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The promise of precision medicine in rheumatology

Joel M Guthridge[1,2], **Catriona A Wagner**[1], **Judith A James**[1,2,*]

[1]Arthritis and Clinical Immunology, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA

[2]Departments of Medicine and Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

Abstract

Systemic autoimmune rheumatic diseases (SARDs) exhibit extensive heterogeneity in clinical presentation, disease course, and treatment response. Therefore, precision medicine – whereby treatment is tailored according to the underlying pathogenic mechanisms of an individual patient at a specific time – represents the 'holy grail' in SARD clinical care. Current strategies include treat-to-target therapies and autoantibody testing for patient stratification; however, these are far from optimal. Recent innovations in high-throughput "omic" technologies are now enabling comprehensive profiling at multiple levels, helping to identify subgroups of patients who may taper off potentially toxic medications or better respond to current molecular targeted therapies. Such advances may help to optimize outcomes and identify new pathways for treatment, but there are many challenges along the path towards clinical translation. In this review, we discuss recent efforts to dissect cellular and molecular heterogeneity across multiple SARDs and future directions for implementing stratification approaches for SARD treatment in the clinic.

Introduction

Systemic autoimmune rheumatic diseases (SARDs) are characterized by dysregulated immunity and inflammatory responses, resulting in damage and destruction to joints, connective tissues, skin, blood elements, and other target organs. Patients with SARDs are often initially treated with generalized immunosuppressive treatment regimens associated with considerable toxicity and side effects[1]. If their condition does not adequately respond to these treatments, targeted immunotherapies or biologic disease-modifying antirheumatic drugs (bDMARDs) are often used. No validated predictive biomarkers of treatment response exist, and clinical decisions are made based on symptoms, treatment guidelines, provider experience, and medication access. As a result, treatment responses to bDMARDs vary considerably and are often suboptimal[2]. In addition, no FDA-approved bDMARDs exist for some SARDs, such as primary Sjögren's syndrome (pSS), partly due to disease

*Corresponding Author: Judith A. James, MD, PhD, Arthritis and Clinical Immunology, Oklahoma Medical Research Foundation, 825 N.E. 13th Street, Oklahoma City, Oklahoma 73104, Tel (405) 271-4987, Fax (405)271-7063, Judith-James@omrf.org.

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heterogeneity complicating clinical trials and care. Therefore, stratification of patients based on the underlying disease mechanisms may be necessary to identify novel therapeutic targets, design effective clinical trials, and select the optimal therapeutic interventions.

Unlike traditional clinical care, precision medicine tailors targeted therapies to the molecular characteristics of individual patients. Molecularly-informed treatment approaches are already used to select effective treatments for cancer patients according to tumor-specific genomic markers[3]. For example, solid tumor patients with neurotrophic tyrosine receptor kinase (*NTRK*) gene fusions can be treated with tropomyosin receptor kinase inhibitors – such as entrectinib and larotrectinib – independent of cancer type[4]. Through advances within the rheumatology field, genetic analyses have enabled a deeper understanding of the mechanisms of monogenic autoinflammatory diseases (characterized by dysregulated innate immunity), allowing for molecular diagnosis and the use of targeted therapies, such as IL-1 receptor antagonists for inflammasome-mediated autoinflammatory diseases[5].

The use of precision medicine for SARDs has been more challenging, as SARDs are polygenic and involve a complex interplay between genetic and environmental factors. However, some aspects of precision medicine are currently used in rheumatology to guide treatment and monitor disease activity. As a long-standing example, in gout patients, medication doses are titrated until patients achieve a serum urate level less than 6mg/dl to limit toxicities while optimizing treatment[6]. In SARDs, including pSS, systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), systemic sclerosis (SSc), and anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV), testing for autoantibodies (those directed against self-proteins, signifying autoimmunity) is often used as one component to aid diagnosis or for patient stratification. For example, the presence of antibodies against citrullinated peptides is associated with extra-articular manifestations of RA and helps guide treatment decisions[7], while positivity for antibodies against nuclear proteins or anti-double-stranded DNA is used to determine eligibility for belimumab treatment in SLE[8,9]. Laboratory testing of autoantibodies and other serological markers, such as complement, complement split products, and panels of soluble mediators, is also used to measure SLE disease activity and flare[10]. Furthermore, molecular stratification in rheumatology clinical trials has already shown some benefits. In a recently published phase II trial of iberdomide (whose targets include the transcription factor Aiolos) in moderate-to-severe SLE, prespecified stratification analyses demonstrated a higher response in patients with elevated Aiolos or type I IFN gene signatures at baseline[11]. Despite many advances, the implementation of precision medicine in SARDs is still in its infancy.

Recently, technological advances in molecular characterization and computational biology approaches (Box 1) have allowed for the identification of patient subgroups within each SARD (Supplementary Table 1), suggesting that diverse molecular pathways contribute to disease pathogenesis. Therefore, as in cancer and autoinflammatory diseases, tailoring treatments to the underlying molecular pathways of each disease using precision medicine is a promising approach to treating SARDs. In this review, we discuss recent studies dissecting the molecular heterogeneity of SARDs; we also consider the promise and the challenges of translating these findings into improved outcomes and precision clinical care.

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Patient stratification in rheumatologic disease

Rheumatoid arthritis (RA)

RA is a chronic joint disease with synovial inflammation resulting in joint damage and disability. Several studies have identified peripheral blood biomarkers for RA[12]. However, molecular signatures in the synovial tissue may not be reflected in the peripheral blood[13]; therefore, recent studies have focused on deciphering heterogeneity within the synovium. A pivotal study in 2014 used synovial gene expression to identify four RA patient clusters with lymphoid, myeloid, low inflammatory, or fibroid gene signatures[14] and since then, similar patient clusters have been observed using RNA sequencing[15]. Recent studies have expanded on these findings in treatment-naive patients with RA, identifying three patient clusters: "lympho-myeloid," with abundant B cells and monocytes/macrophages, "diffuse-myeloid", with predominantly monocytes/macrophages and low numbers of B cells, and "pauci-immune", with elevated fibroblasts and low levels of inflammation[16,17]. Notably, observational studies suggest that these synovial phenotypes may predict response to treatment, as a myeloid signature is associated with response to TNFα inhibition[14], while the pauci-immune signature is associated with inadequate response to TNFα inhibition[18].

Approximately half of RA patients have low levels or a lack of CD20+ B cells in the synovium[14], consistent with the heterogeneous response to the anti-CD20 monoclonal antibody rituximab in RA patients[19]. In the first biopsy-driven multi-center clinical trial in RA (R4RA), patients classified as CD20 B cell-poor by RNA sequencing of synovial biopsies exhibited a better treatment response to the IL-6 receptor inhibitor tocilizumab compared to rituximab[20]. Tocilizumab and rituximab were similarly efficacious in B cell-high patients[20]. Although further validation is necessary, these data suggest that synovial immune signatures may be able to predict clinical responses and, in the near future, guide treatment decisions in RA.

Recent single-cell analyses have focused on defining novel disease-associated cell subsets within the inflamed synovium of RA patients to identify new molecular pathways, biomarkers, and therapeutic targets, as previously reviewed[21,22]. For example, single-cell RNA-sequencing analyses identified an expanded population of Thy1+ fibroblasts in the sublining of RA synovium regulated by Notch signaling[23–26]. The synovial sublining fibroblasts express chemokines and cytokines, such as IL-6, and induce inflammation with minimal effects on bone and cartilage destruction[25,27]. Furthermore, expansion of a novel cell type that shares molecular features with synovial sublining fibroblasts, termed pre-inflammatory mesenchymal (PRIME) cells, is observed in the blood one week prior to RA flare, following activation of B cells[28]. Peripheral PRIME cells decrease during disease flare, suggesting that PRIME cells migrate to the synovium, where they differentiate into sublining fibroblasts and contribute to disease pathogenesis[28].

Unique populations of peripheral CD4+ T cells have also been demonstrated in RA synovial tissue. In seropositive RA, a large proportion of synovial CD4+ T cells express PD-1, IL-21, and CXCL13, consistent with a follicular helper T cell (Tfh) phenotype and suggesting a role in B cell responses and antibody production[29]. However, unlike Tfh cells, the PD-1+ cells in RA synovial tissue, termed peripheral helper T cells (Tph), are primarily CXCR5-

negative and express chemokine receptors (CCR5 and CCR2) that induce migration to inflamed tissues[29]. Therefore, Tph cells may promote ectopic lymphoid structure formation and autoantibody production within the synovium. Taken together, these studies have identified novel pathogenic subsets within the inflamed tissue of RA. Expanding research on these unique cell subsets and determining whether they are differentially expressed in synovial patient clusters may help define predictive biomarkers and tailor therapies for RA patients.

Psoriatic arthritis (PsA)

Psoriatic arthritis (PsA) is a common component of the psoriasis spectrum disorders, occurring in up to 30% of psoriasis patients. PsA can affect the axial skeleton, peripheral joints, and entheses[30]. Recent advances have established the role of Th1 and Th17 cells and their associated cytokines – such as TNFα, IL-12, IL-23, and IL-17 – in PsA pathogenesis[31]. This in turn has resulted in the approval of several targeted therapeutics for PsA, including TNF inhibitors and bDMARDs targeting IL-12 and IL-23 (ustekinumab), IL-23 (guselkumab, risankizumab) and IL-17 (ixekizumab, secukinumab)[32]. However, treatment decisions are challenging given the broad array of approved therapeutics and the scarcity of predictive molecular biomarkers. In addition, treatment responses can differ between the skin and joints. For example, while approximately 50% of PsA patients achieve 75% improvement in psoriasis following treatment with IL-23 or IL-17 inhibitors, the same percentage only achieve a 20% improvement in joint disease[33,34]. This may reflect distinct gene expression patterns in the skin and synovium, as both IL-17 and IL-23 gene signatures are elevated in the skin compared to the synovium[35,36]. Synovial expression of IL-23 is also dependent on synovial inflammation and histological features, which may contribute to the heterogeneous response in joint disease following treatment with inhibitors of the IL-17–IL-23 axis[36]. Therefore, defining predictive biomarkers of treatment response that reflect heterogeneity in both the skin and joint of PsA patients is imperative to help optimize treatment decisions.

Genetic, transcriptomic, proteomic, and epigenomic approaches have identified potential molecular biomarkers to guide therapy in PsA, as extensively reviewed elsewhere[32,37]. In addition, T cell phenotypes have emerged as a potential tool to predict response to approved bDMARDs. Based on flow cytometry analysis of peripheral blood, Miyagawa et al. demonstrated that PsA could be stratified into four distinct subtypes based T cell phenotyping, namely activated Th1 cell-predominant, activated Th17 cell-predominant, Th1/Th17-high, and Th1/Th17-low[38]. In a proof-of-concept study, the same group stratified bDMARD treatment based on these T cell phenotypes, with ustekinumab administered to the activated Th1 cell-predominant patients, secukinumab to the activated Th17 cell-predominant patients, secukinumab or TNFα inhibitor to the Th1/Th17-high patients, and TNFα inhibitor to the Th1/Th17-low patients[38]. After 6 months of treatment, a higher proportion of patients who received bDMARDs according to T cell phenotype achieved low disease activity in the skin and joints compared to patients who received bDMARDs based on the 2015 European Alliance of Associations for Rheumatology (EULAR) recommendations[38]. Although these findings need to be validated in larger, randomized

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studies, stratifying patients with PsA based on helper T cell profiles may have potential for precision medicine.

Systemic lupus erythematosus (SLE)

SLE is a complex, multi-organ autoimmune disease marked by pathogenic autoantibodies and chronic inflammation. Early studies demonstrated that approximately half of SLE patients have stable, elevated levels of type I IFN causing dysregulated expression of IFN genes, which correlates with disease activity, specific clinical manifestations, and the presence of autoantibodies, which all points to type I IFN as a potential therapeutic target[39]. Indeed, anifrolumab, a monoclonal antibody against type I IFN receptor subunit 1, reduces disease activity and recently received FDA approval for treatment of adults with moderate to severe SLE following the phase II MUSE trial and phase III TULIP-1 and −2 trials[40–42]. However, only about 50% of patients responded to anifrolumab in the TULIP-2 trial[40]. In addition, although a phase IIb clinical trial demonstrated greater efficacy of anifrolumab in patients with high IFN gene signatures[41], the phase III trial showed a similar treatment response in patients with high and low IFN gene signatures[40]. These nuanced data highlight the need for additional stratification approaches to identify predictive molecular signatures of drug response, as well as additional novel therapeutic targets in SLE.

Several studies have molecularly stratified SLE patients into more homogenous groups based solely on transcriptomics[43–49], epigenomics[50–52], serological profiles[53–56], or cellular phenotyping[57] approaches. In a longitudinal study, Banchereau et al. profiled the blood transcriptome of pediatric SLE patients, revealing seven patient subgroups based on different immune signatures – including type I IFN, neutrophils and myeloid cells, and plasmablasts – that correlated with disease activity [45]. Using the same pediatric data[45] and an additional adult SLE patient cohort, Toro-Dominguez et al. selected genes that correlated with disease activity to cluster patients, identifying three disease clusters characterized by a lymphocyte or neutrophil signature[46]. Furthermore, the associated drug-induced gene expression signatures differed between the lymphocyte- and neutrophil-driven clusters, suggesting that the clusters respond differently to treatments[58]. In another study of adult SLE patients, blood transcriptome profiles identified different subgroups of SLE based on disease activity with different molecular pathways, including immune activation, oxidative phosphorylation, and cell metabolism[47]. Notably, neutrophil signatures were associated with active nephritis in all three studies[45–47].

Multiplex analysis of 10 serum cytokines and chemokines also revealed distinct groups of SLE patients[56]. Patients with active SLE were characterized mainly by elevated SLE-associated cytokines, such as IFNα and BlyS, or CXCL10 and CXCL13, while patients with inactive SLE predominantly expressed low levels of these cytokines[56]. In addition, DNA methylation patterns are variable in SLE patients and correlate with disease activity[50], and changes in DNA methylation of naïve CD4+ T cells are associated with clinical manifestations of SLE[51,52]. Immune cell phenotyping of juvenile-onset SLE patients also revealed subsets of patients with distinct immune cell profiles, with significant differences in CD8 T cell phenotypes associated with disease activity[57].

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A recent cross-sectional analysis integrated gene expression modules, autoantibody specificities, soluble mediator levels, and clinical data with unsupervised machine learning to identify seven SLE patient clusters with distinct molecular pathways[59]. Clinical features were similar across the clusters, suggesting that different mechanisms may contribute to similar clinical manifestations[59]. Three of the clusters had elevated IFN signatures, consistent with previous studies[45]. In a separate study, single-cell RNA sequencing of peripheral blood mononuclear cells from pediatric SLE patients identified eight cell subgroups that contributed to this IFN signature, including subpopulations of CD4+ and CD8+ T cells, conventional and plasmacytoid dendritic cells, monocytes, NK cells, and plasma cells[60]. Hierarchical clustering of these cell subgroups identified six patient clusters and demonstrated that expansion of these cell groups correlated with increased disease activity[60].

In addition, patients with lupus nephritis (LN; a complication of SLE) exhibit an elevated IFN signature in most active leukocytes as well as keratinocytes and tubular cells from skin and renal biopsies, and an elevated IFN signature in tubular cells is associated with non-response to treatment[61,62]. Of note, a recent study from the Accelerating Medicines Partnership RA/SLE Network demonstrated that the majority of patients with high urinary expression of IFNγ-inducible chemokines have the most aggressive form of LN (known as proliferative LN)[63]. However, a subset of patients with proliferative disease clustered with those with non-proliferative disease, suggesting that stratifying LN patients by chemokine expression may better define classes of LN with similar molecular pathways compared with the current histology-based classification system[63]. In addition, the same group found that reduced urinary IL-16 levels are associated with response to treatment in LN patients[64]. Together, these studies provide a rationale for molecular stratification approaches for defining classes and treatment responses in LN.

Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV)

AAV is characterized by inflammation within, and eventual destruction of, small and medium-sized blood vessels. ANCAs are detected in most AAV patients, most commonly targeting myeloperoxidase (MPO) and proteinase-3 (PR3). AAV is traditionally divided into three disease groups – granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis (EGPA) – based on clinical and histological features[65]. Specifically, GPA causes a medium and small vessel vasculitis that can be severe, with pulmonary, renal, and other vascular involvement. Up to 90% of severe GPA patients exhibit cytoplasmic ANCA staining associated with anti-PR3 antibodies[66]. In contrast, MPA is characterized by necrotizing vasculitis of small and medium blood vessels without granulomatous inflammation, often with less severe renal involvement, and perinuclear ANCA staining, associated with anti-MPO antibodies[66].

To detect additional biomarkers for the treatment of AAV, McKinney et al. genetically profiled purified leukocytes isolated from GPA and MPA AAV patients[67]. CD8 T cell gene expression profiles divided AAV patients into two subgroups that predicted prognosis[67]. The poor prognosis group exhibited a genetic signature enriched for genes involved in the IL-7R pathway, TCR signaling, and genes expressed by memory T cells[67]. Furthermore, a

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CD8 T cell exhaustion gene expression signature is associated with a better prognosis in AAV patients[68]. Together, these studies suggest that CD8+ memory T cells may contribute to disease severity in a subset of AAV and that targeting this pathway may be an effective treatment. In addition, patients with PR3-ANCA positive and MPO-ANCA positive AAV spanned both transcriptional clusters[67,68], indicating further molecular heterogeneity within patients with the same ANCA specificity.

In 2012, a genome-wide association study (GWAS) of patients with GPA and MPA found that ANCA specificity distinguished two distinct disease subsets, independent of clinical classification[70]. Specifically, single nucleotide polymorphisms in the human leukocyte antigen (HLA)-DPB1 region and genes encoding PR3 (*PRTN3*) and α1-antitrypsin (*SERPINA1*) were associated with presence of ANCAs specific for PR3 (PR3-ANCA positive AAV), while SNPs in the HLA-DQ region were associated with presence of ANCAs specific for MPO (MPO-ANCA positive AAV)[70]. ANCA specificity may also reveal subsets of EGPA patients with different disease mechanisms and responses to treatments[69]. Therefore, PR3- and MPO-ANCA positive AAV may represent distinct disease entities with different disease mechanisms and responses to treatment. However, although *post hoc* analyses of clinical trials found that patients with PR3-ANCA positive AAV were more likely to respond to rituximab compared to cyclophosphamide[71,72] and were at increased risk of relapse following treatment with mycophenolate mofetil compared to cyclophosphamide[73], neither study showed evidence that treatment effects differed by ANCA subtype[71,73]. Therefore, prospective studies are needed to determine the value of ANCAs as predictive biomarkers of treatment response.

Systemic sclerosis (SSc)

SSc is a connective tissue disease resulting in progressive fibrosis and vascular abnormalities. The hallmark symptom of SSc is skin fibrosis; however, fibrosis can occur in multiple organs, such as the lung, heart, and kidney, which determines clinical outcome[74]. Historically, SSc is clinically classified based on the extent of skin fibrosis — as limited cutaneous SSc (lcSSc) if fibrosis is limited to the fingers, distal extremities and face, or as diffuse cutaneous SSc (dcSSc) if fibrosis extends to the trunk and proximal extremities[75,76]. Furthermore, the classical SSc-specific autoantibodies are differentially associated with major clinical subsets, organ manifestations, and overall prognosis, helping in the diagnosis and classification of SSc patients. Specifically, anti-centromere autoantibodies are associated with lcSSc and usually better prognosis, while anti-topoisomerase autoantibodies are associated with dcSSc, interstitial lung disease, organ fibrosis, and worse prognosis[77]. Anti-RNA polymerase III autoantibodies are also associated with dcSSc, as well as renal crisis and earlier mortality[78]. However, the disease course and major organ manifestations are variable within each subset[76,79,80], indicating that classification of SSc based on cutaneous involvement alone does not fully capture the heterogeneity of the disease.

Compared to healthy controls, dcSSc patients express distinct inflammatory and fibrotic gene expression signatures in both lesional and non-lesional skin biopsies[81,82]. Extending on these findings, Milano et al. found that SSc patients could be divided into four subsets based on gene expression signatures[83]. One subset, characterized by expression of cell

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proliferation genes and labelled "diffuse-proliferation" or "fibroproliferative" was solely comprised of dcSSc patients — while another group was termed "limited" as it consisted of only lcSSc patients[83]. However, dcSSc and lcSSc patients were not found exclusively in these two groups; they were also found within molecular subgroups displayingincreased expression of genes associated with innate and adaptive immune responses (termed "inflammatory" subgroup) or with similar gene expression patterns to healthy controls (termed "normal-like")[83]. These subsets have been confirmed in additional cohorts and affected organs[84–90]. Together, these studies suggest that distinct molecular signaling pathways can lead to SSc, which is not reflected by clinical classification.

Further analysis into the molecular pathways underlying these subsets demonstrated that the fibroproliferative subset was strongly associated with platelet-derived growth factor and cell proliferation gene signatures[86,89], while the inflammatory subset was enriched for IFN response, B cell receptor signaling, monocyte chemotaxis, M2 macrophage activation, adaptive immune response, and NFκB-activating innate immune response pathways[86,89]. Interestingly, consensus analysis found that extracellular matrix remodeling and *TCRβ* gene expression modules were conserved within both the inflammatory and the fibroproliferative subsets[89]. These findings may indicate that the two subsets have different mechanisms that converge on the same outcome or that the subsets are dynamic and interconnected. However, no statistically significant differences exist in disease duration between the inflammatory and fibroproliferative subsets[83], and longitudinal analyses have demonstrated that these subsets are stable for at least 12 months[84].

Several studies have suggested the utility of these four molecular signatures in the targeted treatment of SSc. Patients in the inflammatory subgroup are more likely to respond to mycophenolate mofetil and abatacept[85,91,92], and exhibited worsening skin and lung fibrosis following dasatinib[93]. In addition, a small study of two patients in the fibroproliferative subgroup demonstrated an imatinib mesylate-responsive signature[94]. To allow stratification of SSc patients in the clinic, Franks et al. developed a machine-learning classifier based on the four previously-defined molecular signatures that could identify molecular subsets for individual patients with an accuracy of 85.4%[95]. However, more studies are needed to determine whether this classifier can accurately identify patients likely to respond to a specific therapy.

Primary Sjögren's Syndrome (pSS)

pSS is characterized by lymphocytic infiltration of the exocrine glands, primarily the lacrimal and salivary glands, resulting in severe and persistent dryness of the mouth and eyes. Approximately half of pSS patients also experience extraglandular manifestations, such as arthritis, lung disease, and lymphoma[96]. Elevated type I and type II IFN signatures have been demonstrated in the blood and salivary glands of subsets of pSS patients[97–102], which is associated with increased prevalence and titers of anti-Ro/SSA and anti-La/SSB autoantibodies and higher disease activity[100,103]. Bodewes et al. identified three subgroups of pSS patients based on three modules of systemic (measured in whole blood) IFN genes previously identified in SLE: no IFN activation, only type I IFN activation, and both type I and type II IFN activation[104]. Similarly, the salivary gland patterns of IFN expression are

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heterogeneous between pSS patients, with patients stratifying as type I IFN-predominant, type II IFN-predominant, or mixed type I and type II IFN[105].

To gain a more comprehensive understanding of pSS heterogeneity, recent studies have focused on broader panels of whole blood gene expression signatures, other soluble mediator levels, and autoantibodies to profile pSS patients. Clustering of previously identified transcriptional modules revealed three pSS patient clusters that differed in the expression of IFN and inflammation modular network signatures[106]. However, these clusters did not differ in demographics or clinical characteristics, including the prevalence of autoantibodies[106]. Using a similar approach, Soret et al. identified 4 groups of pSS patients with differing type I and type II IFN responses[107]. Multi-omic characterization of these clusters demonstrated different patterns of immune dysregulation, including B cell activation or neutrophil infiltration. Therefore, although patient clusters have not been used in clinical trials of pSS, patient subsetting may be of benefit as more therapeutic agents are investigated.

Challenges for implementing precision medicine in SARDs

Several challenges to the implementation of current and evolving findings in clinical care are shared across the SARDs. An unmet need in rheumatology is for more precise measures of treatment response in clinical trials and practice. Currently, due to the heterogeneity and multisystem manifestations of SARDs, disease improvement is measured using composite indices of multiple clinical parameters. Determining the best treatment outcome measure is crucial to the success of a clinical trial, as evident by the phase III TULIP-1 and –2 clinical trials of anifrolumab in SLE. The first trial, TULIP-1, failed to meet its primary endpoint as measured by the SLE Responder Index but met some secondary endpoints, including the BILAG-Based Composite Lupus Assessment (BICLA)[42]. Thus, in a second clinical trial, TULIP-2, the primary endpoint was changed to BICLA response, resulting in a significantly higher percentage of patients with a response compared to placebo[40]. However, all of the currently utilized measures of treatment response have several pitfalls, including limitations in the features they include, the inability to measure partial response, and inaccuracies in defining mild and moderate flares[108]. In addition, many of these systems are complex, require physician training, and may not be feasible in real-world clinical settings. Therefore, including molecular signatures that reflect the underlying mechanisms of disease activity may help improve clinical assessment tools for SARDs, resulting in more accurate measurements of treatment response and more meaningful endpoints for patients.

The majority of transcriptomic stratification studies in SARDs have used heterogeneous populations of cells from whole blood or peripheral blood mononuclear cells; however, gene expression differences may be due to the proportion of individual cell types sampled. Therefore, single-cell analyses may reveal additional and more accurate pathotypes and molecular signatures of disease, especially for lower abundance cell subsets. In addition, peripheral blood analyses may not reflect local inflammation and molecular heterogeneity within the tissues, as suggested in RA[13]. One approach has been to purify single populations of immune cells prior to transcriptome analysis[44,67,68], demonstrating gene expression differences not observed in peripheral blood mononuclear cell analysis[44]. Another study

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showed SLE heterogeneity at the single-cell level using single-cell RNA sequencing[60], which identified novel cell populations that may be targeted therapeutically. Most recently, two studies combined single-cell RNA sequencing with genotype data to demonstrate cell type-specific gene expression in autoimmune diseases, including SLE[109,110]. Importantly, stratification of SLE patients based on the expression of genes differentially expressed in at least one cell type between SLE patients and controls revealed two patient clusters that differed in myeloid cell activation and expression of the IFN-induced protein IFITM3, as well as the presence of disease flare and anti-Smith antibodies (which are characteristic of SLE)[110]. Currently, there are practical limitations to the use of cell- and tissue-specific analyses in the clinic; however, these analyses will provide further important information on cell- and tissue-specific mechanisms within patient clusters, potentially identifying new biomarkers and therapeutic targets.

Overall, the disease course of SARDs is complex with a wide range of disease stages, including preclinical, active disease, disease flare, suppression, and remission, with potentially different underlying molecular mechanisms and signatures (Fig. 1). Most studies only analyze patients with active disease and severe symptoms. However, some studies have demonstrated relative stability of patient clusters for up to two years[46,84,111], while others have found that standard-of-care treatments are associated with changes in transcriptomic profiles[45,112,113]. Therefore, more longitudinal studies incorporating multiple disease stages are required to understand how the molecular signatures change with disease progression, medication use or treatment sequencing, organ damage, and external triggers, such as infections or vaccination. Furthermore, these studies may also provide insights into preventing disease onset and flare.

The prevalence, clinical and serological manifestations, and outcomes of SARDs vary among racial groups[114] and sex[115], suggesting that the underlying disease mechanisms may also differ between these patients. Recent studies have found that SLE gene expression signatures differ significantly in self-identified African American, Native American, Asian, and European American patients[113,116]. However, race-based subgroups may still contain further genetic heterogeneity and differences in clinical outcomes according to race could likely reflect other factors beyond biologic ones. Therefore, caution is warranted when associating race and underlying biologic mechanisms. In addition, many stratification approaches focus exclusively on female and European ancestry cohorts, or use combined cohorts without considering race or sex effects. Therefore, studies need to be conducting on diverse cohorts so that study populations adequately represent clinic patients and are more widely generalizable.

Future Directions

Despite recent advances, much work remains to realize the potential of precision medicine in the rheumatology clinic. Although recent studies have demonstrated the feasibility of identifying relatively homogenous molecular subsets within SARDs and the clinical benefit of stratifying patients prior to treatment[20] (Fig. 2, Supplementary Table 1), no standard method to stratify patients currently exists. Therefore, studies within and across diseases must be integrated to identify common underlying molecular signatures that can most

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effectively subtype patients. Furthermore, additional heterogeneity within the identified molecular clusters may need to be resolved. Dissecting this heterogeneity will require deeper layers of individual molecular profiling, incorporating genetic risk, cell-specific transcriptional signatures, epigenomics, mass spectrometry, tissue and in vitro molecular signatures, and environmental factors (Box 1). These evolving technologies are especially important for SARDs such as pSS, in which there are no approved bDMARDs, and AAV, in which extensive "omic" approaches have not yet been applied.

Multiomics approaches in biofluids such as blood, urine, synovial fluid, and saliva, or in the target tissue requires implementing artificial intelligence and machine learning to uncover signals or biomarkers that stratify patients by predicted treatment response, potential for adverse events, or disease course. These molecular biomarkers or variables would need to be developed into specific algorithms to stratify patients and then validated in additional studies and cohorts. Longitudinal studies would also need to be performed to evaluate the impact of therapeutics on individual molecular signatures or patient clusters. In addition, to be practical for use in the clinic, any algorithm should be refined to be fast, inexpensive, reliable, and accessible from a relevant and practical tissue source. Further investigation into the application of novel individualized patient treatment response screening assays using peripheral blood or ex-vivo tissue explants[117] or pluripotent stem cell organoid cultures[118], are also warranted.

Some features are shared across different SARDs, including affected tissues, presence of autoantibodies, susceptibility genes (such as the HLA region), molecular signatures, and type I IFN signatures[119] (Fig. 2). In addition, therapies targeting similar pathways are effective for multiple SARDs, suggesting that these diseases share pathogenic mechanisms. In a recent study from the PRECISE systemic autoimmune disease consortium, stratification of patients with seven autoimmune diseases (including SLE, RA, SSc, and pSS) revealed four patient clusters with inflammatory, lymphoid, IFN, or healthy-like molecular signatures, based on transcriptome and methylome data[111]. Importantly, all disease groups were represented in each cluster, suggesting that stratification was independent of clinical diagnosis[111]. Similar findings have been observed using immune cell phenotypes[120,121] and soluble mediator profiles[122,123]. Together, these data demonstrate that SARDs share common molecular pathways; therefore, classifying these patients based on molecular signatures instead of clinical diagnosis may benefit treatment selection in the future. Further studies analyzing pathways across active SARD patients and meta- and cross-disease analyses spanning shared datasets are required to confirm and refine these shared molecular signatures.

Conclusion

While precision medicine is still in its infancy in rheumatic disease, advancements in molecular profiling technologies and stratification techniques gives hope for new therapeutic options and personalized treatments in SARDs. The discovery of cellular and molecular subgroups across SARDs provide meaningful explanations for the vast heterogeneity in rheumatic disease. Further clinical response trials and longitudinal outcomes studies using these identified subgroups may help determine the best stratification algorithms to optimize

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treatments. In addition, larger, longitudinal studies incorporating multiple SARDs for deep molecular profiling are necessary to determine signature stability, overlap among the diseases, and more precise patient stratification for translation to clinical care. Finally, sound computational approaches that compare patient subgroup and drug-effect gene expression profiles could guide the repurposing of existing drugs[124]. In closing, we see promise in recent molecular stratification strategies to guide drug discovery and increase treatment effectiveness in rheumatology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Box 1.

Current and emerging approaches for molecular characterization of systemic autoimmune rheumatic disease patients

Current

- Autoantibodies
- Clinical Imaging
- Clinical lab testing: Complement levels and split products
- Soluble mediators: Cytokines, chemokines, and soluble receptors
- Transcriptomics: Molecular signatures
- Genetics: Disease associated variants
- Immunophenotyping: Flow cytometry
- Tissue histology

Emerging

- Genetics: Genetic load, polygenic risk scores, extended HLA haplotypes
- Transcriptomics: Cell-specific expression/signatures (scRNA-seq)
- Immunophenotyping: Single-cell proteomics (CyTOF), Proteogenomics (CITE-seq), Repertoire immunomics
- Perturbomics (Multi-omic evaluation after stimulation or other perturbation conditions)
- Spatial tissue analytics: Multiplex tissue imaging (CODEX, serial IHC)
- Imaging mass cytometry (Hyperion, IonPath)
- Epigenomics (sorted cell and single-cell): DNA methylation, histone modification, chromatin conformation (ATAC-seq), protein-DNA interactions (CUT&RUN)
- Mass spectroscopy (biofluid) and imaging mass spectrometry (tissue): Proteomics, metabolomics, lipidomics, and glycomics
- Environmental factors: Microbiomics, Exposomics

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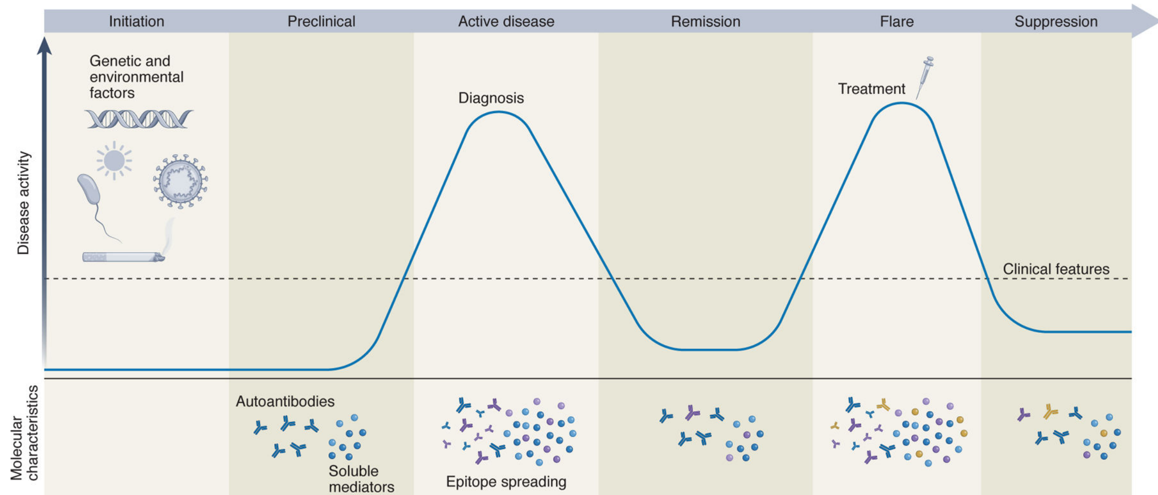

Fig. 1. Molecular mechanisms and signatures may differ according to disease stages.

The disease course of systemic autoimmune rheumatic diseases is complex with various disease stages. In patients with a genetic predisposition, autoimmunity may be initiated following environmental triggers, such as viral infection, the microbiome, low vitamin D, and smoking. Patients experience a preclinical phase following autoimmunity initiation with soluble mediator and autoantibody (autoab) production but no clinical symptoms. As the disease progresses, patients experience periods of active disease, in which clinical features are present, and the patients are diagnosed. This stage is characterized by increases in the levels of autoantibodies and soluble mediators, as well as alterations in the soluble mediator profiles and epitope spreading. Active disease can be followed by periods of remission and disease flare, as well as disease suppression following treatment. Changes in levels or the profiles of soluble mediators and antibodies may occur in each of these disease phases, highlighting the need for longitudinal studies incorporating multiple disease stages.

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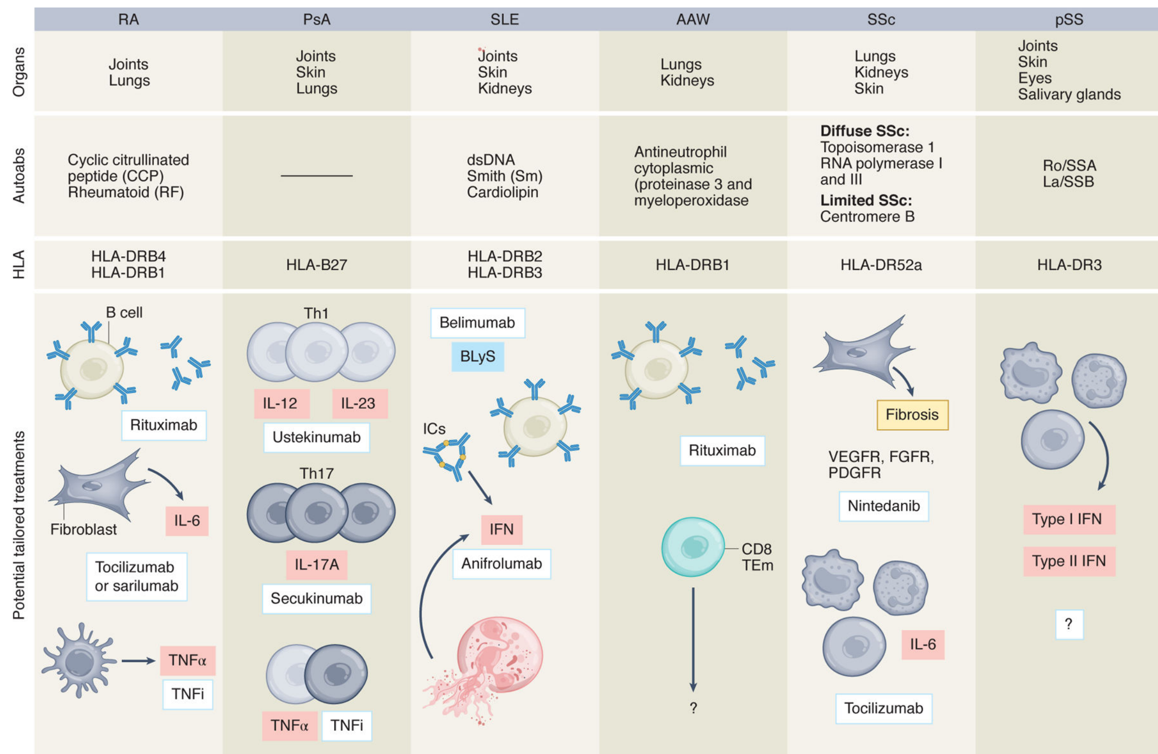

Fig. 2. Potential tailored treatment regimens based on recently identified molecular subgroups. Systemic autoimmune rheumatic diseases (SARDs) share common features, including the dominant affected organs, presence of autoantibodies (autoabs), association with human leukocyte antigen (HLA) regions, and FDA-approved biologic disease-modifying antirheumatic drugs (bDMARDs). For some SARDs, molecular subgroups suggest the potential for precision use of these bDMARDs. **(A)** Patients with rheumatoid arthritis (RA) can be stratified according to B cell, myeloid cell, and fibroblast molecular synovial signatures. Patients with a myeloid cell signature respond well to TNF inhibition[12], while those with low B cells respond better to the IL-6 receptor inhibitor tocilizumab than to B cell depletion with rituximab[18] (which may be more effective in patients with a B cell signature). (**B**) Patients with psoriatic arthritis (PsA) can be stratified based on T cell phenotypes. A proof-of-concept study[32] tailored treatments based on these T cell phenotypes – whereby patients with a Th1 cell predominant phenotype received the IL-12p40 inhibitor ustekinumab, those with a Th17 cell-predominant phenotype received the IL-17 inhibitor secukinumab, and those with a mixed phenotype received TNF inhibitors – and showed better outcomes compared to the current treatment recommendations. **(C)** Subsets of patients with systemic lupus erythematosus (SLE) with B cell/plasmablast, neutrophil, and IFN signatures have been identified. Both plasmablasts and neutrophils can contribute to the IFN signature via immune complex (IC) formation or uncleared neutrophil extracellular traps (NETs), respectively, suggesting that these patients may respond to the IFN receptor antagonist anifrolumab. Furthermore, patients with a plasmablast signature may respond to the BLyS inhibitor belimumab. **(D)** Patients with anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) differ in their ANCA specificity, which may be associated with differing responses to rituximab[61,62]. Patients with AAV who have poor

prognoses exhibit CD8 effector memory T cell gene expression profiles; however, the role of these cells in AAV is unknown. **(E)** Patients with systemic sclerosis (SSc) can be grouped according to the expression of cell proliferation, platelet-derived growth factor (PDGF), and fibrotic gene signatures or inflammatory gene signatures — suggesting the use of nintedanib (which inhibits the growth factor receptors FGFR, PDGFR, and VEGFR), or tocilizumab, respectively. **(F)** Although no FDA-approved bDMARDs exist for primary Sjögren's syndrome (pSS), patients can be stratified based on the predominance of type I or type II IFN, suggesting that th

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