Type I interferon–mediated autoimmune diseases: pathogenesis, diagnosis and targeted therapy

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Abstract

Type I interferons (IFN-Is) are a group of molecules with pleiotropic effects on the immune system forming a crucial link between innate and adaptive immune responses. Apart from their important role in antiviral immunity, IFN-Is are increasingly recognized as key players in autoimmune CTDs such as SLE. Novel therapies that target IFN-I appear effective in SLE in early trials, but effectiveness is related to the presence of IFN-I biomarkers. IFN-I biomarkers may also act as positive or negative predictors of response to other biologics. Despite the high failure rate of clinical trials in SLE, subgroups of patients often respond better. Fully optimizing the potential of these agents is therefore likely to require stratification of patients using IFN-I and other biomarkers. This suggests the unified concept of type I IFN–mediated autoimmune diseases as a grouping including patients with a variety of different traditional diagnoses.

Rheumatology key messages

- Type I interferons play a causal role in a range of diseases, most notably in autoimmune connective tissue diseases.
- Biologics that target type I interferons appear effective in SLE and are in phase III trials.
- Assays for type I interferons can stratify interferon and non-interferon therapies but need further research.

Introduction

Autoimmune rheumatic diseases are characterized by a breakdown of immune tolerance leading to inflammation and irreversible end-organ tissue damage. Diverse cellular components and molecules contribute to the development of autoimmunity and their roles vary between individuals as well as diseases. However, common themes may be used to classify, diagnose and target therapy to groups or subsets of patients. The use of anti-TNF and B cell–depleting therapies has led to a rethinking of diagnosis and investigation in terms of ultimate therapy. Dysregulation of type I IFN (IFN-Ⅰ) is a common factor in multiple autoimmune rheumatic diseases and is recently of increased interest due to appreciation that it may define clinical phenotypes and therapy responses as well as the potential to treat with direct IFN-Ⅰ blockade [1, 2].

IFNs are generally classified into three families—IFN-I, IFN-II and IFN-III—which differ in their immunomodulatory properties, their structural homology and the group of cells from which they are secreted [3, 4]. IFN-Is (IFN-α, -β, -ω, -ε, -κ) compose the largest family and, alongside IFN-III (IFN-Ⅰ), activate intracellular signalling pathways that mediate immune responses against viruses and tumours [3, 5, 6]. Although most cells are capable of producing IFN-Ⅰs, in most situations the majority comes from dedicated danger-sensing cells called plasmacytoid dendritic cells (pDCs). IFN-Ⅰs act on all nucleated cells during viral invasion to inhibit viral replication [4]. They also have potent immunostimulatory properties, including inducing the maturation and activation of myeloid DCs (mDCs), favouring the Th1 phenotype and promoting B cell activation, antibody production and Ig class switching [7–9]. These immunostimulatory properties underlie their roles in autoimmunity. In contrast, although there is overlap in the gene sets whose expression they induce, IFN-Ⅰ (IFN-γ) is functionally distinct. It is produced mainly by NK cells and certain T cell subsets and regulates aspects of immune responses like phagocytosis and antigen presentation [10]. IFN-Ⅰ activity is commonly measured in patients using the presence or absence of expression of IFN stimulated genes (ISGs) (referred to as an IFN signature) or the level of expression (an IFN score). However, novel assays may be superior.

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In this review we examine our current knowledge on IFN-Is in multiple autoimmune diseases, their measurement and therapeutic targeting.

Production and regulation of IFN-Is

IFN-I production

pDCs were first described in the 1950s and their primary role is the production of IFN-I in response to pathogen-associated molecular patterns or danger signals [11, 12]. Their development from common DC progenitors is upregulated by several transcription factors [signal transducer and activator of transcription 3 (STAT3), MTG16, IRF8], while other factors (STAT5) inhibit pDC differentiation [13]. pDCs produce IFN-I after sensing viral antigens or, in autoimmunity, endogenous nucleic acids via toll-like receptors (TLRs), predominantly TLR7 and TLR9. Upon binding of TLRs to these antigens within endosomal compartments, the myeloid differentiation primary response protein 88-IRF7 pathway is activated, and eventually the secretion of IFN-I is mediated via the nuclear factor κB (NF-κB) signalling pathway [14, 15]. However, TLR-independent pathways of sensing nucleic acids mediated by other transcription factors might have an important role in the early development of autoimmunity [16]. An important aspect of the function of pDCs in autoimmune disease is that the uptake of viral or endogenous nucleic acids can be facilitated by Fc receptors, while host-derived DNA can form complexes with ANA, being internalized via FcγRIIA [17, 18]. The pleiotropic effects of IFN-I can be seen in Fig. 1.

Regulation of IFN-I production

The balance of immune responses induced by IFNs is regulated at multiple stages to limit the toxicity to the host by preventing tissue damage and autoimmunity [19]. These include regulation of IFN production and response to target cells.

The IFN regulatory factor (IRF) family of transcription factors is crucial for the promotion of IFN production [20]. IRFs have heterogeneous functions in the regulation of both innate and adaptive immunity and are associated with the recognition of pathogen-associated molecular patterns from TLRs [21]. A wide range of regulatory receptors, including BDCA-2, ILT7, NCR2 and CD32 (FcγRIIa), are expressed on the cell surface of human pDCs, which modulate the intracellular signalling pathways in response to TLR ligands [13, 18, 22].

Although pDCs are the main source of IFN-I, other cells such as epithelial cells or fibroblasts can secrete these cytokines [23]. IFN production by neutrophils may be important in autoimmunity [24]. NK cells can induce the secretion of IFN-α by pDCs stimulated by RNA-containing immune complexes, while monocytes play an inhibitory role [25]. Oestrogen might also favour IFN-I production through activation of the TLR-7 signalling pathway, consistent with female predominance of these diseases [26].

IFN-I effects on target cells

Outcomes of IFN-I signalling may be as diverse as promotion of cell survival and promotion or prevention of apoptosis [27–29]. Although all IFN-I ligands signal through the same receptor (IFNAR), they result in different biological outcomes [30]. This is important for therapy, as either ligands or receptors may be targeted. The IFNAR2 subunit of the receptor has a surface-bound (IFNAR2b) and a soluble form, both with regulatory activity [31]. In contrast, IFN-II (IFN-γ) signals via the IFNGR receptor. IFN-III signals via a receptor that combines a unique subunit (IFNLR1) with one also used by IL-10 family cytokines, and its expression is much more restricted to cells of epithelial origin and DCs [32]. Interestingly, our group found that IFN-III signalling could also vary between cells: skin fibroblasts respond to IFN-III (not only keratinocytes as previously thought), but they do so via mitogen-activated protein kinase instead of STAT1 [33]. There is considerable overlap between the genes whose expression is induced by these pathways. This makes measurement of activity using gene expression, as in an IFN signature, complex. IFN-I and -II, variations in circulating immune cells (e.g. lymphopenia seen in lupus) and changes in other immune functions could all influence results.

Early evidence about the link of IFN-I to autoimmunity was seen in patients receiving immunotherapy with IFNs for chronic viral infections or malignant carcinoid tumours [34, 35]. Interestingly, the presence of autoantibodies prior to IFN therapy considerably increased the risk for autoimmune phenomena that often characterize SLE, RA and PM, suggesting that IFN-I might contribute to the development of clinical manifestations from a pre-clinical stage. Nevertheless, autoimmunity may remit after cessation of treatment, implying that regulatory factors control autoimmune responses and the transition to clinically overt disease is much more complicated [36].

While the mechanisms behind the dysregulation of the IFN system are complex and remain unclear, advances have been made in understanding their role in systemic autoimmune diseases.

SLE and IFN-Is

SLE is a prototypic IFN-I-mediated autoimmune disease whose clinical manifestations are diverse in the organs affected, severity and response to targeted and non-targeted therapies [37]. Its pathogenesis is similarly complex, but a defining feature is an immune response against endogenous nuclear antigens, with ANA being central to diagnosis, activity and tissue inflammation [38]. ANA positivity may precede clinical symptoms by years, and only the proportion of such individuals develop organ inflammation, suggesting that autoantibodies are an incomplete explanation for pathology [39].

Increased levels of serum IFN-α were described in patients with SLE >30 years ago and were associated with disease activity and specific clinical manifestations such as fever, arthralgia, rash and leukopenia [40, 41]. High dose IFN-α treatment can induce a variety of
neuropsychiatric adverse effects, while similar symptoms in neuropsychiatric SLE are linked to IFN-α production. Higher levels of IFN-α were detected in cerebrospinal fluid, but decreased when the manifestations of lupus psychosis subsided [42]. IFN-I might contribute to LN [43]. In murine lupus models, IFN-α exacerbated GN by increasing immune complex deposition in the kidneys [44]. Although SLE patients have reduced numbers of circulating pDCs, these are increased intraglomerularly [45]. In cutaneous lupus erythematosus (CLE) there is a unique IFN environment in the skin. Keratinocytes can produce IFN-III, enhancing IFN-I production [46]. Patients with active CLE also have detectable serum levels of IFN-α/C21 [47]. Genes in the IFN pathway and regulation of innate immune responses are prominent in SLE susceptibility. These include variants in HLA and Fcγ receptor genes IRF5, STAT4, PTPN22, TNFAIP3, BLK, BANK1, TNFSF4 and ITGAM [48]. Intriguingly, high IFN-I activity seems to be a heritable risk factor that is clustered in specific families in both SLE patients and their healthy first-degree relatives [49]. The risk haplotypes in the IFN regulatory factors IRF5 and IRF7 are associated with increased IFN-I activity and the risk is dependent on particular auto-antibodies [50–55]. The risk haplotype of IRF5 is also associated with a risk of progression to clinical disease in ANA-positive individuals [56]. Gene variants in IFIH1 (a cytoplasmic dsRNA sensor that activates IFN-α signalling) correlate to anti-dsDNA antibodies and increased sensitivity to IFN-α [57]. In addition, IRF8 is strongly related to increased cardiovascular risk in mouse models as well as SLE patients [58, 59].

What is the environmental trigger for induction of IFN-I production? It has been proposed that nucleic acids from common viruses like EBV could initiate IFN-α production via activation of intracellular TLR7 and TLR9, leading to disease in genetically predisposed individuals [60]. An alternative theory suggests that self-derived nucleic acids comprise the major inducer of IFN-α secretion in SLE via the intracellular receptors responsible for antiviral immunity [61]. Nucleic acid-autoantibody complexes can be internalized by Fc receptors and recognized by endosomal TLR7 and TLR9, inducing aberrant IFN-α production by pDCs [18, 62]. Autoantibodies against RNA-associated proteins such as snRNP, Ro(SSA) and La(SSB) can also augment immune responses [63, 64]. The RNA binding protein Ro60 has been recently shown to regulate IFN-stimulated gene expression [65].

Expansion of plasmablasts and plasma cells is a hallmark of SLE that is positively correlated with disease activity, and IFN-I enhances the differentiation of B cells to plasmablasts [66, 67]. Using an in vitro model, our collaborators showed that IFN-I promotes the differentiation of plasma cells and also confers a unique phenotype: IFN-I-stimulated plasma
cells, including those derived from SLE patients, secrete ISG15, through which they have pro-inflammatory effects independent of antibody secretion [88].

In mice, TLR9 and myeloid differentiation primary response protein 88 signalling are crucial for the switching of autoreactive IgM anti-self B cells to the pathogenic IgG2a and 2b subclasses [69]. T cells are directly affected by IFN-α, promoting the generation of effector and memory CD8+ T cells [70]. Therefore, innate immunity may moderate adaptive immune responses against self-antigens.

Although immune complexes potentiate pDCs, other cells could amplify this. There is increasing interest in the role of neutrophils in autoimmunity. The presence of neutrophils in inflamed kidney tissue was reported long ago in both experimental models and patients with autoimmune conditions affecting the kidneys [71, 72]. Neutrophils undergo a special type of cellular death (NETosis), in which they release web-like structures, known as neutrophil extracellular traps (NETs), composed of chromatin and granule proteins that can bind and kill microorganisms [73]. NETs also contain nuclear material, DNA and histones and anti-microbial agents [cathelicidin (LL37), high mobility group box 1] that prevent the degradation of nuclear acids. Many cytokines, including IFN-α, can actually act as priming factors on mature neutrophils, allowing the formation of NETs upon subsequent stimulation with complement factor 5a [74]. As a consequence, neutrophils could be in the centre of another positive feedback loop between induction and maintenance of IFN-I perpetuating immune responses.

Other autoimmune and inflammatory diseases

Although dysregulation of the IFN-I system has been well studied in SLE, there is evidence of increased IFN-I activity in many other rheumatic and inflammatory disorders, potentially sharing common molecular pathways.

SS

Primary SS (pSS) is an autoimmune disorder characterized by autoantibodies against RNP, Ro(SSA) and La(SSB) [75]. Non-HLA variants such as IRF5 and STAT4 (IFN-related) were reported as risk loci in a large genome-wide association study [76]. ISG expression is upregulated in both humans and mouse models, especially in those with detectable autoantibodies, and many studies have tried to correlate these findings with disease pathogenesis [77]. As in SLE, autoantigens of apoptotic origin provide the immunogenic stimulus for the initiation of pathogenic responses [78]. RNA-containing immune complexes can activate pDCs in salivary glands and enhance the production of IFN-α, while IFN-α itself can upregulate the expression of ISGs in the target organs [79, 80]. Early studies clearly identified an IFN signature in salivary glands from patients with pSS; IRF7, IRF8 and IRF9 were significantly upregulated [81, 82]. Peripheral blood mononuclear cells (PBMCs) also expressed an IFN signature and closely correlated to anti-Ro(SSA) and anti-La(SSB) titres [83, 84]. A subgroup of pSS patients with a monocyte IFN signature also presented greater disease activity alongside higher B cell activating factor mRNA expression [85].

Inflammatory myositis

In myositis, pDCs infiltrate tissues and might secrete aberrant amounts of IFN-I; ISGs are significantly upregulated in both inflamed muscles and PBMCs [86-88]. Serum IFN-α is correlated with serum muscle enzyme levels in untreated disease among patients with JDM and inversely correlated with the duration of untreated disease [89]. Additionally, anti-Jo1 and anti-Ro(SSA) autoantibodies were associated with higher expression of ISGs in PBMCs and greater disease activity in patients with DM [90].

Other systemic autoimmune diseases

Other CTDs associated with ANA have some evidence for involvement of IFN-I, at least in subsets of patients. An IFN signature similar to SLE and myositis was identified in patients with scleroderma [88]. APS was reported as a side effect in patients receiving IFN-α therapy for unrelated diseases [91, 92]. We found that patients with early incomplete forms of CTDs (of whom a proportion progressed to SLE or other diseases) had increased IFN activity [93]. Further, we found that a subgroup of patients with established UCTDs of >12 months duration also had increased IFN activity [94].

RA

The IFN signature was studied in RA as a biomarker for disease activity and response to therapy. In pre-clinical RA, individuals with arthralgia and elevated IFN-I signature were at greater risk to develop arthritis [95]. IFN-I also predicted therapy response and, interestingly, had an opposite predictive value for two targeted therapies. Patients with a high IFN-I signature had a poor response to rituximab [96, 97]. Although RA patients with a high IFN signature presented greater disease activity, in a recent study, a higher IFN score in neutrophils correlated with a good response to anti-TNF treatment [98, 99]. IFN-I status may predict complications of RA. Increased IFN-regulated transcripts, including IFIT, IFIT2 and IFIT7, in a subset of RA patients were associated with upregulated pathways related to coagulation, complement activation and fatty acid metabolism [100].

Outside systemic autoimmunity: roles for IFN-I in other diseases

IFN-I influences host immune response to cancers as well as response to radiotherapy [101]. Intratumoural IFN-I can enhance antitumour immunity as well as having beneficial anti-angiogenic effects [102]. IFN-I has complex roles in chronic infection. It is a mediator of antiviral defence, and
evasion of IFN-I affects the pathogenicity of HIV and CMV infection, although unhelpful immunosuppressive effects of IFN-I have also been described [103–105]. IFN-I may mediate atherosclerosis, which is of particular interest given the prevalence of this complication in autoimmune rheumatic diseases [106].

Interferonopathies

Interferonopathies are a heterogeneous group of disorders, mainly presenting an autosomal recessive inheritance pattern, characterized by constitutive upregulation of IFN-I. Acardi–Goutier syndrome (AGS), the most well-studied interferonopathy, usually presents an early onset during childhood, with symptoms resembling those of SLE [107]. The IFN signature in peripheral blood has been reported to be universal in AGS patients with mutations in \( \text{TREX1}, \text{IFIH1}, \text{RNASEH2A}, \text{RNASEH2C}, \text{ADAR1} \); each mutation in these genes has been correlated with different clinical manifestations [108–110]. These monogenic diseases, culminating in the dysregulation of IFN-related responses, strongly support the linkage between IFN-I and autoimmunity.

Therapeutic targeting of the IFN-I pathway

Given its pleotropic roles in diverse diseases, blockade of IFN-I has the potential to become a versatile treatment in rheumatology and beyond (Table 1).

The most direct approach, with the greatest use in human clinical trials, is the mAb blockers of IFN-\( \alpha \) and its receptor. However, the traditional lupus therapy HCQ has relatively selective effects on IFN-I by blocking TLR7 and TLR9 activation [111]. A number of small molecule or oligonucleotide inhibitors of TLRs for potential use in SLE and other autoimmune diseases are in pre-clinical or phase I development [112]. IFN signalling may also affect the efficacy of glucocorticoids. Glucocorticoids present decreased activity to inhibit the IFN pathway in pDCs activated via TLR-dependent pathways in SLE patients and lupus mouse models [113, 114].

New therapeutic approaches targeting IFN-\( \alpha \) (but not other forms of IFN-I) directly by neutralizing mAb (sifalimumab, rontalizumab, AGS-009) have shown encouraging results. Phase I clinical trials have confirmed their safety, tolerability and ability to partially inhibit the overexpression of ISGs [115–117]. The inhibition of IFN-\( \alpha/\beta \)-inducible genes in whole blood was dose dependent and the expression of genes for B cell activating factor, IL-10, IL-1\( \beta \) and GM-CSF were also suppressed [118]. In a phase Ib, randomized, double-blind, placebo-controlled study, sifalimumab achieved its primary endpoint by reducing disease activity in patients with SLE with an acceptable safety profile. Efficacy was confirmed in the high IFN signature subgroup. In the low IFN signature group, differences were not significant, although this may be related to patient numbers. Immunological parameters such as complement levels and anti-dsDNA antibodies remained unchanged [119]. Surprisingly, in a recent phase II study, rontalizumab proved superior in comparison with the control only in the group of patients with low IFN signature. The low IFN signature group had similar clinical characteristics at baseline, but less serological activity. This group also had higher trough concentrations of rontalizumab, suggesting that the inefficacy in the high IFN signature group may have been due to underdosing. However, this was not reflected in attenuation of ISG expression, which was similar in both groups [120].

Given the multiple forms of IFN-I, targeting the shared IFNAR1 receptor may more effectively block IFN signalling [121, 122]. Anifrolumab, an anti-IFNAR1 mAb, met its primary endpoints of a reduction in global disease activity score in patients with SLE and the level of suppression of the IFN signature was clearly associated with increased anifrolumab concentrations [123]. Blocking IFNAR1 with anifrolumab reduced ISG expression more than blocking IFN-\( \alpha \) with sifalimumab. Anifrolumab demonstrated better efficacy in the high IFN signature subset and is now in phase III clinical trials. Overall, the relationship between clinical response and IFN signature status was different in each trial. This is shown in Fig. 2.

Other strategies directly target pDCs, the main source of IFN-I. Early, transient depletion of pDCs in BXSB lupus-prone mice before disease initiation led to reduced expansion of T and B cells, reduced production of auto-antibodies and amelioration of GN [124]. In NZB/NZW lupus-prone mice, inhibition of Bcl-2, a necessary molecule for pDC survival, resulted in selective depletion of pDCs and a reduction of IFN-\( \alpha \) production [125].

### Table 1 Pharmaceutical agents targeting the IFN-I pathway

<table>
<thead>
<tr>
<th>Pharmaceutical agent</th>
<th>Manufacturer</th>
<th>Definition</th>
<th>Target</th>
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</thead>
<tbody>
<tr>
<td>Sifalimumab</td>
<td>MedImmune</td>
<td>Fully human mAb</td>
<td>IFN-( \alpha )</td>
</tr>
<tr>
<td>Rontalizumab</td>
<td>Genetech</td>
<td>Recombinant humanized mAb</td>
<td>IFN-( \alpha )</td>
</tr>
<tr>
<td>AGS-009</td>
<td>Argos Therapeutics</td>
<td>Humanized IgG4 mAb</td>
<td>IFN-( \alpha )</td>
</tr>
<tr>
<td>Anifrolumab</td>
<td>MedImmune</td>
<td>Fully human mAb</td>
<td>IFN-( \beta/\alpha ) receptor</td>
</tr>
<tr>
<td>IFN-( \alpha/\beta )-Kinoid</td>
<td>Neovacs</td>
<td>Oligonucleotide antagonist</td>
<td>IFN-( \alpha )</td>
</tr>
<tr>
<td>IMO-3100</td>
<td>Idera Pharmaceuticals</td>
<td>Oligonucleotide antagonist</td>
<td>TLR7/9 inhibition</td>
</tr>
<tr>
<td>DV1179</td>
<td>Dynavax</td>
<td>Oligonucleotide antagonist</td>
<td>TLR7/9 inhibition</td>
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</table>
Furthermore, proteasome inhibitors (carfilzomib, bortezomib) managed to suppress IFN-α production by TLR-activated pDCs by inhibiting pDC survival and function in lupus mice models [126]. More recently, the pDC inhibitory receptor BDCA-2 (CD303) has been used to block IFN-I production in pre-clinical studies [127].

Finally, the immunization of SLE patients presenting mild to moderate disease with IFN-α-kinoid (IFN-K), a drug composed of inactivated IFN-α coupled to a carrier protein, induced anti-IFN-α antibodies and significantly improved disease biomarkers in all patients [128]. Interestingly, higher titres of anti-IFN-α antibodies were found in IFN signature/C150 positive patients, which were also linked to a reduction of the IFN score.

**Measuring IFN activity in patients**

While IFN-I is known to mediate clinical manifestations of SLE, assays for IFN activity have not yet become routinely used in the care of SLE patients in the same way as B cell biomarkers such as autoantibody titres and complement levels.

The measurement of IFN-α itself by ELISA has been used to monitor disease activity and predict response to IFN-I targeted therapies [23]. Although early studies reported elevated levels of IFN-α in the sera of SLE patients, the sensitivity of the method appears low, since IFN-I levels are either undetectable or dependent on several factors, including ethnic background, age and sex [129, 130]. Indirect methods have also been used to measure IFN-I activity. For example, human Wish epithelial cell line cells were cultured with 50% patient plasma and the expression level of certain ISGs was then evaluated [131, 132].

In research cohorts, 60–80% of lupus patients exhibit an increased expression of ISGs in PBMCs (IFN signature). In childhood-onset SLE the IFN signature is almost universally observed [133]. IFN scores are similar but are generally used to refer to a continuous parameter derived from quantitative PCR rather than the absence or presence of increased expression. IFN signatures and scores consistently correlate with B cell biomarkers of activity, such as titres of anti-dsDNA, anti-Ro, anti-U1RNP, anti-Sm autoantibodies and lower complement (C3) levels [134]. IFN-I assays showed an association with disease activity in cross-sectional studies [133, 135, 136]. However, these were inconsistent and other studies failed to demonstrate any association [137, 138]. Longitudinal analyses of ISG expression in SLE patients have also produced more complex results: although patients with higher IFN scores had greater disease activity, scores of individual patients could not predict flares [139]. This discrepancy might be due to the choice of ISGs or the methods used to derive unidimensional IFN scores from genome-wide micro-array data [140]. Some studies have suggested that higher ISG expression is associated with particular organ involvement in SLE. For instance, five IFN-I-inducible genes (LY6E, OAS1, OASL, MX1, ISG15) were highly expressed in patients with active renal or neurological disease, but not in other manifestations.

![Fig. 2 IFN gene signature and response to IFN-targeted therapies](https://www.rheumatology.oxfordjournals.org/lookup/doi/10.1093/annrheumdis/56.10.1662)

Three phase II studies of IFN-blocking biologics: 

(A) Rontalizumab: biomarker-negative patients responded better than biomarker-positive patients. This suggests that biomarker-negative patients may not have IFN-I-independent disease. (B) Sifalimumab: efficacy was confirmed in biomarker-positive patients (n = 350). The number of biomarker-negative patients was smaller (n = 81), so it is difficult to compare response rates. The difference between placebo response rates appears more striking than the difference between the placebo and active treatment arms within each biomarker category. (C) Anifrolumab: the most marked difference in response between biomarker-positive (n = 229) and negative (n = 76) patients, but this was largely due to a low placebo response rate in biomarker-positive patients.
## Table 2: Effect of the IFN-I gene signature on response to targeted therapies in autoimmune diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Drug</th>
<th>Target</th>
<th>Assay</th>
<th>Clinical response if IFN-I biomarkers high</th>
<th>Description</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>RA</td>
<td>Rituximab</td>
<td>B cells</td>
<td>PBMC IFNGS</td>
<td>Worse</td>
<td>A three-ISG qPCR IFN score (OAS-1, ISG-15, Mx-1) was used to classify patients as high or low. IFN-high patients had lower change in DAS28 and EULAR response rate</td>
<td>Thurlings et al. [96]</td>
</tr>
<tr>
<td>RA</td>
<td>Rituximab</td>
<td>B cells</td>
<td>Whole blood IFNGS</td>
<td>Worse</td>
<td>A cluster of eight ISGs on micro-array (LY6E, HERC5, IFI44L, ISG15, MxA, MxB, EPS771, RSAD2) was associated with a lower change in DAS28 and EULAR response rate</td>
<td>Raterman et al. [97]</td>
</tr>
<tr>
<td>RA</td>
<td>TNF blockers</td>
<td>TNF-α</td>
<td>Reporter cell assay</td>
<td>Better</td>
<td>Patients with a high IFNGS expression (IFIT-1, PKR, Mx-1) in reporter cells had a higher EULAR response rate</td>
<td>Mavragani et al. [132]</td>
</tr>
<tr>
<td>RA</td>
<td>Infliximab</td>
<td>TNF-α</td>
<td>Neutrophil IFNGS</td>
<td>Better</td>
<td>A higher IFN response gene expression (178 ISGs in total) in RA neutrophils correlates with a greater change in DAS28 and EULAR response rate</td>
<td>Wright et al. [99]</td>
</tr>
<tr>
<td>SLE</td>
<td>Rontalizumab</td>
<td>IFN-α</td>
<td>PBMC or whole blood IFNGS</td>
<td>Worse</td>
<td>Patients with a low three-gene IFNGS (HERC5, EPS71, CMPK2) treated with rontalizumab had a higher SRI-4 response rate and reduction in oral steroids compared with placebo or IFNGS-high patients</td>
<td>Kalunian et al. [120]</td>
</tr>
<tr>
<td>SLE</td>
<td>Sifalimumab</td>
<td>IFN-α</td>
<td>Whole blood IFNGS</td>
<td>Same/better</td>
<td>Patients with a high four-gene IFNGS (IFI27, IFI44, IFI44L, RSAD2) had a lower placebo response rate and similar or slightly better SRI-4 rate on sifalimumab compared with placebo</td>
<td>Khamashta et al. [119]</td>
</tr>
<tr>
<td>SLE</td>
<td>Anifrolumab</td>
<td>IFNAR</td>
<td>Whole blood IFNGS</td>
<td>Better</td>
<td>Patients with a high IFNGS had a lower placebo response rate but much greater response on anifrolumab compared with placebo. Patients with low IFNGS had no improvement on anifrolumab compared with placebo</td>
<td>Brohnaw et al. [123]</td>
</tr>
<tr>
<td>IIM</td>
<td>Rituximab</td>
<td>B cells</td>
<td>Serum IFN-regulated chemokine score</td>
<td>Better</td>
<td>IFN-regulated chemokines before treatment correlated with an improvement in disease activity measures in refractory myositis patients treated with rituximab. High levels of myeloid type I IFN gene expression (37 ISGs in five distinct clusters) in skeletal muscle predict better responses to rituximab in PM/DM</td>
<td>López de Padilla et al. [151]</td>
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</table>

DAS28, 28-joint DAS; IFNGS, type I IFN gene signature.
Traditional organ-based classifications of autoimmune diseases are based on individual clinical features. Due to overlap in pathogenic mechanisms, they are usually treated with the same range of therapies. However, response rates vary significantly. Recent data on IFN-I indicate higher response rates when diagnostic groups are subdivided by biomarkers rather than clinical features. In this respect, the high IFN-I SLE patients may be more closely related to high IFN-I SS patients than low IFN-I SLE patients. Existing classifications therefore appear increasingly arbitrary when considering ultimate therapeutic strategies. Reclassification of patients according to the dominant pathogenic mechanism may be more appropriate instead of the pattern of organs affected. This is analogous to the classification of bacterial infections according to microbial agent (and therefore the antibiotic required) rather than the site of infection.

Given the pleiotropic effects of IFN-I on all cells, the varying transcriptional response of individual circulating populations may also be important. Although high-density oligonucleotide microarray has proven to be valuable to investigate the genetic mechanism in the pathogenesis of SLE, most of these studies have used unseparated leukocytes or whole blood [141]. A recent study investigated ISG expression in multiple sorted cell types, including monocytes, DCs, NK cells and B and T lymphocytes, from SLE patients and showed distinct profiles in different cell types [142]. A distinct gene expression profile has been recently identified in both classical and non-classical monocytes from SLE patients [143]. Genome-wide DNA methylation analyses of CD4+ T cells from SLE patients revealed a persistent hypomethylation of certain ISGs (e.g. IFIT1, IFIT3, MX1, STAT1, IFI44L, USP18, TRIM22, BST2), suggesting that epigenetic modifications could influence the responsiveness of autoreactive T cells [93, 140, 144, 145].

The IFN signature might contribute to the early stages of disease development, as the expression of certain genes has been linked to certain autoantibody profiles in patients with incomplete lupus erythematosus, suggesting that the IFN signature might be used as a biomarker for individuals with a higher risk for disease progression [146]. The results confirmed a different IFN signature in peripheral B cells, T cells and myeloid cells leading to the upregulation of distinct transcriptional factors, which favour a pro-inflammatory phenotype. Interestingly, cytosolic nucleic acid sensing pathways were mostly upregulated in myeloid cells.

**Conclusion: a case for an IFN-centred classification of autoimmune disease?**

IFN-I activity is a common feature in most CTDs as well as other diseases such as RA. However, this is a variable
feature: IFN-I appears to be one of many routes to autoimmunity. IFN-I blocking therapies are a new therapeutic class with positive phase II data in SLE. IFN-I activity predicts response to both IFN-I and other targeted therapies in many autoimmune diseases (Table 2).

The regulation and function of IFN-I are complex processes. pDCs have multiple regulatory mechanisms, and other cells contribute to IFN-I production. Hence, while IFN-I may be viewed as a single mediator and target, it operates as part of a complex network involving almost every component of the immune system. Therapeutic strategies that target ligands, receptors, TLRs or pDCs may have markedly different clinical effects.

Variable IFN-I activity within each CTD, with associated differences in response to therapy, suggest that reclassification of these diseases according to pathogenic mechanisms may be more appropriate than existing organ-based classifications. This concept is illustrated in Fig. 3. Similar concepts have been suggested by other classes of targeted therapy. The presence of autoantibodies produced by short-lived plasma cells defines diseases amenable to B cell targeted therapies (e.g. seropositive RA, SLE with high serological activity, ANCA-associated vasculitis and anti-synthetase syndromes) [147]. The presence of TNF-mediated tissue inflammation, with or without adaptive immune involvement, defines diseases amenable to TNF-blocking therapy (e.g. seropositive and seronegative RA, SpAs, Crohn’s disease and sarcoidosis) [148].

The PRECISESADS European consortium aims to reclassify systemic autoimmunity using omics for improved diagnosis and therapy [149]. The UK MASTERPLANS consortium aims to stratify SLE according to therapy response [150]. There are challenges to such approaches. First, for IFN-I, the complexities of its function may mean that reducing IFN-I activity to a unidimensional signature or score may not fully describe differences between individuals. Second, IFN illustrates a problem in many biomarker studies. Due to the close crosstalk between B cells, IFN and other mediators in established active disease, it may be difficult to determine which of many positive markers defines the key mediator for an individual.

Because of these challenges, even if phase III trials of IFN-I blocking therapies were to fail to reproduce the efficacy seen in phase II studies (as previously found for other biologics in SLE), further research would be needed to establish the role of these agents.

Overall, IFN-I represents an important biomarker and pathway for therapeutics in autoimmunity whose ramifications have yet to be fully elucidated. We propose that a research agenda should include better understanding of the clinical phenotype of IFN-I-mediated diseases, including severity, cardiovascular and other complications and level of response to conventional therapies, in order to quantify the potential benefits of IFN-I blocking therapy; establishment of the efficacy of IFN-I blocking therapy in a wider range of autoimmune diseases; and improved biomarkers that can accurately establish key pathogenic mediators in complex autoimmune diseases to fully realize the potential illustrated by IFN-I signatures.

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