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Type I Interferon-Mediated Autoimmune Diseases:
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Abstract

Type I interferons (IFN-I) 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-I are increasingly recognized as key players in autoimmune connective tissue diseases such as systemic lupus erythematosus (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 biomarkers. This suggests the unified concept of Type I Interferon Mediated Autoimmune Diseases, as a grouping including patients with a variety of different traditional diagnoses.

Key Messages

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

Conflicts of Interest

Dr Vital has received honoraria from Roche and research grants paid to his employer from Roche and AstraZeneca.

Professor Emery has received consultant fees from BMS, Abbott, Pfizer, MSD, Novartis, Roche and UCB. He has received research grants paid to his employer from Abbott, BMS, Pfizer, MSD and Roche.

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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 features 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 interferons (IFN-I) is a common factor in multiple autoimmune rheumatic diseases and is of increased interest recently 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 3 families –IFN-I, IFN-II and IFN-III– which differ in their immunomodulatory properties, their structural homology and the group of cells they are secreted from (3, 4). IFN-I (IFN-α, -β, -ω, -ε, -κ) consist of the largest family and alongside IFN-III (IFN-λ) activate intracellular signaling pathways which mediate immune responses against viruses and tumors (3, 5, 6). Although most cells are capable of producing IFN-I, in most situations the majority comes from dedicated danger-sensing cells called plasmacytoid dendritic cells (pDCs). IFN-I 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 dendritic cells (DCs), favoring Th1 phenotype and promote 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-II (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 presence or absence of expression of interferon stimulated genes (ISGs) (referred to as an interferon signature) or level of expression (an interferon score). However, novel assays may be superior.

In this review, we examine our current knowledge on IFN-I in multiple autoimmune

diseases, their measurement, and therapeutic targeting.

Production and regulation of type I IFNs

IFN-I production

Plasmacytoid dendritic cells (pDCs) were first described in the 1950s and their primary role is the production of IFN-I in response to pathogen-associated molecular patterns (PAMPs) or danger signals (11, 12). Their development from common DC progenitors is upregulated by several transcription factors (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 within endosomal compartments to these antigens, the myeloid differentiation primary response protein 88 (MYD88)-IRF7 pathway is activated, and eventually the secretion of IFN-I is mediated via nuclear factor-κB (NFκB) signaling pathway (14, 15). However, TLR-independent pathways of sensing nucleic acids mediated via other transcription factors might have an important role in early development of autoimmunity (16). An important aspect of pDCs' function 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 antinuclear antibodies (ANA) being internalized via FcγRIIA (17, 18). The pleiotropic effects of IFN-I can be seen in Figure 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 interferon regulatory factor (IRF) family of transcription factors is crucial for the propagation of IFN production (20). IRFs have heterogeneous functions in the regulation of both innate and adaptive immunity and are associated with the recognition of PAMPs from TLRs (21). pDCs constitutively express IRF7, which modulate the intracellular signaling 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 secretion of IFN-α by pDCs stimulated by RNA-containing immune complexes, while monocytes play an inhibitory role (25). Oestrogens might also favor IFN-I production through activation of signaling pathways (26, 27).

IFN-I effects on target cells

Outcomes of IFN-I signaling may be as diverse as promotion of cell survival and promotion or prevention of apoptosis (29-31). Although all IFN-I ligands signal through the same receptor (IFNAR), they result in different biological outcomes (32). 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 (33). 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 dendritic cells (34). Interestingly, our group found that IFN-III signaling could also vary between cells: skin fibroblasts respond to IFN-III (not only keratinocytes as previously thought) but they do so via MAPK instead of STAT1 (35). 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 interferon signature, complex. IFN-II and III, 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 given in patients receiving immunotherapy with IFNs for chronic viral infections or malignant carcinoid tumors (36, 37). Interestingly, the presence of autoantibodies prior to IFN therapy considerably increased the risk for autoimmune phenomena that often characterize SLE, RA, polymyositis, suggesting that type IFNs might contribute to the development of clinical manifestations from a preclinical 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 (38).

Whilst 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.

Systemic Lupus Erythematosus and type I IFNs

SLE is a prototypic type I interferon-mediated autoimmune disease whose clinical manifestations are diverse in 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 a 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 over 30 years ago and were associated with disease activity and specific clinical manifestations such as fever, arthralgia, rash, and leukopenia (42, 43). 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 lupus nephritis (43). In murine lupus models IFN-α exacerbated glomerulonephritis by increasing immune complex deposition in kidneys (47). Patients with SLE have reduced numbers of pDCs in blood, but increased intraglomerularly (45). In cutaneous lupus erythematosus 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-λ1 (50).

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 being clustered in specific families in both SLE patients and their healthy first-degree relatives (49). The risk haplotypes in the interferon regulatory factors IRF5 and IRF7 are associated with increased IFN-I activity and the risk is dependent on particular autoantibodies (53-

58). The risk haplotype of IRF5 is also associated with risk of progression to clinical disease in ANA positive individuals (56). Gene variants in IFIH1 (a cytoplasmic dsRNA sensor that activates IFN-α signaling) correlate to anti-dsDNA antibodies and increased sensitivity to IFN-α (60). In addition, IRF8 is strongly related to increased cardiovascular risk in mouse models as well as SLE patients (61, 62).

What is the environmental trigger for induction of IFN-I production? It has been proposed that nucleic acids from common viruses like Epstein-Barr virus (EBV) could initiate the 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), La (SSB), can also augment immune responses (18, 66). The RNA binding protein Ro60 has been recently shown to regulate IFN-stimulated gene expression (65).

Expansion of plasmablasts/plasma cells is a hallmark of SLE positively correlated with disease activity and IFN-I enhances the differentiation of B cells to plasmablasts (70, 71). Using an in-vitro model, our collaborators showed that IFN-I promotes differentiation of plasma cells and also confers a unique phenotype: IFN-I stimulated plasma cells, including those derived from SLE patients, secrete ISG15, via which they have pro-inflammatory effects independent of antibody secretion (72).

In mice, TLR9 and MyD88 signaling are crucial for switching of autoreactive of 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 potently trigger 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 reported long ago in both experimental models and patients with autoimmune conditions affecting the kidneys (75, 76). Neutrophils undergo 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 (78). NETs also contain nuclear material, DNA and histones, and antimicrobial agents (LL37, HMGB1) that prevent nuclear acids from degradation. 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 IFN-I system has been most well studied in SLE, there is evidence of increased IFN-I activity in many other rheumatic and inflammatory disorders, potentially sharing common molecular pathways.

Sjögren's Syndrome

Primary Sjögren's Syndrome (pSS) is an autoimmune disorder characterized by autoantibodies against ribonucleoproteins, 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 (84). ISG expression is upregulated in both humans and mouse models, especially in those with detectable autoantibodies, and many studies 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 (87, 88). Early studies clearly identified an IFN signature in salivary glands from patients with pSS; IRF7, IRF8, and IRF9 were significantly upregulated (91, 92). PBMCs also expressed an IFN signature and closely correlated to anti-Ro(SSA) and anti-La(SSB) titers (93, 94). A subgroup of pSS patients with monocyte IFN signature also presented higher disease activity alongside higher BAFF mRNA expression (95).

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 (96-98). Serum IFN-α is correlated to serum muscle enzyme levels in untreated disease among patients with juvenile dermatomyositis and inversely correlated to the duration of untreated disease (99). Additionally, anti-Jo1 and anti-Ro(SSA) autoantibodies were associated with higher expression of ISGs in PBMCs and higher disease activity in patients with dermatomyositis (100).

Other Systemic Autoimmune Diseases

Other connective tissue diseases associated with ANA also have some evidence for involvement of IFN-I, at least in subsets of patients. An interferon signature similar to SLE and myositis was identified in patients with scleroderma (98). Antiphospholipid syndrome was reported as a side effect in patients receiving IFN-α therapy for unrelated diseases (101, 102). We found that patients with early incomplete forms of connective tissue diseases (of whom a proportion progressed to SLE or other diseases) had increased interferon activity (93). Further, we found that a subgroup of patients with established undifferentiated connective tissue diseases of more than 12 months duration also had increased interferon activity (94).

Rheumatoid Arthritis

IFN signature was studied in RA as a biomarker for disease activity and response to therapy. In preclinical 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, it had opposite predictive value for two targeted therapies. Patients with high IFN-I signature had a poor response to rituximab (106, 107). Although RA patients with high IFN signature presented higher disease activity, in a recent study higher IFN score in neutrophils correlated with a good response to anti-TNF treatment (108, 109). IFN-I status may predict complications of RA. Increased IFN-regulated transcripts, including IFIT, IFIT2, and IRF7, in a subset of RA patients were associated with upregulated pathways related to coagulation, complement activation and fatty acid metabolism (110).

Outside systemic autoimmunity: roles for type I interferon in other diseases

IFN-I influences host immune response to cancers as well as response to radiotherapy (101). Intratumoral IFN-I can enhance antitumor immunity as well as having beneficial anti-angiogenic effects (102). IFN-I has complex roles in chronic infection. It is a mediator of anti-viral defense, and evasion of IFN-I affects the pathogenicity of HIV and CMV infection, although unhelpful immunosuppressive effects of IFN-I have also been described (113-116). 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, which are characterized by constitutive upregulation of IFN-I. Aicardi-Goutieres syndrome (AGS), the most well studied interferonopathy, usually presents an early onset during childhood with symptoms resemble those of SLE (107). IFN signature in peripheral blood has been reported to be universal in AGS patients with mutations in TREX1, IFIH1, RNASEH2A, RNASEH2C, ADAR1, while each mutation in these genes has been correlated with different clinical manifestations (119-121). These monogenic diseases culminating in the dysregulation of IFN-related responses strongly support the linkage between IFN-I and autoimmunity.

Therapeutic targeting of type I IFN pathway

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

The most direct approach, with greatest use in human clinical trials, is the monoclonal antibody blockers of IFN-α or its receptor. However, the traditional lupus therapy hydroxychloroquine 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 or other autoimmune diseases are in pre-clinical or Phase I development (112). IFN signaling 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 (126, 127).

New therapeutic approaches targeting directly IFN-α (but not other forms of IFN-I) by neutralizing monoclonal antibodies (sifalimumab, rontalizumab, AGS-009) have shown encouraging results. Phase I clinical trials confirmed their safety, tolerability and their ability to partially inhibit the overexpression of ISGs (128-130). The inhibition of IFN-α/β-inducible genes in whole blood was dose-dependent and the expression of genes for BAFF, IL-10, IL-1β, GM-CSF were also suppressed (118). In a phase IIb, randomized, double-blind, placebo-controlled study, sifalimumab achieved its primary endpoint by reducing disease activity in patients with SLE with acceptable safety profile. Efficacy was confirmed in the IFN signature high subgroup. In the IFN signature low 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 superiority in comparison with the control only in the group of patients with low IFN signature. The IFN signature low 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 IFN signature high group may have been due to under-dosing. 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 signaling (134, 135). Anifrolumab, an anti-IFNAR1 monoclonal antibody, met its primary endpoints of reduction in global disease activity score in patients with SLE and the level suppression of IFN signature was clearly associated with increased anifrolumab concentrations (123). Blocking IFNAR1 with anifrolumab reduced ISG expression more than blocking IFN-α with sifalimumab. Anifrolumab demonstrated better efficacy in the IFN signature high 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 Figure 2.

Other strategies directly targeted 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 autoantibodies and amelioration of glomerulonephritis (137). In NZB/NZW lupus-prone mice, inhibition of Bcl-2, a necessary molecule for pDC survival, resulted in selectively depletion of pDCs and

reduction of IFN-α production (138). Furthermore, proteasome inhibitors (carfilzomib, bortezomib) managed to suppress the IFN-α production by TLR-activated pDCs by inhibiting pDC survival and function in lupus mice models (139). More recently, the pDC inhibitory receptor BDCA-2 (CD303) has been used to block IFN-I production in pre-clinical studies (140).

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 (141). Interestingly, higher titers of anti-IFN-α antibodies were found in IFN signature positive patients, which were also linked to the reduction of IFN score.

Measuring Interferon 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 titers and complement levels.

The measurement of IFN-α itself by ELISA was used to monitor disease activity and predict response to IFN-I targeted therapies (23). Although early studies reported elevated levels of IFN-α in sera of SLE patients, the sensitivity of the method appears low, since IFN-I levels are either undetectable or dependent on several factors such as ethnic background, age, and sex (143-146). Indirect methods were also 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 (147, 148)

In research cohorts, 60-80% of lupus patients exhibit an increased expression of ISGs in PBMCs, known as interferon signature. In childhood-onset SLE the IFN signature is almost universally observed (133). Interferon scores are similar but are generally used to refer to a continuous parameter derived from qPCR rather than absence or presence of increased expression. Interferon signatures and scores consistently correlate with B cell biomarkers of activity such as titers of anti-dsDNA, anti-Ro, anti-U1RNP, anti-Sm autoantibodies and lower complement (C3) levels (134). IFN-I assays showed association with disease activity in cross-sectional studies (149, 151, 152). However, these were inconsistent and other studies failed to

demonstrate any association (153, 154). Longitudinal analyses of ISG expression in SLE patients have also given more complex results: although patients with higher IFN scores had greater disease activity, scores of individual patients could not predict flares (155). This discrepancy might be due to the choice of ISGs or methods used to derive unidimensional interferon scores from genome-wide micro-array data (156). 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 (153). However, this is complex to analyze; variations in methodology for measurement of IFN-I activity comparing activity between different organ domains is complex. That study used a categorical (Yes/No) definition of activity for each organ.

Given the pleotropic 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 of pathogenesis of SLE, most of these studies used unseparated leukocytes or whole blood (141). A recent study investigated the ISG expression in multiple sorted cell types, including monocytes, dendritic cells, NK cells, B and T lymphocytes, from SLE patients and showed distinct profiles in different cell types (158). A distinct gene expression profile has been recently identified even in 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 (103, 156, 160-162).

IFN signature might contribute to the early stages of the disease development, as the expression of certain genes has been linked to certain autoantibody profiles in patients with incomplete lupus erythematosus, suggesting that IFN signature might be used as a biomarker for individuals with higher risk for disease progression (163). 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 favor a pro-inflammatory phenotype. Interestingly, cytosolic nucleic acid sensing pathways were mostly upregulated in myeloid cells.

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

IFN-I activity is a common feature in most connective tissue diseases 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 is complex. 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 ligand, receptor, TLR or pDCs may have markedly different clinical effects.

Variable IFN-I activity within each connective tissue disease, with associated differences in response to therapy, suggest that reclassification of these diseases according to pathogenic mechanism may be more appropriate than existing organ-based classifications. This concept is illustrated in Figure 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, spondyloarthropathies, Crohn's disease and sarcoidosis) (148).

The PRECISESADS European consortium aims to reclassify systemic autoimmunity using 'omics for improved diagnosis and therapy (149). Within SLE 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 cross-talk 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.

Due to 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:

1. Understand 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.
2. Establish the efficacy of IFN-I blocking therapy in a wider range of autoimmune diseases.
3. Improved biomarkers that can accurately establish key pathogenic mediators in complex autoimmune diseases to fully realize the potential illustrated by IFN-I signatures.

Table 1: Pharmaceutical agents targeting the Type I interferon pathway

Pharmaceutical agent	Manufacturer	Definition	Target
Sifalimumab	MedImmune, Inc.	Fully human mAb	IFN-α
Rontalizumab	Genetech	Recombinant humanized mAb	IFN-α
AGS-009	Argos Therapeutics	Humanized IgG4 mAb	IFN-α
Anifrolumab	MedImmune, Inc.	Fully human mAb	IFN-α / β receptor
IFN-α-Kinoid	Neovacs	Vaccine	IFN-α
IMO-3100	Idera Pharmaceuticals	Oligonucleotide antagonist	TLR7/9 inhibition
DV1179	Dynavax	Oligonucleotide antagonist	TLR7/9 inhibition

Table 2: Effect of Type I interferon gene signature on response to targeted therapies in autoimmune diseases

Disease	Drug	Target	Assay	Clinical response if IFN-I biomarkers high	Description	Reference
RA	Rituximab	B cells	PBMC IFNGS	Worse	A 3-ISG qPCR interferon 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	Thurlings et al., 2010
RA	Rituximab	B cells	Whole blood IFNGS	Worse	A cluster of 8 ISGs on micro-array (LY6E, HERC5, IFI44L, ISG15, MxA, MxB, EPSTI1, RSAD2) was associated with lower change in DAS28 and EULAR response rate	Raterman et al., 2012
RA	TNF blockers	TNF-α	Reporter cell assay	Better	Patient with high IFNGS expression (IFIT-1, PKR, Mx-1) in reporter cells had higher EULAR response rate	Mavragani et al., 2010

Disease	Drug	Target	Assay	Clinical response if IFN-I biomarkers high	Description	Reference
RA	Infliximab	TNF-α	Neutrophil IFNGS	Better	Higher IFN-response gene expression (178 ISGs in total) in RA neutrophils correlates with a greater change in DAS28 and EULAR response rate	Wright et al., 2015
SLE	Rontalizumab	IFN-α	PBMC or Whole blood IFNGS	Worse	Patients with a low 3-gene IFNGS (HERC5, EPSTI, CMPK2) treated with rontalizumab had higher SRI-4 response rate and reduction in oral steroids compared to placebo or IFNGS high patients	Kalunian et al., 2016
SLE	Sifalimumab	IFN-α	Whole blood IFNGS	Same / Better	Patients with a high 4-gene IFNGS (IFI27, IFI44, IFI44L, RSAD2) had lower placebo response rate, and similar or slightly better SRI-4 rate on sifliamumab in comparison to placebo	Khamashta et al., 2016

Disease	Drug	Target	Assay	Clinical response if IFN-I biomarkers high	Description	Reference
SLE	Anifrolumab	IFNAR	Whole blood IFNGS	Better	Patients with a high IFNGS had lower placebo response rate, but much greater response on anifrolumab compared to placebo. Patients with low IFNGS had no improvement on anifrolumab compared to placebo	Brohawn et al., 2015
IIM	Rituximab	B cells	Serum IFN-regulated chemokine score	Better	IFN-regulated chemokines before treatment correlated with improvement in disease activity measures in refractory myositis patients treated with rituximab	López De Padilla et al., 2015
IIM	Rituximab	B cells	Muscle myeloid IFNGS expression	Better	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	Nagaraju et al., 2016

Legends to Figures

Figure 1. Pleiotropic roles of type I IFNs on cells of immune system

The stimulation of pDCs by increased apoptotic material and immune complexes via TLR-independent and TLR-dependent pathways culminates in the aberrant secretion of type I IFNs (IFN-α/β), which affects the function of multiple cell types. Type I IFNs promote the differentiation of monocytes into mDCs and significantly lower the activation threshold of autoreactive T and B cells. The production of several cytokines from mDCs, such as BLyS and APRIL, alongside type I IFNs enhances survival and activation of B cells, differentiation into plasma cells, antibody production and class-switching from IgM to IgG isotype. The promotion of Th1 and Th17 immune responses is also important mediating end-organ tissue damage in patients with SLE. The enhancement of cytotoxicity of both $CD8^+$ T cells and NK cells is another effect, while NK cells' role has recently acknowledged as positive regulators of IFN by pDCs by secretory molecules (e.g., MIP-1b) or cell-to-cell interactions. Notably, monocytes inhibit the type I IFN-promoting effect of NK cells via the production of several factors, such as TNF-α, PGE2 and ROS. Neutrophils are key players in inducing type I IFN production by pDCs in a DNA- and TLR9-dependent manner. Dying neutrophils are characterized by a special type of cellular death (NETosis); they release web-like structures known as neutrophil extracellular traps (NETs) composed of chromatin and granule proteins that can bind and kill microorganisms. Except for NETs' antimicrobial function, they also contain nuclear material, such as DNA, RNA and histones, and antimicrobial agents (LL37, HMGB-1. β-defensins) preventing nuclear acids from degradation. IFN-α itself can actually act as priming factor on both pDCs and mature neutrophils, which in turn secrete IFN-α and enhance its production by pDCs. Eventually, the formation of new RNA- and/or DNA-containing immune complexes trigger pDCs via activation of intracellular TLR7 and TLR9 respectively and amplify type I IFN production.

SLE: systemic lupus erythematosus; pDC: plasmacytoid dendritic cell; mDC: myeloid dendritic cell; NK: natural killer; Nφ: neutrophils; Mo: monocytes; Th: T helper; IFN: interferon; TLR: toll-like receptor; ICs: immune complexes; NETs: neutrophil extracellular traps; BLyS: B lymphocyte stimulator; APRIL: a proliferation-inducing ligand; TNF-α: tumour necrosis factor; PGE2: prostaglandin E2; ROS: reactive

oxygen species

Figure 2: Interferon gene signature and response to interferon-targeted therapies

Three phase II studies of IFN-I blocking therapies have been published. Definitions of biomarker positive patients and clinical response varied slightly between these studies. However, in each case there was a different relationship between presence of interferon biomarkers and clinical response. (A) shows data for rontalizumab – biomarker negative patients responded better than biomarker positive. This may be because therapy did not effectively neutralize stronger IFN-I activity, but does suggest that biomarker negative patients may not have IFN-I independent disease. (B) shows data for sifalimumab. Efficacy was confirmed in biomarker-positive patients (n=350). Number of biomarker-negative patients was smaller (n=81) so it is difficult to compare response rates. However, the difference between placebo response rates appears more striking than the difference between placebo and active treatment arms within each biomarker category. (C) shows data for anifrolumab. This study had 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. For references see main text.

Figure 3: The concept of therapy-based classification of autoimmune connective tissue diseases.

Traditional organ-based classifications of autoimmune diseases are based on syndromes of individual clinical features. However, these overlap between different diseases and many patients with undifferentiated connective tissue diseases do not fit into any single diagnosis. Due to overlap in pathogenic mechanisms between these diagnoses, they are usually treated with the same range of therapies in individual trials for each diagnosis. However, response rates in these trials are variable. Recent data on IFN-I indicate that response rates are higher if diagnostic groups are subdivided using biomarkers rather than clinical features. From a therapeutic point of view, IFN-I high SLE patients are more closely related to IFN-I high Sjögren's patients than IFN-I low SLE patients. Existing classifications therefore appear increasingly arbitrary when considering ultimate therapeutic strategies. It may be more appropriate to re-classify patients according to the dominant pathogenic

mechanism, and therefore appropriate therapeutic target instead of pattern of organs affected. This is analogous to the classification of bacterial infections according to microbial agent, and therefore antibiotic, rather than site of infection.

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