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Systemic lupus erythematosus (SLE): emerging therapeutic targets.

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Abstract

Introduction: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with a heterogeneous clinical presentation whose etiologies are multifactorial. A myriad of genetic, hormonal, immunologic, and environmental factors contribute to its pathogenesis, and its diverse biological basis and phenotypic presentations make development of therapeutics difficult. In the past decade, tens of therapeutic targets with hundreds of candidate therapeutic agents' targets have been investigated.

Areas covered: We used a PUBMED database search through April 2020 to review the relevant literature. This review discusses therapeutic targets in the adaptive and innate immune system, specifically: B cell surface antigens, B cell survival factors, Bruton's tyrosine kinase, costimulators, IL-12/IL-23, the calcineurin pathway, the JAK STAT pathway, and interferons.

Expert Opinion: Our ever-improving understanding of SLE pathophysiology in the past decade has allowed us to identify new therapeutic targets. Multiple new drugs are on the horizon that target different elements of the adaptive and innate immune system. SLE research remains challenging due to the heterogenous clinical presentation of SLE, confounding from background immunosuppressives being taken by SLE patients, animal models that inadequately recapitulate human disease, and imperfect and complicated outcome measures. Despite these limitations, research is promising and ongoing. The search for new therapies that target specific elements of SLE pathophysiology are discussed as well as key findings, pitfalls, and questions surrounding these targets.

Keywords: lupus, therapeutic target, adaptive immune system, innate immune system, intracellular signaling

Article Highlights

- Emerging therapies for SLE target the innate immune system, the adaptive immune system, and intracellular signaling pathways.
- Of the 42 promising therapeutics discussed in this review, we believe that voclosporin and anifrolumab are the most likely ones to emerge as successful therapies in the near future.
- Conventional wisdom is not always wise, and more is not always better. As an example, whereas rituximab – which robustly depletes B cells – failed in two phase 3 clinical trials, belimumab – which only modestly depletes B cells – emerged as the first FDA-approved biologic for SLE. We need to focus on data rather than on preconceived notions.
- Stratification of patients into clinical trials remains an issue in developing new SLE therapies. Our inability to categorize SLE patients into the appropriate "flavors" undoubtedly has led to failure in clinical trials of potentially beneficial drugs.
- Omics analyses, including profiling of the epigenome, transcriptome, and metabolome, should enhance our knowledge of SLE and help us uncover novel targets and biomarkers.

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with diverse clinical features. Creating therapeutics for SLE has been historically difficult for several reasons. Firstly, the disease is incredibly heterogeneous, making it difficult to identify and analyze patients with similar presentations [1]. Secondly, most SLE patients receive background therapy at the time of study, making it difficult to ascertain whether a new study drug is helpful or not. Thirdly, murine SLE models do not fully recapitulate the human disease because of different underlying genetics and underlying pathophysiology. (Mice are not simply small humans with tails and fur.) Lastly, commonly-used SLE outcome measures – the SLE Responder Index (SRI-4) and the BICLA (BILAG-based Combined Lupus Assessment) – are complicated and imperfect. Indeed, it is naïve to believe that any single outcome measure could adequately characterize the manifold nuances and subtleties of a disease as complex and diversified as

SLE. To date, the US Food and Drug Administration (FDA) has approved only four drugs for the treatment of SLE: aspirin, in 1948; hydroxychloroquine (HCQ) and corticosteroids, in 1955; and belimumab, in 2011. Despite the paucity of currently approved drugs, the future looks bright as many therapeutic targets show potential. In this review, we will discuss emerging new SLE therapeutic targets in the adaptive and innate immune systems.

We reviewed 200+ publications on emerging lupus therapies. PUBMED was the main database, using the keywords "lupus", "emerging", and "therapy." Publications in a language other than English were excluded due to the authors' illiteracy in non-English languages. References of likely interest that were cited in these 200+ were also reviewed. In addition, relevant papers from the senior author's personal library of publications collected over 40+ years were also reviewed.

2. Adaptive Immunity

Adaptive immunity is the antigen-specific host defense system, comprised of T and B lymphocytes and immunoglobulins. Ideally, the adaptive immune system remains largely quiescent, being triggered only upon encounter with "unwelcome" foreign material, such as pathogenic microbes. In SLE, the adaptive immune system is dysregulated, leading to a myriad of abnormalities, including autoantibody production, activation of auto aggressive T effector cells, and decreased regulatory T cells (Tregs). Not surprisingly, B and T cells are major targets of candidate therapeutics.

2.1 B cells

B cells are indispensably involved in SLE pathogenesis. To our knowledge, there has never been a case of SLE in a human completely devoid of B cells, and genetic depletion of B cells prevents development of SLE in SLE-prone MRL/*lpr* mice and NZM 2328 (NZM) mice [(2),(3)]. B cells generate autoantibodies, prime autoreactive T cells, and produce cytokines. Although not all autoantibodies are pathogenic, some form immune complexes with self-antigens, thereby stimulating, under some circumstances and

conditions, a dysfunctional immune response. Different autoantibody specificities are associated with different clinical presentations. For example, anti-ribosomal P antibody is associated with increased risk for neuropsychiatric lupus, and anti-dsDNA antibody is associated with glomerulonephritis. With the notable exception of anti-Ro autoantibodies in neonatal lupus, the evidence for "pathogenicity" for any autoantibody (including anti-dsDNA) associated with SLE is slim-to-none. For example, the injection of anti-DNA IgG-producing hybridomas derived from SLE patients into SCID mice increased proteinuria but did not result in pathologic changes on kidney histology [(4)], consistent with anti-dsDNA antibodies alone often being insufficient to cause nephritis. On the other hand, this does not preclude the pathogenicity of some anti-dsDNA antibodies, in that non-autoimmune BABL/c mice injected with anti-DNA monoclonal antibodies (mAbs) derived from MRL/*lpr* and (NZWxSWR)F1 SLE-prone mice developed Ig deposition in multiple organs (including the kidneys), pathologic glomerular hypercellularity, and proteinuria [(5)].

Aside from generating autoantibodies, B cells serve as antigen-presenting cells (APCs). Neither nephritis nor vasculitis developed in MRL/*lpr* mice rendered devoid of B cells, and activated and memory T cells in these mice were decreased, thereby establishing the indispensability of B cells to SLE and the critical role for B cells in T cell activation [(2),(6)]. In genetically engineered MRL/*lpr* mice that harbored B cells incapable of secreting antibodies, nephritis developed along with spontaneous T cell activation, proving an antibody-independent role for B cells in SLE [(7)].

In addition to producing autoantibodies and activating T cells, B cells secrete numerous cytokines, including proinflammatory cytokines (e.g., lymphotoxin-α, TNF-α, and IL-6) and regulatory cytokines (e.g., IL-10). An imbalance in cytokine secretion could push a hitherto non-inflammatory response into a proinflammatory one and help drive SLE disease.

2.1.1 B cell surface antigens

2.1.1.1 General Biological Properties of B Cell Surface Proteins

Maturation of B cells starts in the bone marrow and continues in peripheral lymphoid organs (e.g., lymph nodes and spleen). During maturation, the profile of B cell surface antigens expressed undergoes an evolution with clinically important ramifications (Figure 1).

CD20, a member of the tetraspan family of integral membrane proteins, is expressed throughout B cell ontogeny, with the exception of early pre-B cells and terminally differentiated plasma cells. Although it has no known ligand, CD20 serves both as a clinically useful marker for B cells and as a therapeutic target [(8)]. CD19, expressed on B cells throughout their ontogeny until terminal differentiation into plasma cells, is a transmembrane protein that physically associates with the B cell antigen receptor (BCR) and potentiates its signaling [(9)]. CD19 also serves both as a clinically useful marker for B cells and as a candidate therapeutic target [(8)]. CD22, a lectin-like adhesion receptor, is a member of the sialoadhesin subclass of the Ig superfamily and is a component of the B-cell activation complex [(10)]. CD22 is expressed on the surface of B-lineage cells from immature B cells until germinal center B cells but is absent from plasma cells and memory B cells. Upon BCR stimulation, CD22's three tyrosine-based inhibitory motifs (ITIMs) are phosphorylated, leading to recruitment of tyrosine phosphatase I (SHP-1) and other effector molecules which limit BCR signaling [(9),(10)].

2.1.1.2 B Cell Surface Proteins and Plasma Cells in Preclinical SLE Studies

2.1.1.2.1 CD20

Treatment of MRL/*lpr* mice transgenic for the human *CD20* gene with high doses of a murine anti-human CD20 mAb depleted B cells, leading to ameliorated clinical and histologic disease and declines in serum autoantibody levels [(11)]. Of note, B cells from autoimmune-prone strains were more difficult to deplete than those from non-autoimmune-prone strains.

2.1.1.2.2 CD19

Treatment of (NZB × NZW) F1 (BWF1) and MRL/*lpr* mice with CD19-targeted chimeric antigen receptor T cells (CAR-Ts) resulted in decreased autoantibody production, decreased proteinuria, and prolonged lifespan [(12)]. Similarly, treatment of autoimmune-prone *Sle1* mice transgenic for the human *CD19* gene with a humanized anti-human CD19 mAb led to B cell depletion, decreased levels of autoantibodies, and decreased levels of inflammatory proteins [(13)].

Anti-CD19 mAb do not necessarily need to deplete B cells to be effective. Inactivation, rather than physical depletion, of B cells has been achieved through XmAb5871 (now known as obexelimab), an anti-CD19 mAb genetically engineered to bind the inhibitory FcγRIIb receptor with high affinity. By co-engaging CD19/BCR and FcγRIIb on human B cells, this mAb strongly inhibits BCR-induced activation of normal human B cells *in vitro* through a SH2-containing inositol polyphosphate 5-phosphatase (SHIP)-mediated pathway [(14),(15)]. Importantly, XmAb5871 inhibits *in vivo* anti-tetanus antibody responses generated in immunodeficient SCID mice engrafted with human peripheral blood mononuclear cells [(16)], proving its ability to affect *in vivo* antibody responses.

2.1.1.2.3 CD22

Genetic variants and polymorphisms of the *CD22* gene have been linked to susceptibility to autoimmune diseases, including SLE [(17)]. Studies in autoimmune-prone mice have also linked the *CD22* gene to SLE [(18),(19)], and CD22 deficiencies associate with increases in autoantibody production [(19), (20)].

2.1.1.2.4 Plasma Cells

Prominent B cell surface antigens are frequently absent from terminally differentiated plasma cells, so most B-cell targeted therapies fail to effectively eliminate plasma cells [(21)]. In contrast, plasma cells are highly sensitive to proteasome inhibitors. Treatment of BWF1 or MRL/*lpr* mice with the proteasome inhibitor bortezomib not only depleted plasma cells but also reduced nephritis and prolonged survival

[(22)]. Similar salutary effects were observed with the proteasome inhibitors carfilzomib and delanzomib in BWF1 and MRL/*lpr* mice [(23),(24)].

2.1.1.3 Emerging Therapeutics Targeting B Cell Surface Proteins and Plasma Cells

Although the SLE LUNAR and EXPLORER trials failed to demonstrate any benefit for the anti-CD20 mAb, rituximab (RTX) in combination with standard-of-care (SOC) medications, the failure may have been due to trial design rather than with the drug itself. Indeed, large uncontrolled studies have pointed to efficacy for RTX in severe refractory SLE [(25),(26)], and obinutuzumab, another CD20 mAb, met its primary end point in the lupus nephritis NOBILITY phase II trial, with a greater percentage of obinutuzumab-treated patients achieving complete renal response than those receiving SOC alone (Table 1) [(27)]. The phase III REGENCY trial (NCT04221477) to evaluate obinutuzumab in lupus nephritis is planned to start in 2020 [(27)], so CD20 may turn out to be a *re-emerging* target in SLE.

Whereas CD20 is not expressed on plasmablasts, long-lived plasma cells, or early B cells, CD19 is (albeit only on a fraction of long-lived plasma cells). Accordingly, targeting CD19 should affect a broader B cell population than targeting CD20. Of note, the anti-CD19 mAb, MEDI551 (inebilizumab), despite its robust B cell-depleting potency, spares regulatory B cells [(28)], buttressing the attractiveness of this agent. MEDI551 has not yet been evaluated in SLE clinical trials.

Two other CD19-targeting agents, however, are currently undergoing evaluation in SLE clinical trials. XmAb5871 (obexelimab), an anti-CD19 mAb with its Fc portion genetically engineered to bind FcγRIIb with high affinity, recently completed a unique phase II trial (NCT02725515) designed to minimize background medications. In this trial, patients with moderate-to-severe, non-organ-threatening SLE discontinued background immunosuppressive medications other than antimalarials and/or ≤10 mg prednisone per day. Subjects received 80 mg of intramuscular methylprednisolone on days 1 and 15 to quiet SLE disease activity, and the 104 patients who achieved the required improvement in disease activity were randomized 1:1 to receive XmAb5871 or placebo every 14 days for up to 16 doses. The

primary endpoint, defined as the proportion of patients with no loss of improvement (LOI) in the efficacy-evaluable population at day 225, was not met.(29) Nevertheless, the secondary endpoint of time to LOI was statistically longer in patients treated with XmAb5871, suggesting that further evaluation may be warranted. Phase III trials for SLE with XmAb5871 are not underway yet.

The other emerging CD19-targeting approach draws from CAR-T therapy utilized in oncology, in which T cells are removed from the patient, genetically engineered to recognize a molecule on a particular cell type (such as a leukemic cell), and infused back into the patient to destroy the targeted cells. CD19-CAR-T cells have been tested to date in one open-labeled uncontrolled single-arm phase I clinical trial in SLE patients in China (NCT03030976). Results have not been released yet.

Epratuzumab is an anti-CD22 mAb that downregulates B cells by initiating phosphorylation of CD22, leading to internalization of CD22 and CD79α and subsequent downregulation of CD19, CD79β, and CD21 from the cell surface via trogocytosis [(30)]. Patients treated with epratuzumab at any tested dose in the phase 2b EMBLEM trial manifested higher proportions of BICLA responders than did placebo-treated patients [(31)]. Surprisingly and disappointingly, epratuzumab did not meet its primary endpoints in the phase 3 EMBODY 1 and 2 trials [(32)], although *post hoc* analyses from the EMBODY trials showed improvements in SLE patients with associated Sjogren's syndrome.

Several factors may have influenced the disappointing results of EMBODY 1 and 2. Firstly, there was a high discontinuation rate; about one-third of patients discontinued the study prior to week 48 and were categorized as non-responders. Corticosteroid dosage was another issue in these studies; 40% of patients did not reduce their corticosteroid dosage, and another 40% of patients increased their corticosteroid dosage or had missing data. These issues likely led to spuriously high placebo rates, thereby diminishing the difference in response between placebo and drug.

With regard to plasma cells, retrospective review of 12 patients with refractory SLE treated with bortezomib revealed all to have improved in several clinical parameters, including rash, proteinuria,

arthritis, and serositis; however, two patients developed severe neuropathy which led to treatment discontinuation [(33)]. A phase 2 trial (NCT02102594) was terminated due to recruitment difficulties. Chronic use of bortezomib has significant toxicity, including, but not limited to, neuropathy and infection. Its toxicity may be why it has been difficult to recruit patients for trials. On the other hand, targeting of the immunoproteasome (a specific proteasome found in immune cells only) with KZR-616 was well tolerated and showed evidence of disease suppression in released results from a phase 1b trial (NCT03393013) [(34)]. The phase 2 portion of this study in active proliferative lupus nephritis is in the enrollment phase

2.1.2. B Cell Survival Factors

2.1.2.1 General Biological Properties of BAFF and APRIL

The two B-lineage cell survival factors that have received the bulk of attention are BAFF and APRIL. BAFF is a 285-amino acid type-II transmembrane protein member of the TNF ligand superfamily and binds to three receptors: BCMA, TACI, and BR3. Overexpression of BAFF leads to increases in B cells, whereas genetic depletion or pharmacologic neutralization of BAFF leads to reductions in B cells [(35)].

APRIL is a 250–amino acid member of the TNF ligand and superfamily with substantial homology to BAFF and binds to TACI and BCMA but not to BR3. Although APRIL is a vital survival factor for plasma cells, neither its overexpression nor its deficiency has a major effect on B cell numbers [(36)]. Since APRIL can form heterotrimers with BAFF [(36)], one of the physiologic roles for APRIL may be to downregulate BAFF activity.

2.1.2.2 BAFF and APRIL in Preclinical SLE Studies

A role for BAFF in SLE is irrefutable. Development of SLE in SLE-prone NZM mice bearing a disrupted *Baff* gene is profoundly diminished, and these mice are resistant to IFN-α-driven disease that develops in NZM wild-type mice [(3)]. Moreover, BAFF antagonist treatment of MRL/*lpr* or BWF1 mice stops

progression of SLE disease [(36)]. In humans, an association between BAFF and SLE has been documented repeatedly, with increased levels of BAFF in at least half of SLE patients [(37)]. Indeed, patients with elevated BAFF expression tend to accrue greater organ damage over time than patients with more modest BAFF expression [(38)].

While the evidence that BAFF overexpression has a role in SLE is compelling, the same cannot be said for APRIL. APRIL-transgenic mice did not display clinical autoimmune features [(39)], and no amelioration of SLE features was appreciated in APRIL-deficient NZM mice [(40)]. In NZM 2410 mice, treatment with an inhibitor of both BAFF and APRIL was more immunosuppressive but not more efficacious than treatment with BAFF inhibitor alone [(41)]. Whereas there was a modest delay in development of proteinuria and death in BWF1 mice treated with an anti-APRIL mAb [(42)], this delay well have been due to reduction in BAFF activity by removal of circulating BAFF/APRIL heterotrimers [(36)].

In humans, genome wide association studies (GWAS), meta-analyses, and candidate gene and replication studies have failed to document any association between SLE and APRIL [(43)]. Whereas one study of Japanese SLE patients suggested an association between development of SLE and the G67R polymorphism in the *APRIL* gene [(44)], a larger study of European Americans failed to demonstrate this association [(45)]. At this point, the preponderance of evidence suggests that, unlike BAFF, APRIL does not have a key role in SLE.

2.1.2.3 Emerging Therapeutics Targeting B Cell Survival Factors

As indicated above, the anti-BAFF mAb, belimumab, is FDA-approved for SLE. Four other BAFF antagonists either have undergone or are still undergoing evaluation in clinical trials.

One such antagonist is atacicept, a fusion protein between one of the BAFF receptors (TACI) and the Fc portion of IgG. It binds and neutralizes both BAFF and APRIL, raising the possibility that it could be

more potent (and efficacious) than drugs targeting BAFF alone. In the APRIL-SLE trial, patients were randomized to receive atacicept (75 mg or 150 mg) or placebo twice weekly for 48 weeks [(46)]. Enrollment in the 150 mg arm was terminated early due to two deaths, and there was no difference between atacicept 75 mg and placebo for the primary outcome of flare rate or time to first flare. Nevertheless, statistically significant decreases in flare rate and time to first flare were noted among patients that had received the higher atacicept 150 mg dose. In the phase IIb ADDRESS II study, patients with active SLE were randomized to 75 mg of atacicept, 150 mg of atacicept, or placebo [(47)]. Although the primary endpoint (SRI-4 response) rate was not met, there was a trend towards increased response with atacicept, especially in patients with high levels of disease and serological activity. Importantly, the safety profile of atacicept was acceptable in both ADDRESS II and its extension study. In contrast to the APRIL-SLE trial, there was no increased frequency of adverse events or serious infections when compared to placebo [(47)]. Phase III SLE trials are being contemplated [(48)].

A second BAFF antagonist is the peptibody, blisibimod. Like belimumab, blisibimod binds only to BAFF. The primary endpoint of SRI-6 was not achieved in phase III trials, but blisibimod was associated with steroid reduction, decreased proteinuria, and biomarker response [(49)]. Whether blisibimod undergoes further clinical evaluation remains uncertain.

A third BAFF antagonist is tabalumab, a human IgG4 anti-BAFF mAb. Tabalumab was studied in two large double-blind randomized controlled phase III trials. In the first, the primary and secondary clinical endpoints were not met, although significant reductions in anti-dsDNA antibodies were seen in the treated group [(50)]. In the second trial, in which patients received one of two tabalumab doses or placebo, the more frequent dosing group achieved its primary outcome. [(51)] However, the response rate did not appear to be greater than that previously observed in the belimumab trials. Moreover, key secondary endpoints, including time to severe flare, corticosteroid-sparing effect, and fatigue, were not met. Given the lack of robustness of tabalumab in these trials, the sponsor has abandoned further development of tabalumab for SLE.

A fourth BAFF antagonist, telitacicept (also called RC18) is, like atacicept, a recombinant fusion protein of the extracellular domain of the TACI receptor and the Fc domain of human IgG1. Its phase 2b trial met its primary endpoint of a SRI-4 response across all doses. (52) A phase 3 trial is in the recruitment phase (NCT04082416).

2.1.3 Bruton's Tyrosine Kinase

2.1.3.1 General Biological Properties of Bruton's Tyrosine Kinase

Bruton's tyrosine kinase (Btk) is a Tec family tyrosine kinase expressed in B and myeloid cells. It was first identified as the genetic defect in X-linked agammaglobulinemia, a disorder in which B cells in the bone marrow fail to mature beyond the pre-B cell stage, resulting in a markedly decreased number of or absence of mature B lymphocytes and immunoglobulins [(53)]. Btk is an important proximal component of BCR signaling pathways; it is required for TLR-induced IL-10 expression by B cells, for synergy between the BCR and TLRs in enhancing IL-6 expression, and for integrin-mediated adhesion of B lineage cells and their response to chemokines [(53)].

2.1.3.2 Bruton's Tyrosine Kinase in Preclinical SLE Studies

Introduction of the *Xid* mutation in the *Btk* gene into multiple murine models, including BWF1 [(54)], BXSB [(55)], MRL/*lpr* [(56)], motheaten [(57)], and *gld* [(58)], leads to reduced autoantibody levels. Furthermore, BWF1 [(54)], BXSB [(55)], and MRL/lpr [(56)] mice also do not develop renal disease when they bear the *Xid* mutation. (56) Moreover, small molecule inhibitors of Btk lead to reduced kidney damage and mortality in BWF1 and BXSB.Yaa mice [(53)].

2.1.3.3 Emerging Therapeutics Targeting Bruton's Tyrosine Kinase

The Btk inhibitor, MSC2364447C, is undergoing phase 2 trials (NCT02975336), and another Btk inhibitor, BIIB068, has completed a phase 1 trial (NCT02829541); results are not available yet. Fenebrutinib (GDC-0853) recently completed a phase 2 trial but it did not meet its primary endpoint of SRI-4 response or secondary endpoint of BICLA response (NCT02908100). Phase 3 trials are not planned.

2.2. T Cells

As with B cells, T cells are indispensably involved in SLE pathogenesis. To our knowledge, there has never been a case of SLE in a human completely devoid of T cells. In mice, athymic BWF1 mice do not develop SLE, but disease can be reconstituted following engraftment of a thymus [(59)]. In addition, depletion of $CD4^+$ T cells protects from disease [(60)], and deletion of the *CD4* gene attenuates SLE in MRL/*lpr* mice [(61)].

$CD8^+$T cells can also promote and prevent autoimmunity. They can promote autoimmunity through secretion of inflammatory cytokines and dysregulated apoptosis. They can also prevent autoimmunity through differentiation into Tregs and through destruction of self-reactive cells. Altered gene expression profiles of $CD8^+$T cells have been linked to SLE.(62)

2.2.1. Costimulators

2.2.1.1 Biological Properties of Costimulators

T cell activation is generally dependent on costimulation. Costimulators serve as “second signals”, building on the “first signal” delivered through the T cell antigen receptor (TCR). The best known costimulators for T cells are CD80 (B7-1) and CD86 (B7-2) expressed on APCs, and each binds to CD28 on T cells. Engagement of the TCR in the absence of CD28–CD80/CD86 interactions does not lead to T cell activation.

CD40 on APCs and CD40L on T cells represent another set of well-known costimulatory molecules. Binding of CD40L to CD40 activates APCs to upregulate expression of CD80/86 costimulators and to secrete cytokines (such as IL-12) that promote activation.

On the flip side of costimulators are checkpoint inhibitors which downmodulate ongoing immune responses. The best known of these are CTLA-4, which is homologous to CD28 and has a higher affinity for CD80/CD86 than does CD28, and PD-1, which has garnered vast attention in the field of cancer immunotherapy. PD-1 is also being studied in preclinical SLE, and its deficiency in murine models has led to development of lupus-like disease. (63)

2.2.1.2 Costimulators in Preclinical SLE.

Alternations in CD28–CD80/CD86 interactions have profound effects in murine SLE. Deficiency of CD80 exacerbated disease in MRL/*lpr* mice, deficiency of CD86 ameliorated disease in MRL/*lpr* mice (64), and treatment of BWF1 mice with CTLA-4-Ig blocked autoantibody production and prolonged survival [(65)].

Alterations of CD40–CD40L interactions also profoundly affect murine SLE. CD40L is ectopically expressed in SLE-prone BXSB mice, and CD40L-transgenic mice spontaneously produce anti-DNA antibodies and develop glomerulonephritis [(66)]. Simultaneous blockade in BWF1 mice of both CD28–CD80/86 and CD40–CD40L axes led to a better outcome than those following blockade of a single axis [(67)].

2.2.1.3 Emerging Therapeutics Targeting Costimulators,

Abatacept, a fusion protein of the Fc region of IgG1 with the extracellular domain of CTLA-4, is approved by the FDA for the treatment of rheumatoid arthritis. This success of CTLA-4-Ig in murine SLE notwithstanding, abatacept in a phase IIb trial failed to meet its primary endpoint of a decreased proportion of patients with new flare. However, *post hoc* analyses documented decreased BILAG A flares

(most notably polyarthritis flares) among abatacept-treated patients [(68)]. Abatacept also failed to meet its primary endpoint, time to complete response, in a phase II/IIII trial as an adjunct treatment for class III or IV nephritis [(69)]. However, patients who received abatacept experienced greater reduction in protein-to-creatinine ratio and improvements in biomarkers than those who did not [(69)]. Although abatacept may not be an effective drug for lupus nephritis, it may be promising for arthritis, and a phase 1/2 trial for its usage in SLE arthritis is ongoing (NCT02429934).

Although toralizumab and ruplizumab, both anti-CD40L mAbs, were discontinued in clinical trials due to thromboembolic events [(70), (71)], CDP7657 (dapirolizumab pegol), a monovalent PEGylated Fab of the corresponding anti-CD40L mAb, was specifically engineered to prevent platelet activation to circumvent the adverse effects of its predecessors [(70)]. A phase 2b trial of this drug showed improvements in disease activity, although the primary objective of establishing a dose-response relationship was not met [(72)]. A phase 3 trial is planned for later this year (NCT04294667). On the flip side of the CD40—CD40L axis, BI655064 and CFZ-533 are anti-CD40 mAbs that are currently undergoing phase 2 clinical trials (NCT0338554, NCT02770170 and NCT03610516, NCT03656562, respectively).

2.3 Cytokines

Cytokines are secreted proteins, produced by cells belonging to the adaptive immune system and the innate immune system, which promote, mediate, and regulate immune and inflammatory reactions. Not surprisingly, cytokine dysregulation contributes to the pathogenic state in SLE. Due to their key roles in immune regulation, virtually any cytokine could potentially be a therapeutic target in SLE. BAFF and APRIL as targets were discussed above. As discussed below, IL-12, IL-23, and IL-17 have garnered considerable attention as potential targets as has treatment with IL-2.

2.3.1 General Biological Properties of IL-12 and IL-23

The IL-12 and IL-23 are heterodimeric cytokines that share a p40 subunit. IL-12 stimulates the JAK/STAT pathway (further discussed below) and promotes both the differentiation of naïve $CD4^+$ T cells into IFN-γ-producing Th1 cells and the differentiation of T follicular helper (Tfh) cells. Both Th1 and Tfh cells are expanded in SLE. (73)

IL-23 indirectly signals through JAK/STAT by stabilizing the expression of genes controlling T cell activation, which leads to the differentiation of $CD4^+$ T cells into Th17 cells [(74),(75)].

2.3.1.1 Contributions of IL-12 and IL-23 to SLE

Genetically, SLE risk is associated with the IL-12/IL-12R pathway [(76)]. SLE patients harbor higher serum levels of IL-12 than do controls, and p40 subunit serum levels are positively correlated with SLE disease activity (as measured by SLEDAI) and negatively with serum C3 levels [(77)].

The IL-23 pathway is also dysregulated in SLE. In mouse models, treatment with IL-23 promoted nephritis, whereas treatment with anti-IL-23 antibody abrogated nephritis [(78), (79)]. In human SLE, IL-23R is upregulated on T cells from SLE patients with active, but not inactive, disease [(80)]. Moreover, addition of IL-23 to co-cultures of B and T cells led to induction of autoantibodies, especially anti-dsDNA [(80)].

2.3.1.2 Emerging Therapeutics Targeting IL-12/IL-23

A phase II trial of ustekinemab, an anti-IL-12/IL-23 mAb (already FDA-approved for psoriasis, psoriatic arthritis, Crohn's disease, and ulcerative colitis) documented efficacy in SLE. In this trial, 62 patients received ustekinumab, and 42 patients received placebo. Baseline clinical and laboratory features, background immunosuppression, and disease activity were similar in both groups [(81)]. At week 24, 62% of the patients in the ustekinumab achieved an SRI-4 response as opposed to 33% of the patients in the placebo group [(81)]. Despite these exciting and robust results, phase III trials were discontinued in June 2020 based on a pre-planned futility analysis. [(82)] With these disappointing results, ustekinumab

joins the ranks of many other medications that were "sure bets" for SLE but failed late stage trials. Given its striking success in phase II trials, however, it follows that ustekinumab may still be useful in a distinct subset of patients.

2.3.2 General Biological Properties of IL-17

IL-17 (IL-17A) Is a proinflammatory cytokine produced mainly by T_H17 cells that is essential in host defense against bacteria and fungi, but also in autoimmune disease pathogenesis [(83)]. It belongs to the IL-17 family which contains six structurally related cytokines: IL-17A (IL-17), IL-17B, IL-17C, IL-17D, IL-17E (IL-25) and IL-17F [(84)]. IL-17 upregulates inflammatory gene expression either through induction of *de novo* gene transcription or by the stabilization of proinflammatory mRNA transcripts [(84)].

2.3.2.1 Contributions of IL-17 to SLE

IL-17 has been shown to have a role in both murine and human SLE studies [(83)]. In a murine model of pristane–induced lupus, IL-17 deficient mice were protected from development of autoantibodies and glomerulonephritis [(85)]. In SLE human patients, increased serum levels of IL-17 and increased numbers of IL-17 producing cells have been noted [(83)]. Increased IL-17 was also found in target organs of SLE including skins, lungs, and kidneys, suggesting that IL-17 may play a role in local tissue damage [(83)]. Double negative T cells from SLE patients produce significant amounts of IL-17, and IL-17^+ and double negative T cells have been found in kidney biopsies of patients with lupus nephritis [(86)]. Plasma levels of IL-17 have also been shown to positively correlate with proteinuria and anti-dsDNA antibodies in patients with lupus nephritis, although another study showed no association between increased IL-17 serum levels and lupus nephritis [(87)].

2.3.2.2 Emerging Therapeutics Targeting IL-17

Tibulizumab is an engineered bispecific dual-antagonist antibody against BAFF and IL-17 that is composed of an anti-IL-17 single chain variable fragment derived from ixekizumab fused via a glycine-rich linker to the anti-BAFF mAb tabalumab [(88)]. Tibulizumab potently antagonized both BAFF and IL-17 in cell-based and *in vivo* systems, and it suppressed B cell development and survival in cynomolgus monkey [(88)]. Human clinical trials have not yet begun.

2.3.3 General Biological Properties of IL-2

IL-2 is a pleiotropic cytokine that is produced by $CD4^+$ T cells shortly after their activation. Due to differential expression of the β and the γ chains of the IL-2R on target cells, low concentrations of IL-2 selectively activate Tregs (which have a higher affinity for IL-2), whereas high concentrations expand not only Tregs, but effector T cells, NK cells, and $CD8^+$ T lymphocytes as well [(89)].

2.3.2.1 IL-2 in Preclinical SLE Studies

Mice deficient in IL-2 develop a multitude of autoimmune conditions in association with hyperactivity of T (especially $CD4^+$) and B cells [(90),(91),(92)]. Conversely, MRL/*lpr* mice developed reduced autoantibody titers, reduced kidney and synovial inflammation, and greater longevity following infection with an IL-2-expressing recombinant vaccinia virus [(93)]. Along similar lines, neutralization of IL-2 in clinically healthy BWF1 mice accelerates SLE progression, whereas treatment of these mice with IL-2 ameliorates disease [(94)].

2.3.2.2 IL-2 as an Emerging Therapeutic

IL-2 therapy shows promise not only in murine SLE but in human SLE as well. Treatment with low dose IL-2 of a 36 year-old female with refractory SLE led to Treg expansion, a decrease in circulating anti-dsDNA antibodies, and a rapid and robust reduction in disease activity [(95)]. Buoyed by this success, a combined phase I/II trial of recombinant human IL-2 in active and refractory SLE documented reduction

in SLEDAI in 10 of the 12 patients treated. In 8 of these patients, complete disappearance of clinical manifestations, as assessed by the SELENA-SLEDAI score, was noted [(96)]. Other phase II trials are ongoing; phase 3 trials have not yet been scheduled [(89)].

3. Intracellular Signaling

3.1 Biological Roles of mTOR, Calcineurin, and JAK/STAT Pathways

The mTOR (mammalian target of rapamycin) pathway serves as the core component of at least two multi-protein complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), each of which having distinct functions. mTORC1 promotes anabolic cellular metabolism and controls cell growth, while mTORC2 responds to growth factors and controls cell metabolism, survival, and the organization of the actin cytoskeleton. It stands to reason that dysregulation of mTOR can have profound consequences for the host.

Another vital intracellular signaling pathway revolves around calcineurin. In T cells, antigen recognition induces early signaling events, including the tyrosine phosphorylation of molecules in the TCR complex and recruitment of adapter proteins to the site of T cell antigen recognition. These events lead to the activation of several biological intermediates which leads to increased cytosolic calcium. This stimulates calcineurin which, in turn, results in activation of the transcription factor nuclear factor of activated T cells (NFAT). When NFAT is dephosphorylated, it migrates to the nucleus where it binds to and activates the promoters of several genes, including IL-2 and components of IL-2R.

The JAK/STAT pathway is another key intracellular signaling pathway in response to cytokines binding to their receptors. The STATS (signal transducer and activator of transcription) are present as inactive monomers in the cytoplasm and are recruited to the cytoplasmic tails of cross-linked cytokine receptors, where they subsequently undergo phosphorylation by Janus Kinases (JAKs). Phosphorylated STAT

proteins dimerize and move to the nucleus, where they bind to specific sequences in the promoter regions of specific genes and stimulate transcription.

There are four JAKS and seven STATs [(97)]. Different JAK-dependent cytokine receptors signal through different JAKs [(97)]. Certain *JAK and STAT* genes have been linked with specific diseases. For example, hematopoietic growth factors signal through JAK2, and gain of function (GOF) mutations in *JAK2* cause hematologic diseases such as polycythemia vera, essential thrombocythemia, and myelofibrosis [(98)].

3.1.1 Contribution of Calcineurin, mTOR, and JAK/STAT pathways to SLE

Activated mTOR in SLE is associated with expansion of T_H17 and $CD3^+CD4^-CD8^-$ double negative T cells and contraction of Tregs. Rapamycin, the prototypic mTOR inhibitor, ameliorates nephritis and increases IL-2 production is MRL/*lpr* mice [(99)].

SLE T cells exhibit increased calcium-dependent NFAT activity upon activation [(100)]. In SLE T cells, NFAT enters the nucleus and upregulates *CD40L* [(100)]. Dipyridamole, a calcineurin inhibitor, eliminated skin manifestations and proteinuria in MRL/*lpr* mice [(101)].

TYK2 is closely associated with SLE. It is part of the JAK that binds to IFNAR, and GWAS have shown that polymorphisms in TYK are associated with SLE. (102)

STAT3 is highly phosphorylated in the nucleus of SLE T cells [(103)]. Knocking out STAT3 in T cells abrogated development of nephritis in MRL/*lpr* mice, and pharmacologic inhibition of STAT3 ameliorated nephritis in these mice [(104)].

3.1.2 Emerging Therapeutics Targeting mTOR, Calcineurin and JAK/STAT Pathways

Rapamycin, also known as sirolimus, improved clinical and laboratory parameters in an open-label clinical trial of nine patients with refractory SLE (Table 2) [(105)]. In a recent phase 1/2 single-arm open-label trial, rapamycin was given to patients with active SLE who were refractory to conventional

medications but did not have life-threatening or severe renal and hematologic manifestations of lupus [(106)]. In this trial, the primary endpoint was met, with decreases in both SLEDAI and BILAG disease scores. Given the positive results, double-blind placebo-controlled phase 3 trials would certainly be welcome.

Tacrolimus, a calcineurin inhibitor, has also been trialed in SLE and is already approved for lupus nephritis in Japan. Calcineurin inhibitors have an anti-proteinuric effect, rendering them very appealing for SLE therapy [(107)]. In a phase 4 RCT, tacrolimus plus prednisolone was non-inferior to mycophenolate mofetil (MMF) plus prednisolone as induction therapy for lupus nephritis [(108)]. In a large randomized Chinese trial, combination therapy with MMF, tacrolimus, and corticosteroids was found to be superior to cyclophosphamide and corticosteroids in inducing remission [(109)]. Indeed, a recent meta-analysis concluded that tacrolimus is effective and safe drug for induction therapy in lupus nephritis.

Voclosporin is a next-generation calcineurin inhibitor that more potently binds calcineurin and has a faster elimination of its metabolites as compared to cyclosporine A [(110)]. In the phase 2 double-blind RCT, AURA–LV, voclosporin achieved its primary end point of complete renal remission at both 24 and 48 weeks. [(110)]. In the AURORA phase III trial, voclosporin achieved its primary endpoint of renal response at 52 weeks. In ethnic subgroup analysis, the drug reached its endpoint for both Hispanic/Latino and non-Hispanic/Latino patients. It also achieved all of its secondary endpoints, and all subgroup analyses favored voclosporin (111). Based on these exciting results, the FDA has granted priority review for a new drug application for voclosporin. (112)

Regarding JAK inhibitors, there are three drugs already approved by the FDA for a non-SLE indication (RA): baricitinib, tofacitinib, and upadacitinib. Baricitinib, an oral selective JAK1 and JAK2 inhibitor, demonstrated efficacy (predominantly on arthritis) in SLE at a dose of 4 mg/day (but not 2 mg/day) in a phase II double-blind RCT [(113)]. Of note, baricitinib did not improve CLASI (cutaneous lupus disease severity index) compared to placebo. Whereas the 4 mg/day dose of baricitinib has not been approved for

RA due to an increased risk of deep venous thrombosis (DVT), there was only one DVT recorded in the phase 2 SLE study. Based on these encouraging results, phase 3 trials for baricitinib in SLE are currently enrolling subjects (NCT03843125, NCT03616964, NCT03616912). Tofacitinib is a JAK 1/3 inhibitor that was studied in a phase 1B trial (NCT02535689) in patients with mild to moderate SLE, stratified by the presence or absence of STAT4 risk allele. Results have not been released yet. Further phase I/II trials are in the recruiting stage (NCT03159936 and NCT03288324). A phase 2 trial investigating ABBV-105 (a Btk inhibitor) and upadicitinib alone or in combination for the treatment of moderate to severe SLE is in the recruitment phase (NCT03978520).

TYK2 inhibitors are also under active investigation for SLE. BMS-986165 is undergoing phase 2 trials for SLE (NCT03252587) and for lupus nephritis (NCT03943147). PF06700841 is a JAK1/TYK2 inhibitor that is in the recruitment stage for a phase 2 trial for patients with moderate to severe SLE (NCT03845517).

4. Innate Immunity

The innate immune system comprises all of the host's protective devices and mechanisms that are not part of the adaptive immune system. This includes epithelial barriers, phagocytic cells, natural killer cells, the complement system, the coagulation pathways, and many cytokines. Activation of innate immunity has been repeatedly implicated in the pathogenesis of SLE. GWAS have identified over fifty gene loci that predispose to SLE [(114), (115), (116)], with at least twenty-one of them being associated with the innate immune response.

4.1 Interferons

4.1.1 Biological Properties of Interferons

Interferons (IFNs) are anti-viral proteins produced and released by host cells in response to viruses, bacteria, ultraviolet light, or microbial nucleic acids. IFNs can be divided into three families: type I, type

II, and type III. Type I IFNs are comprised of IFN-α, IFN-β, IFN-κ, IFN-δ, IFN-ε, IFN-τ, IFN-ω, and IFN-ζ , with IFN-α and IFN-β having been studied the most [(117)]. Type I IFNs can be secreted by most nucleated cells when their pattern recognition receptors are activated, but the predominant type I IFN producer is the plasmacytoid dendritic cell (pDC).

In contrast to the very large type I IFN family, IFN-γ is the sole member of the type II IFN family. It is produced mainly by $CD4^+$ cells, $CD8^+$ cells, and NK cells, with contributions from B cells, NKT cells, and professional APCs. IFN-γ triggers a cascade resulting in the induction of genes for inflammatory cytokines and apoptotic factors [(117)]. Of note, IFN-γ also activates STAT3 homodimers which lead to production of not only proinflammatory cytokines but also to IL-10, an anti-inflammatory cytokine [(118)].

Type III IFNs are the most recently described IFNs and include IFNλ-1 (also called IL-29), IFNλ-2 (IL-28A), IFNλ-3 (IL-28B), and IFNλ-4. They are thought to directly affect an antiviral immune response at epithelial surfaces in the early stages of viral infection and skew the balance of Th1 and Th2 cells to Th1 phenotype [(119)]. Type III IFNs are predominantly produced by APCs and epithelial cells. They signal via a heterodimeric receptor and recruit IRF9 and active ISGF3 which drive IFN-stimulated genes [(117)].

4.1.2 Contribution of IFNs to SLE

Type I IFN is widely accepted to have a vital role in SLE. IFN-α in particular is likely a key driver of SLE pathogenesis by promoting increased antigen presentation, development of pathogenic $CD4^+$ T cells, and suppression of natural regulatory T cells (nTregs) [(120)]. Type 1 IFN-driven autoimmune disease, including not only SLE but Aicardi-Goutières syndrome as well, may be triggered by aberrant DNA from reverse-transcribed cellular RNA in the setting of defective checkpoint mechanisms (121)(122)

In autoimmune-prone NZB mice, deletion of the IFN-α/β receptor retards development of serological and clinical disease [(123)]. Long-term treatment of male BXSB mice with anti-IFNα receptor antibodies has a similar salutary effect [(124)]. In humans, some SLE patients have detectable levels of IFN-α in the serum, while normal individuals have little to none [(125)]. Several studies have shown evidence of an "IFN-response signature" in active SLE, indicative of an increased expression of type I IFN-regulated genes [(126), (127), (128)].

In contrast to IFN-α, the role of IFN-β in SLE is controversial, as it may have anti-inflammatory properties and inhibit IL-1, IL-10, and inflammasome activation [(129)]. That notwithstanding, IFN-β may contribute to tissue injury by inducing PD-1 and altering T cell function [(130)].

Type II IFN (IFN-γ) is genetically associated with SLE [(131)]. At the protein level, IFN-γ is increased in SLE peripheral blood mononuclear cells and correlates with disease activity [(132)]. IFN-γ also drives BAFF production [(133)] and promotes germinal center formation, thereby amplifying the ongoing (auto)immune response [(134)]. Increased expression of IFN-γ in transgenic mice leads to a SLE-like syndrome characterized by increased pathogenic autoantibodies and glomerulonephritis [(135)]. T cells that secrete IFN-γ are a hallmark of SLE in MRL*/lpr* mice, and deletion of the IFN-γ receptor in these mice resulted in decreased production of inflammatory cytokines, prevented development of the characteristic lymphadenopathy and splenomegaly, prevented kidney destruction, and extended overall survival [(136)].

Type III IFNs may also be involved in SLE [(117)]. IFNλ and the IFNλ receptor are expressed in cutaneous LE skin lesions, and exposure of human keratinocytes to IFNλ induced expression of proinflammatory cytokines [(137)]. Persistently increased levels of IFNλ associate with a poor histological response to immunosuppressive therapy in lupus nephritis [(138)].

4.1.3 Emerging Therapeutics Targeting IFNs

In addition to high-dose corticosteroids and HCQ which downregulate IFNs, several therapies specifically targeting type I and type II IFNs are being investigated. Three anti-IFN-α mAbs, rontalizumab, sifalimumab (formerly known as MEDI-545), and AGS-009, have been tested in clinical trials (Table 3). Rontalizumab did not meet its efficacy endpoints in phase II studies and is no longer being evaluated for SLE [(139)]. Although sifalimumab achieved its endpoint, the absolute effect was modest and is also no longer being developed for SLE [(140)]. AGS-009 led to marked decreases in IFN-α levels in the phase 1 trial, but phase 2 trials have not been planned [(141)].

IFNα-Kinoid (IFN-K) is a vaccine composed of IFN-α2b coupled onto a carrier protein that induces polyclonal IFN-α neutralizing antibodies. It was recently evaluated in a phase 2b study and significantly reduced the IFN gene signature, although its clinical co-primary endpoints of neutralization of the IFN gene signature and BICLA response were not met [(142)]. Neovacs, the company that is developing this vaccine, announced that its clinical advisory board would support the design of a phase III study, although none is ongoing currently [(143)].

An alternative approach to targeting the ligand (IFN) is targeting the receptor (IFNAR1). The phase-III trial of the humanized mAb, anifrolumab, disappointingly did not achieve its primary endpoint (proportion of patients who achieved an SLE-responder index-4 [SRI-4] response at week 52) in the phase III TULIP-1 trial [(144)]. Nevertheless, several of its secondary endpoints, including reduction in corticosteroids, CLASI (The Cutaneous Lupus Erythematosus Disease Area and Severity Index) responses, and BICLA (BILAG-based Combined Lupus Assessment) responses, were met [(144)]. A second phase-III trial, TULIP II, using BICLA as the primary endpoint, was successful [(145)], so anifrolumab is now undergoing open-label extension of both TULIP-1 and TULIP-2 phase III trials (NCT02794285).

Type I IFN is also being targeted through an indirect approach. Reverse transciptase inhibitors have been studied in preclinical SLE since retroelements have been shown to trigger type I IFN-driven inflammatory disease. (122) By inhibiting reverse transciptase, type I IFN will be inhibited.

With regard to type II IFN, AMG 811 is a human anti-IFN-γ IgG1 mAb that was assessed in phase I studies for mild-to-moderate stable SLE, SLE nephritis, and discoid lupus [(146),(147)]. In the phase Ib, randomized, multiple-dose escalation study (NCT00818948), AMG 811 demonstrated an acceptable safety profile, but no effects on SELENA-SLEDAI scores, proteinuria, C3 or C4 complement levels, or anti-dsDNA antibodies were observed [(148)]. Similarly, AMG 811 did not demonstrate any clinical benefit for discoid lupus in its phase 1 study (NCT01164917) [(146)]. The changes in CLASI score, physician's assessment of skin disease, or patient's self-assessed skin disease did not differ between the treatment and placebo groups. Due to these disappointing results, phase 2 trials are not underway. The disappointing AMG 811 results suggest that targeting type II IFN in SLE may not be as fruitful as targeting type I IFN.

4.2 Toll-Like Receptors (TLRs)

4.2.1 Biological Properties of TLRs

TLRs are single-pass membrane-spanning pattern recognition receptors expressed by many cell types that recognize pathogen–associated molecular patterns of microbes. They are located in different cellular compartments; some are on the cell surface, others are in the endoplasmic reticulum, and others are in the cytoplasm. When engaged, TLRs recruit adapter proteins (proteins that mediate other protein-protein interactions) within the cytosol of the immune cell, ultimately activating transcription factors that, depending on the intracellular signal pathways triggered, stimulate expression of genes that encode proinflammatory or anti-inflammatory proteins.

4.2.2 Role of TLRs in SLE

The TLR family can be divided into extracellular and intracellular, although there is some overlap. TLR1, TLR2, TLR4, TLR5, TLR6, and TLR11 are largely located on the cell surface and recognize PAMPs (pattern associated molecular patterns) [(149)]. TLR3, TLR4, TLR7, TLR8, and TLR9 are largely expressed intracellularly in endosomal or lysosomal compartments and the endoplasmic reticulum [(149)].

Of the extracellular TLRs, *TLR2* and *TLR4* mRNA are expressed to greater degrees in PBMCs (peripheral blood mononuclear cells) of SLE patients than of healthy subjects [(150)]. Conversely, deficiency in TLR2 and/or TLR4 downmodulates serological and pathological abnormalities in murine SLE models [(151)].

Regarding TLR5, its expression correlates with IFN-α mRNA in the PBMCs of SLE patients [(150)]. Additionally, the stop code polymorphism allele C1174T of the *TLR5* gene is associated with protection from development of SLE [(152)]. The roles of the other intracellular TLRs (TLR1, TLR6, and TLR11) in SLE remained to be delineated.

Regarding intracellular TLRs, poly I:C (a synthetic immunostimulant that interacts with TLR3) aggravates lupus nephritis in MRL/*lpr* mice through interactions with TLR3 on glomerular mesangial cells and APCs [(153)]. Poly I:C injection does not increase anti-dsDNA antibodies, and ablation of TLR3 does not affect the formation of autoantibodies, suggesting that the role for TLR3 role in SLE is B-cell independent [(153)].

The role for TLR7 is well established in SLE mouse models. The Y-linked autoimmune acceleration (*Yaa)* cluster includes a duplication of the *TLR7* gene, and mice expressing this cluster display autoimmune phenotypes [(154)]. In male BXSB mice, autoreactive B cell responses to RNA-related antigens are due to *TLR7* gene replication [(155)]. In these same mice, ablated TLR7 signaling results in

decreased autoantibody production [(156)]. A similar paradigm, that TLR7 enhances autoimmunity and its ablation abates it, is also seen in pristane-induced mice and MRL/*lpr* mice [(157), (158), (159)].

Although TLR8 is phylogenetically similar to TLR7, few studies have been published on its role in SLE. Recent studies in C57BL/6 mice revealed that TLR8 in dendritic cells may restrain TLR7-mediated lupus manifestations [(160)] and that TLR8 deletion accelerates autoimmunity in SLE-prone mice through a TLR-7 dependent mechanism [(161)].

The role of TLR9 is more controversial; multiple SLE-prone mouse studies have shown that TLR9 is needed for B cell production of anti-dsDNA, anti-chromatin, and anti-nucleosome autoantibodies. Paradoxically, deletion of TLR9 in these models did not attenuate disease but rather exacerbated it, thereby suggesting a protective role for TLR9 [(159)]. Further studies are needed to delineate TLR-9's role in SLE [(161)].

MyD88 is a common adaptor protein for most TLR signaling, and MyD88-knockout MRL/*lpr* mice fail to develop nephritis and exhibit a longer lifespan than wild-type mice [(162)]. In addition, the IL-IR-associated kinases are also potential targets for SLE therapy in that they act as a scaffold for MyD88 in TLR signaling. Indeed, IRAK-4 deficient patients and MyD88-deficient patients do not develop autoimmune diseases [(163)].

4.2.3 Emerging Therapeutics Targeting TLRs

Oligonucleotides, small molecule inhibitors (SMIs), mAbs, and microRNA (miRNA) regulation may target TLRs and be used as SLE therapeutics. IRS-954 is an oligonucleotide inhibitor of TLR7 and TLR9 that inhibits *in vitro* induction of IFN-α by human pDCs in response to viruses and immune complexes from SLE patients [(164)]. Treatment of BWF1 mice with this oligonucleotide led to decreased serologic, pathologic, and clinical disease, resulting in increased survival [(164), (165)]. Multiple synthetic oligonucleotides are in preclinical evaluation for SLE [(166), (167), (168), 203].

SMIs are oral agents that target endosomal TLRs and downstream proteins. The quinazoline derivative CpG-52364 has completed its phase-I clinical trials (NCT00547014), although results have not yet been published. It blocks activation of TLR 7/8/9, does not cause general immunosuppression, and was shown to be safer and more efficacious than HCQ in animal studies [(170)]. Multiple other SMIs are in preclinical evaluation for SLE [(171)]. IMO-9200 was found to be safe in phase-I clinical trials as an inhibitor of TLR 7/8/9. (172) Anti-TLR antibodies are also in preclinical studies for SLE.

MiRNAs are small noncoding regulatory RNAs that affect posttranscriptional regulation by promoting degradation of mRNA. Under expression of miRNAs are associated with over-activation of TLR signaling in SLE [(173), (174)]. MiR-155-5p, miR-203-5p, and miR-149-5p regulate TLR signaling by targeting MyD88 [(175)]. IRAK proteins are also regulated by different miRNAs. Since miRNAs can regulate >40% of human mRNAs that encode immune genes, therapeutics that alter miRNAs could be of great clinical importance [(176)].

5. Conclusions

In this review, we examined promising targets in adaptive and innate immunity as well as intracellular signaling. Due to the heterogenous presentation and complicated pathophysiology of SLE, there are almost countless targets that are being investigated, including other cytokines, the complement pathway, the microbiome, and more. Additionally, clinical trials are ongoing which combine biologics, such as BLISS-BELIEVE which is investigating the use of combination rituximab with belimumab for treatment of SLE (NCT03312907). As we continue to advance our understanding of SLE, we hope that new agents will emerge for our patients. While there will likely never be one “magic bullet” for the treatment of SLE, one can be optimistic that new therapies will emerge in the not-too-distant future.

6. Expert Opinion

6.1 Safe bets – an oxymoron

Political scientists and sports aficionados have known for decades that a "safe bet" is not necessarily safe. The Presidential elections of Harry Truman over Thomas Dewey in 1948 and Donald Trump over Hillary Clinton in 2016 are proof-positive for the former, and the Super Bowl victory of the Joe Namath-led New York Jets and the improbable triumph in the World Series of the "Miracle" New York Mets, both in 1969, make the case for the latter.

SLE clinical trialists also realize that "safe bets" are often far from safe. Although phase II trial results with epratuzumab were very encouraging, phase III trial results landed with a thud (31) (32) . Although phase II trial results with ustekinumab were resounding, the subsequent phase III trial could not even make it to its planned finish line. The only "safe bet" in SLE is that there is no safe bet (81) (82).

6.2 Which Emerging Targets hold the most promise?

With the caveats that prognostication in SLE is inherently fraught with danger and that our crystal ball is never perfectly clear, we cautiously anticipate anifrolumab becoming an important member of the rheumatologist's therapeutic armamentarium. TULIP II was a successful phase III trial, and despite TULIP I not reaching its primary endpoint, it nevertheless reached many of its important secondary endpoints.

We also cautiously anticipate that voclosporin will emerge as a successful therapy. Long before voclosporin entered the world of SLE, tacrolimus and rapamycin were being used off-label with some success for SLE. In the results released from its phase III trial, voclosporin appears to smell like a rose. However, the devil is in the details, so we eagerly await publication of the actual data.

On the flip side, we are not as sanguine for the novel B cell-targeting agents. The two RTX-based RCTs to date failed, and while belimumab is FDA-approved for SLE, its effect is modest, although its recent success (again, only modest) as an adjunct therapy in lupus nephritis does shine a very favorable light on this non-novel B cell-targeting agent. (177) A myriad of pathogenic pathways, including B cell-

independent pathways, contributes to SLE, so a patient who has failed to respond to a B cell-targeting agent would *a priori* more likely respond to an agent that targets a B cell-independent pathway than to a second (or third) B cell-targeting agent. The reported early dramatic success of telitacicept (52) desperately needs to be validated and replicated, as it is not clear why this BAFF/APRIL antagonist should have great success while the related BAFF/APRIL antagonist, atacicept, has not (47). History in SLE has repeatedly documented that if something appears to be too good to be true, it likely is too good to be true.

6.3 Key pitfalls surrounding emerging targets

The key and recurring booby trap that ensnares any emerging therapeutic target for SLE is the mammoth and seemingly limitless heterogeneity of SLE. When we study SLE in mouse models, we study syngeneic lines. That is, every "patient" is virtually identical to the next. When studying human SLE, however, every patient is akin to a different mouse line, so the vast genetic diversity across the spectrum of human SLE renders development of therapeutics very difficult, to say the least. Moreover, when an investigator studies murine SLE, the "patients" are all housed in the same room, all fed identical menus, and all evaluated by the same investigative team. This is a far cry from human SLE, for which disparate environmental exposures and varied degrees of assessments are the rule. Indeed, given the daunting challenges faced by SLE trialists, it is quite remarkable that any drugs have been able to achieve success.

6.4 Future Perspectives

By now it should be clear that the future of SLE therapeutics lies in precision medicine and aggressively stratifying patients by immune mechanism, genetic profile, biomarkers, and histological findings [(178)]. Novel technologies, such as modular repertoire analysis, epigenetic profiling, and omics analyses will greatly help us in this endeavor [(178)]. While narrowing entry criteria into clinical trials by virtue of specific molecular, genetic, and/or immune properties may decrease the generalizability of results, such restriction will ultimately immeasurably help the discrete subsets of patients for whom current therapy is

inadequate. GWAS studies have already illuminated the field and have identified many SLE susceptibility genes. While the majority of these genes are implicated in well-known SLE dysregulated pathways, such as B and T cell signaling and the type I IFN pathway, others, such as JAZF1, PXK, XKR6, UHRF1BP1, and WDFY4, do not have any known function. Better understanding the roles of these genes will likely identify heretofore unappreciated pathogenic pathways. (179)

Indeed, better profiling of the epigenome, transcriptome, and metabolome will enhance our knowledge of SLE and help us uncover novel targets. The shotgun approach of many of our current drugs target both pathogenic and nonpathogenic cells, so not only is their efficacy sub-optimal, but they lead to unnecessary and avoidable toxicities. Through omics profiling, we should be able to identify detrimental mediators with great precision and develop therapeutics that spare normal cells.

Omics profiling, however, will not obviate the need for many (if not all) of our current state-of-the-art drugs, including hydroxychloroquine, azathioprine, mycophenolate, and, of course, corticosteroids. Belimumab, the one biologic already FDA-approved for SLE, will likely take on a more prominent role, especially in light of its success in lupus nephritis (177). The roles in SLE for biologics or non-biologics already approved by the FDA for diseases other than SLE and the roles for novel biologics or non-biologics not yet approved by the FDA for any condition remain to be seen. Our crystal ball is far too foggy to offer reliable predictions.

Treating SLE remains a challenge. As shown in this review, however, there are many exciting targets on the horizon. As we further study these targets and more rationally stratify patients, SLE will continue to become increasingly treatable. We look forward to the day when this will become reality rather than simply wishful thinking.

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48. ABSTRACT NUMBER: 2642 Atacicept Dose Rationale for a Phase 3 Study in Patients with High Disease Activity and Auto-Antibody Positive SLE Jinshan Shen1, Orestis Papasouliotis2, Eileen Samy1, Philipp Haselmayer3, Peter Chang4, Victor Ona1 and Amy H. Kao1, 1EMD Serono Research & Development Institute, Inc. (a business of Merck KGaA, Darmstadt, Germany), Billerica, MA, 2Merck Institute for Pharmacometrics, an affiliate of

Merck KGaA, Darmstadt, Germany, Lausanne, Switzerland, 3Merck KGaA, Darmstadt, Germany, 4EMD Serono Research & Development Institute, Inc. (a business of Merck KGaA, Darmstadt, Germany), BIllerica, MA Meeting: 2018 ACR/ARHP Annual Meeting. In.

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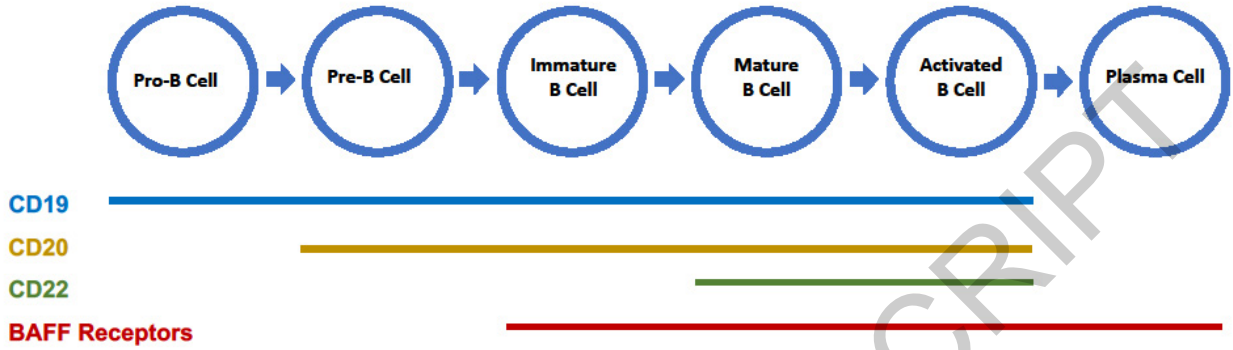

Figure 1. Expression of specific surface markers distinguish the different stages of B cell development.

Table 1: Emerging Targets and Corresponding Therapeutic Agents in Adaptive Immunity in SLE						
Molecular Target	Biological Effect	Biological Agent	Status	FDA approved for indications other than SLE	FDA indications	References
B cells						
CD20	B cell depletion	Rituximab	Phase III trials failed	Yes	Chronic lymphocytic leukemia, Granulomatosis with polyangiitis, Microscopic polyangiitis, Non-Hodgkin lymphomas, Pemphigus vulgaris , Rheumatoid arthritis	25, 26
		Obinutuzumab	Successful Phase II trial, Phase III trial planned	Yes	Chronic lymphocytic leukemia, Follicular lymphoma	27 NCT04221477
CD19	B cell depletion	MEDI551 (Inebilizumab)	Not in clinical trials for SLE yet	Yes	Neuromyelitis optica spectrum disorder	28
		XmAb5871 (obexelima		No		29 NCT02725515

		b) CD19-CAR-T cell therapy	Phase II trial did not meet its primary endpoint; Phase III trials not currently planned In phase 1 clinical trials; awaiting results	Yes	Select non-Hodgkin lymphomas, refractory acute lymphoblastic leukemia	NCT03030976
CD22	B cell inactivation and modest B cell depletion	Epratuzumab	Phase III trial did not meet its primary endpoint	No		31, 32
Plasma Cells						
Proteasome	Inhibits proteasome Inhibits immunoproteasome	Bortezomib KZR-616	Phase II trial terminated due to recruitment difficulties Phase II trial in enrollment phase	Yes No	Mantle cell lymphoma, Multiple myeloma	33 NCT02102594 34 NCT03393013
BAFF	B cell depletion (modest)	Blisibimod Tabalumab	Phase III trial did not meet its primary endpoint First phase III trial did not meet its	No No		49 50, 51

			primary or secondary endpoints. Second phase III trial met its primary endpoint, but did not meet key secondary endpoints. Further trials are not currently planned.			
BAFF + APRIL	B cell and plasma cell depletion	Atacicept Telitacicept	APRIL-SLE phase II/III trial terminated due to increased infections. ADDRESS II Phase IIb trial did not meet its primary endpoint; further phase III trials are being contemplated Met its phase 2B endpoint; recruiting for phase III	No No		46, 47, 48 52 NCT04082416
Bruton tyrosine kinase	Blockade of B cell maturation	MSC2364447C	Undergoing Phase II trials	No No		NCT02975336

		BIIB068 Fenebrutinib (GDC-0853) ABBV-105	Completed a phase I trial; results not yet released Phase II trial did not meet primary or secondary endpoint; Phase III trials not planned Undergoing recruitment for a Phase II trial	No No		NCT02829541 NCT02908100 NCT03978520
T cells						
CD28	Blockade of T cell activation/differentiation	Abatacept	A Phase IIb trial and a Phase II/IIII trial did not meet their primary endpoints; a phase I/II trial for SLE arthritis is ongoing	Yes	Juvenile idiopathic arthritis, Psoriatic arthritis, Rheumatoid arthritis	68, 69 NCT02429934
CD40L	Blockade of T cell activation/differentiation	Toralizumab Ruplizumab CDP7657 (dapirolizu	Discontinued in clinical trials due to thromboembolic events Discontinued in clinical trials due to thromboem	No No No		70 70, 71 70, 72 NCT04294667

		mab pegol),	bolic events Phase III trial planned			
CD40	Blockade of T cell activation/differentiation	BI655064 CFZ-533	Undergoing Phase II trials Undergoing Phase II trials	No No		NCT03385 54 , NCT02770 170 NCT03610 516 , NCT03656 562
Cytokines						
IL-12/IL-23	Binds to the p-40 subunit of both IL-12 and IL-23 and blocks inflammation	Ustekinum ab	Undergoing Phase III trials	Yes	Crohn's disease, Plaque psoriasis, Ulcerative colitis	81, 82
IL-17 and BAFF	Binds to BAFF and IL-17 and blocks inflammation	Tibulizuma b	No clinical trials planned yet	No		88
Tregs	Activates Tregs and increases tolerance	Low-dose IL-2	Undergoing Phase II trials	No		95, 96

Table 2: Emerging Targets and Corresponding Therapeutic Agents in Intracellular Signaling pathways in SLE						
Molecular Target	Biological Effect	Biological Agent	Status	FDA approved for indications other than SLE	FDA Indications	References
mTOR	Inhibits mTOR	Rapamycin (Sirolimus)	Phase 1/2 trial met its primary endpoint	Yes	Lymphangioleiomyomatosis, Renal transplantation (rejection prophylaxis)	105, 106
Calcineurin	Inhibits Calcineurin	Tacrolimus	Phase 4 RCT showed noninferiority to MMF for induction therapy for LN	Yes	Organ rejection prophylaxis	107, 108, 109
		Voclosporin	Phase III trial met its endpoint	No		110, 111
JAK	Inhibits JAK/STAT pathway	Baricitinib	Phase 3 trials are ongoing	Yes	Rheumatoid arthritis	113 NCT03843125 NCT03616964
		Tofacitinib	Awaiting results from a complete phase 1B trial; further phase I/II	Yes	Rheumatoid arthritis, Psoriatic arthritis, Ulcerative colitis	NCT03616912 NCT02535689 NCT03159936 NCT03288324

		Upadicitinib BMS-986165 PF06700841	trials are in the recruitment stage Undergoing recruitment for a Phase II trial Undergoing phase II trials Undergoing phase II trials	Yes No	Rheumatoid arthritis	 NCT03978520 NCT03252587 NCT03943147 NCT03845517

Table 3: Emerging Targets and Corresponding Therapeutic Agents in Innate Immunity in SLE					
Molecular Target	Biological Effect	Biological Agent	Status	FDA approved for indications other than SLE	
Interferons					
Type 1 IFN		Reverse transcriptase inhibitors	Preclinical studies	HIV Hepatitis B	122
IFN-α	Blocks inflammation induced by IFN-α	Rontalizumab	Phase II trial did not meet its endpoints; no longer being evaluated for SLE	No	139
		Sifalimumab		No	140
		AGS-009	Met its primary endpoint in Phase IIb trial, but with modest effect; no longer being evaluated for SLE	No	141
		IFN-K	Had a good safety profile in Phase I trials; no phase II trials planned yet	No	142
			Did not meet its clinical primary endpoints in a phase IIb study; no phase III trials currently		
IFNAR	Blocks inflammation induced by IFN-α	Anifrolumab	Did not meet its primary endpoint in TULIP I phase III trial but met its	No	144, 145 NCT02794285

			primary endpoint in TULIP II phase III trial; Currently undergoing open-label extension of both phase III trials		
Type II IFN	Blocks inflammation induced by IFN-γ	AMG 811	Did not demonstrate any clinical benefit in phase Ib studies; phase II studies are not planned	No	146, 147, 148 NCT00818948 NCT01164917
TLRs					
TLR7 and TLR9	Blocks inflammation induced by TLRs	IRS-954	In preclinical studies	No	164, 165
TLR7, TLR8, and TLR9	Blocks inflammation induced by TLRs	CpG-52364 IMO-9200	Has completed phase-I clinical trials; awaiting results Had a good safety profile in a Phase I trial; no further trials planned yet	No No	171 NCT00547014 17