in gene expression patterns of meta-analysis derived-3 transcriptome and IL-17 pathway. *Arthritis Rheumatol. Abstr. Suppl.* **65**, S495 (2013).
99. Langley, R. G. *et al.* Secukinumab in plaque psoriasis — results of two Phase 3 trials. *N. Engl. J. Med.* **371**, 326–338 (2014).
100. Suarez-Farinas, M. *et al.* Suppression of molecular inflammatory pathways by Toll-like receptor 7, 8, and 9 antagonists in a model of IL-23-induced skin inflammation. *PLoS ONE* **8**, e84634 (2013).
101. Acosta-Rodriguez, E. V., Napolitani, G., Lanzavecchia, A. & Sallusto, F. Interleukins 1β and 6 but not transforming growth factor-β are essential for the differentiation of interleukin 17-producing human T helper cells. *Nat. Immunol.* **8**, 942–949 (2007).
102. Balak, D. M. W. *et al.* Results from a randomized, double-blind, placebo-controlled, monotherapy trial of IMO-8400 demonstrate clinical proof-of-concept for Toll-like receptor 7. 8 and 9 antagonism in psoriasis (poster). *73rd Annual Meeting of the American Academy of Dermatology* **1805** (2015).
103. Ngo, V. N. *et al.* Oncogenically active *MYD88* mutations in human lymphoma. *Nature* **470**, 115–119 (2011).
104. Wang, J. Q., Jeelall, Y. S., Beutler, B., Horikawa, K. & Goodnow, C. C. Consequences of the recurrent *MYD88*^{L265P} somatic mutation for B cell tolerance. *J. Exp. Med.* **211**, 413–426 (2014).
105. Crow, Y. J. Type I interferonopathies: Mendelian type I interferon up-regulation. *Curr. Opin. Immunol.* **32**, 7–12 (2015).
106. Lee-Kirsch, M. A. *et al.* Mutations in the gene encoding the 3′–5′ DNA exonuclease TREX1 are associated with systemic lupus erythematosus. *Nat. Genet.* **39**, 1065–1067 (2007).
107. Rice, G. I. *et al.* Gain-of-function mutations in *IFIH1* cause a spectrum of human disease phenotypes associated with upregulated type I interferon signaling. *Nat. Genet.* **46**, 503–509 (2014).
108. Funabiki, M. *et al.* Autoimmune disorders associated with gain of function of the intracellular sensor MDA5. *Immunity* **40**, 199–212 (2014).
109. Crampton, S. P., Deane, J. A., Feigenbaum, L. & Bolland, S. *Ifih1* gene dose effect reveals MDA5-mediated chronic type I *IFN* gene signature, viral resistance, and accelerated autoimmunity. *J. Immunol.* **188**, 1451–1459 (2012).
110. Liu, Y. *et al.* Activated STING in a vascular and pulmonary syndrome. *N. Engl. J. Med.* **371**, 507–518 (2014).
111. Sharma, S. *et al.* Suppression of systemic autoimmunity by the innate immune adaptor STING. *Proc. Natl Acad, Sci. USA* **112**, E710–E717 (2015).
A provocative study showing that STING deficiency in mice unexpectedly exacerbates lupus-like disease.
112. Huang, L. *et al.* Cutting edge: DNA sensing via the STING adaptor in myeloid dendritic cells induces potent tolerogenic responses. *J. Immunol.* **191**, 3509–3513 (2013).
113. Zhang, X. *et al.* Cyclic GMP-AMP containing mixed phosphodiester linkages is an endogenous high-affinity ligand for STING. *Mol. Cell* **51**, 226–235 (2013).
114. Civril, F. *et al.* Structural mechanism of cytosolic DNA sensing by cGAS. *Nature* **498**, 332–337 (2013).
115. Diner, E. J. *et al.* The innate immune DNA sensor cGAS produces a noncanonical cyclic dinucleotide that activates human STING. *Cell Rep.* **3**, 1355–1361 (2013).
116. Kranzusch, P. J., Lee, A. S.-Y., Berger, J. M. & Doudna, J. A. Structure of human cGAS reveals a conserved family of second-messenger enzymes in innate immunity. *Cell Rep.* **3**, 1362–1368 (2013).
117. Lamphier, M. *et al.* Novel small molecule inhibitors of TLR7 and TLR9: mechanism of action and efficacy *in vivo*. *Mol. Pharmacol.* **85**, 429–440 (2014).
118. Baum, R. *et al.* Cutting edge: AIM2 and endosomal TLRs differentially regulate arthritis and autoantibody production in DNase II-deficient mice. *J. Immunol.* **194**, 873–877 (2015).
119. Gehrke, N. *et al.* Oxidative damage of DNA confers resistance to cytosolic nuclease TREX1 degradation and potentiates STING-dependent immune sensing. *Immunity* **39**, 482–495 (2013).
120. Yu, P. *et al.* Nucleic acid-sensing toll-like receptors are essential for the control of endogenous retrovirus viremia and ERV-induced tumors. *Immunity* **37**, 867–879 (2012).
121. Ciancanelli, M. J. *et al.* Infectious disease. Life-threatening influenza and impaired interferon amplification in human IRF7 deficiency. *Science* **348**, 448–453 (2015).
122. Alsina, L. *et al.* A narrow repertoire of transcriptional modules responsive to pyogenic bacteria is impaired in patients carrying loss-of-function mutations in *MYD88* or *IRAK4*. *Nat. Immunol.* **15**, 1134–1142 (2014).
123. White, M. J. *et al.* Apoptotic caspases suppress mtDNA-induced STING-mediated type I IFN production. *Cell* **159**, 1549–1562 (2014).
124. Zeng, M. *et al.* MAVS, cGAS, and endogenous retroviruses in T-independent B cell responses. *Science* **346**, 1486–1492 (2014).
125. Kuemmerle-Deschner, J. B. *et al.* Canakinumab (ACZ885, a fully human IgG1 anti-IL-1β mAb) induces sustained remission in pediatric patients with cryopyrin-associated periodic syndrome (CAPS). *Arthritis Res. Ther.* **13**, R34 (2011).
126. Ruperto, N. *et al.* Two randomized trials of canakinumab in systemic juvenile idiopathic arthritis. *N. Engl. J. Med.* **367**, 2396–2406 (2012).
127. West, A. P. *et al.* Mitochondrial DNA stress primes the antiviral innate immune response. *Nature* **520**, 553–557 (2015).
References 9, 123 and 127 uncover physiological circumstances in which mitochondrial DNA is released as a cell-intrinsic danger signal leading to cGAS-mediated immune activation.
128. Khamashta, M. *et al.* Safety and efficacy of sifalimumab, an anti IFN-α monoclonal antibody, in a Phase 2b study of moderate to severe systemic lupus erythematosus (SLE). *Arthritis Rheum.***66**, 3531 (2014).
129. Morehouse, C. *et al.* Target modulation of a type I interferon (IFN) gene signature with sifalimumab or anifrolumab in systemic lupus erythematosis (SLE) patients in two open label Phase 2 Japanese trials. *Arthritis Rheumatol. Abstr. Suppl.* **66**, S313–S314 (2014).
130. Lauwerys, B. R. *et al.* Down-regulation of interferon signature in systemic lupus erythematosus patients by active immunization with interferon α-kinoid. *Arthritis Rheum.* **65**, 447–456 (2013).
131. Zimmer, R., Scherbarth, H. R., Rillo, O. L., Gomez-Reino, J. J. & Muller, S. Lupuzor/P140 peptide in patients with systemic lupus erythematosus: a randomised, double-blind, placebo-controlled Phase IIb clinical trial. *Ann. Rheum. Dis.* **72**, 1830–1835 (2013).
132. Reilly, S. M. *et al.* An inhibitor of the protein kinases TBK1 and IKK-ε improves obesity-related metabolic dysfunctions in mice. *Nat. Med.* **19**, 313–321 (2013).
133. Chaudhary, D., Robinson, S. & Romero, D. L. Recent advances in the discovery of small molecule inhibitors of interleukin-1 receptor-associated kinase 4 (IRAK4) as a therapeutic target for inflammation and oncology disorders. *J. Med. Chem.* **58**, 96–110 (2015).
134. Balasubramanian, W. R. *et al.* Novel IRAK-4 inhibitors exhibit highly potent anti-proliferative activity in DLBCL cell lines with activating *MYD88 L265P* mutation. *AACR Annual Meeting Abstract* **3646** (2015).
135. Vajda, E. G., Niecestro, R., Zhi, L. & Marschke, K. B. IRAK4 inhibitors display synergistic activity when combined with BTK or PI3K inhibitors in B cell lymphomas. *AACR Annual Meeting Abstract* **785** (2015).
136. Laska, M. J. *et al.* Polymorphisms within Toll-like receptors are associated with systemic lupus erythematosus in a cohort of Danish females. *Rheumatol.* **53**, 48–55 (2014).
137. Assmann, T. S., Brondani Lde, A., Bauer, A. C., Canani, L. H. & Crispim, D. Polymorphisms in the *TLR3* gene are associated with risk for type 1 diabetes mellitus. *Eur. J. Endocrinol.* **170**, 519–527 (2014).
138. Garcia-Ortiz, H. *et al.* Association of *TLR7* copy number variation with susceptibility to childhood-onset systemic lupus erythematosus in Mexican population. *Ann. Rheum. Dis.* **69**, 1861–1865 (2010).
139. Kawasaki, A. *et al. TLR7* single-nucleotide polymorphisms in the 3′ untranslated region and intron 2 independently contribute to systemic lupus erythematosus in Japanese women: a case-control association study. *Arthritis Res. Ther.* **13**, R41 (2011).
140. Piotrowski, P., Lianeri, M., Wudarski, M., Olesinska, M. & Jagodzinski, P. P. Contribution of toll-like receptor 9 gene single-nucleotide polymorphism to systemic lupus erythematosus. *Rheumatol. Int.* **33**, 1121–1125 (2013).
141. Cen, H. *et al.* Association of *IFIH1* rs1990760 polymorphism with susceptibility to autoimmune diseases: a meta-analysis. *Autoimmunity* **46**, 455–462 (2013).
142. Tansley, S. L. *et al.* Anti-MDA5 autoantibodies in juvenile dermatomyositis identify a distinct clinical phenotype: a prospective cohort study. *Arthritis Res. Ther.* **16**, R138 (2014).
143. Nejentsev, S., Walker, N., Riches, D., Egholm, M. & Todd, J. A. Rare variants of *IFIH1*, a gene implicated in antiviral responses, protect against type 1 diabetes. *Science* **324**, 387–389 (2009).
144. Shigemoto, T. *et al.* Identification of loss of function mutations in human genes encoding RIG-I and mda5: implications for resistance to type I diabetes. *J. Biol. Chem.* **284**, 13348–13354 (2009).
145. Crow, Y. J. *et al.* Characterization of human disease phenotypes associated with mutations in *TREX1*, *RNASEH2A*, *RNASEH2B*, *RNASEH2C*, *SAMHD1*, *ADAR*, and *IFIH1*. *Am. J. Med. Genet. A* **167A**, 296–312 (2015).
A comprehensive review on AGS and its underlying genetic causes.
146. Ravenscroft, J. C., Suri, M., Rice, G. I., Szynkiewicz, M. & Crow, Y. J. Autosomal dominant inheritance of a heterozygous mutation in *SAMHD1* causing familial chilblain lupus. *Am. J. Med. Genet. A* **155A**, 235–237 (2011).
147. Richards, A. *et al.* C-terminal truncations in human 3′–5′ DNA exonuclease TREX1 cause autosomal dominant retinal vasculopathy with cerebral leukodystrophy. *Nat. Genet.* **39**, 1068–1070 (2007).
148. Rutsch, F. *et al.* A specific *IFIH1* gain-of-function mutation causes Singleton–Merten syndrome. *Am. J. Hum. Genet.* **96**, 275–282 (2015).
149. Jang, M. A. *et al.* Mutations in *DDX58*, which encodes RIG-I, cause atypical Singleton–Merten syndrome. *Am. J. Hum. Genet.* **96**, 266–274 (2015).
150. Zhang, X. *et al.* Human intracellular ISG15 prevents interferon-α/β over-amplification and auto-inflammation. *Nature* **517**, 89–93 (2015).
151. Eckard, S. C. *et al.* The SKIV2L RNA exosome limits activation of the RIG-I-like receptors. *Nat. Immunol.* **15**, 839–845 (2014).
152. Belot, A. *et al.* Mutations in *CECR1* associated with a neutrophil signature in peripheral blood. *Pediatr. Rheumatol. Online J.* **12**, 44 (2014).
153. Briggs, T. A. *et al.* Tartrate-resistant acid phosphatase deficiency causes a bone dysplasia with autoimmunity and a type I interferon expression signature. *Nat. Genet.* **43**, 127–131 (2011).
154. Schuberth-Wagner, C. *et al.* A conserved histidine in the RNA sensor RIG-I controls immune tolerance to N1-2′O-methylated self RNA. *Immunity* **43**, 41–51 (2015).
155. Mannion N. M. *et al.* The RNA-editing enzyme ADAR1 controls innate immune responses to RNA. *Cell Reports* **9**, 1482–1494 (2015).
156. Liddicoat B.J. *et al.* RNA editing by ADAR1 prevents MDA5 sensing of endogenous dsRNA as nonself. *Science* http://dx.doi.org/10.1126/science.aac7049 (2015).

Acknowledgements
The authors thank E. Bartok, J. Deane, M. Hasan, P. Lötscher, D. Patel, A. Marshak-Rothstein and A. Weber for discussion and helpful suggestions relating to the manuscript.

Competing interests statement
The authors declare competing interests: see Web version for details.

FURTHER INFORMATION
ClinicalTrials.gov: https://clinicaltrials.gov/

ALL LINKS ARE ACTIVE IN THE ONLINE PDF