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Small Molecules in the treatment of systemic lupus erythematosus

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Abstract

Advances in the understanding of the cellular biological events that underlie systemic lupus erythematosus (SLE) have led to the identification of key molecules and signaling pathways that are aberrantly expressed. The parallel development of small molecules drugs that inhibit or interfere with the specific perturbations identified, offers perspective for more rational, effective and less toxic therapy. In this review, we present data from preclinical and clinical studies of such emerging novel therapies with a particular focus on kinase inhibitors and other compounds that modulate signal transduction. Moreover, we highlight the use of chromatin-modifying medications, bringing attention to the central role of epigenetics in SLE pathogenesis.

Keywords

Systemic Lupus Erythematosus; kinase inhibitors; Jak; Syk; HDAC inhibitors

1. Introduction

The existing therapies for systemic lupus erythematosus (SLE) despite being effective in suppressing disease activity, rarely offer long-term remission; moreover medication-related toxicity contributes to morbidity and mortality (1). On the other hand, the heterogeneous nature of SLE, calls for personalized treatments based on the molecular identities of subgroups of SLE patients.

The approval of imatinib for the treatment of chronic myelocytic leukemia (CML) (2) has set the stage for developing a new therapeutic paradigm based on the identification and then targeting of the specific molecular pathways that are aberrant in disease. This novel approach aims at the development of small molecules that inhibit inter- and intra-cellular signaling potentially offering the prospect of rational, individualized and less toxic treatments. In this review we summarize the most recent research on the development of small molecules that target kinases and other signaling molecules that are implicated in SLE pathogenesis or regulate the chromatin structure of pathogenetically important genes in animal models and humans with SLE.

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2. Inhibition of kinases and phosphatases

2.1. Spleen tyrosine kinase (Syk) inhibition

Syk is widely expressed in hematopoietic, stromal, endothelial and epithelial cells. Classically, Syk is recruited at phosphorylated tyrosines on immunoreceptors, including the B-cell receptor (BCR), T-cell receptor (TCR), Fc receptors (FcR), integrins and C-type lectins. Upon binding of the receptor with its ligand, Syk is activated. Through that interaction, activation of Syk leads to cellular responses such as mobilization of intracellular calcium and regulation of gene transcription programs (3–5). The key role of BCR and TCR in antigen recognition and of FcR in handling immune complexes, places Syk in the epicenter of SLE pathogenesis. Notably, the CD3/TCR complex in SLE is “rewired” and instead of signaling through the CD3 ζ chain and ZAP-70, as it does in normal T cells, it signals through the FcR γ chain and Syk (6–8). The FcR γ chain/Syk complex populates lipid rafts, which are pre-clustered in SLE T cells, and contributes to the hyperexcitable T cell phenotype found in SLE (9–11). Therefore, targeting Syk represents a promising therapeutic strategy in SLE.

Fostamatinib (also known as R788), a small molecule inhibitor of Syk, has demonstrated significant efficacy in clinical trials of rheumatoid arthritis with an acceptable toxicity profile (12, 13). R788 is metabolized to R406, which is the active compound. *In vitro* inhibition of Syk with R406, in SLE T cells resulted in suppression of intracellular calcium signaling (11). *In vivo* inhibition of Syk in NZB/NZW, MRL/*lpr* and BAK/BAX mice prevented the development of renal disease, prolonged survival and ameliorated established renal pathology, although it did not reduce the titer of anti-ds-DNA antibody titers. In MRL/*lpr* and BAK/BAX mice fostamatinib also prevented the development of skin lesions (14, 15).

2.2. Janus Kinases (Jak) inhibition

Jak are tyrosine kinases (Jak1, Jak2, Jak3 and Tyk2) bind to cell receptor subunits and mediate the intracellular signaling initiated by interferons (IFN), many interleukins, colony-stimulating factors, and hormones such as prolactin, erythropoietin and growth hormone. Following receptor ligation, Jak become activated and phosphorylate the latent transcription factors known as signal transducers and activators of transcription (STAT). Then STAT, in homo- or heterodimers, translocate into the nucleus where they regulate gene transcription (16). Mutations of Jak or STAT in humans are associated with severe immune dysfunction, revealing the fundamental role of this pathway in the induction and regulation of immune responses (17–21). Tofacitinib, a small molecule that inhibits Jak3, Jak1 and to a lesser degree Jak2 has been proven efficacious in RA in phase III trials and ruxolitinib, which inhibits Jak2, was approved by FDA to treat myelofibrosis (22–25). Notably, a series of Jak-STAT signaling cytokines, especially type I IFNs, IL-10 and IL-6, as well as the hormone prolactin, have been implicated in the pathogenesis of SLE (26–29). In this context, targeting the Jak-STAT pathway has emerged as an attractive approach to manage inflammation and auto-immunity in SLE.

Treatment of lupus-prone mice with JAK2 inhibitors led to prevention or improvement of established disease. In MRL/*lpr* mice, administration of tryphostin AG490 from week 12 to week 20 of age led to a decrease in proteinuria, T cell and macrophage infiltrates, expression of IFN γ , serum level of dsDNA and deposition of IgG and C3 in the kidney (30). A disease prevention protocol with another Jak2 inhibitor, CEP-33779, which was started at the age of 8 weeks up to 21 weeks, prevented the development of nephritis. In addition, administration of CEP-33779 in NZB/W F1 mice with established nephritis was proven superior to the treatment with dexamethasone and cyclophosphamide, resulting in improved survival,

reduced proteinuria, decreased dsDNA antibodies and decrease in the autoantibody producing spleen plasma cells. Finally, several cytokines associated with SLE pathogenesis, including IL-12, IL17A, IL-6, IL-4, TNF α , were also downregulated upon treatment with the Jak2 inhibitor (31).

2.3. Bruton's Tyrosine Kinase (Btk) inhibition

Btk is a cytoplasmic enzyme that is indispensable for signaling through the BCR. BTK mutations in humans cause X-linked agammaglobulinemia characterized by a complete absence of circulating B cells and lack of immunoglobulins (32).

While BTK activation has not been directly studied in SLE, aberrant activation of B cells is a hallmark of disease pathogenesis. Activated B cells contribute to pathogenesis not only by secreting pathogenic autoantibodies but also produce cytokines and serve as antigen presenting cells. Thus, it is expected that blocking B cell activation will modify the expression of the disease (33).

An oral BTK inhibitor (PCI-32765 or ibrutinib) was given in MRL/*lpr* mice for 12 weeks starting at week 8 of age, before disease onset. Treatment resulted in a decrease in proteinuria, a modest decrease in anti-dsDNA antibody titers (not statistically significant), improvement in interstitial nephritis and perivascular inflammation and a statistically significant reduction of the glomerulonephritis (34).

2.4. Calcium/calmodulin-dependent kinase IV (CaMKIV) inhibition

CaMKIV is a serine/threonine kinase that is activated by calcium and then translocates to the nucleus where it phosphorylates transcription factors and regulates their activity. SLE T cells express increased amounts of nuclear CaMKIV, which activates CREM α that binds to *interleukin (IL)-2* promoter suppressing the transcription of the *IL-2* gene. Intriguingly, incubation of normal T cells with SLE serum, increases CREM α binding to the *IL-2* promoter, through CaMKIV. These findings suggest that increased activity of CaMKIV potentially contributes to the decreased production of IL-2 that has been described in SLE (35). In this context, the potential therapeutic effects of CaMKIV inhibition have been investigated.

Administration of the CaMKIV inhibitor KN-93 to MRL/*lpr* lupus-prone mice prevented the development of lupus nephritis and suppressed established disease improving skin lesions and kidney disease parameters. It also resulted in decreased production of inflammatory cytokines such as IFN- γ and TNF- α (36). Furthermore, genetic deletion of CaMKIV in MRL/*lpr* mice led to less kidney damage and decreased proteinuria at 16 weeks of age. *In vitro* experiments also suggested that CaMKIV inhibition results in decreased mesangial cell proliferation and reduced IL-6 production from these cells (37).

2.5. Rho kinase (ROCK) inhibition

ROCK is a serine/threonine kinase which acts downstream of the small GTPase RhoA. ROCK regulates cytoskeletal dynamics and signaling pathways involved in cell proliferation, gene expression, migration and apoptosis. ROCK has been implicated in SLE pathogenesis by phosphorylating ezrin/radixin/moesin (ERM) and IRF4 (38). ERM proteins connect the adhesion molecule CD44 with cortical F-actin resulting in polar cap formation in SLE T cells, and thus enable them to infiltrate target tissues. *In vitro* studies showed that SLE T cells exhibit enhanced expression of CD44 and ERM; inhibition of ERM phosphorylation with a ROCK inhibitor resulted in decreased ability of the SLE T cells to form polar caps, adhere to hyaluronic acid and migrate. Kidney biopsies of patients with lupus nephritis showed the presence of CD44⁺ERM⁺ T cells, making inhibition of ERM

phosphorylation an attractive target for treating SLE (39). In addition, ROCK phosphorylates the transcription factor IRF4, which is required for the induction of IL-17 and IL-21 (40). IRF4 also regulates immunoglobulin isotype switching and differentiation of mature B cells into antibody-secreting plasma cells. Notably, deficiency of IRF4 ameliorates lupus nephritis in the B6/*lpr* murine model (41).

Administration of the nonselective ROCK inhibitor fasudil in MRL/*lpr* and NZB/W F1 mice, resulted in amelioration of their disease. The observed benefit was characterized by marked reduction of IL-17 and IL-21, diminished expansion of plasma cells in spleen, and marked decrease in dsDNA autoantibody production, diminished glomerular deposition of IgG and C3 and improvement of the proteinuria (40, 42). Fasudil has been used with a good safety profile for cerebral vasospasm and cardiovascular disorders. Potential side-effects include thrombocytopenia and bleeding diathesis but rarely have been reported (43, 44). The amelioration of the disease pathology in murine lupus models and the good safety profile in patients make it an attractive medication for future SLE studies.

2.6. Mammalian target of rapamycin (mTOR) inhibition

mTOR is a serine/threonine protein kinase, highly conserved from yeast to humans, that has a central role in cellular metabolism. It senses and integrates many different environmental signals related to the nutritional supply to the cell, availability of energy, redox status, growth factors and stress. Rapamycin, a macrolide produced by *Streptomyces hygroscopicus*, forms a complex with the intracellular 12-KDa FK506-binding protein FKBP12 and inhibits mTOR (45). It has long been used safely and effectively for preventing organ transplant rejection and based on the notion that it inhibits T cell proliferation it was applied in lupus murine models. In the MRL/*Lpr* mouse treatment with rapamycin improved survival, decreased the albuminuria and ameliorated the pathology of the affected organs. Notably anti-dsDNA antibody titers were significantly decreased (46). Similarly, rapamycin treatment was efficacious in preventing and treating established disease in the NZB/W F1 lupus-prone mouse. The same effect on lowering the anti-dsDNA titer and improving the deposition of IgG and C3 in the kidney was noted (47–49). Studies on human SLE T cells showed that the mTOR kinase activity is upregulated (50). Rapamycin was given off-label in 9 patients with active SLE refractory to conventional immunosuppression. The treatment was well tolerated and led to amelioration of the disease and a decrease in prednisone requirements. The authors investigated the effect of rapamycin on calcium signaling in the patients' T cells and they showed that treatment resulted in normalization of baseline intracellular calcium and CD3/CD28 induced calcium flux (51). A prospective, open label, phase II study of rapamycin in the treatment of SLE is currently ongoing.

2.7. Phosphatidylinositol-3 kinases (PI3K) inhibition

The class I PI3Ks are lipid kinases that generate lipid second messengers involved in a network of signaling pathways that controls cell growth and survival. They are further subdivided in class IA and IB. The class IA contains three catalytic isoforms (p110 α , p110 β , p110 δ) and is activated downstream of TCR, BCR, co-stimulatory and cytokine receptors. The class IB has p110 γ as the only catalytic subunit and is activated by G-protein coupled receptors such as chemokine receptors.

PI3K γ and PI3K δ are the two isoforms mainly expressed in cells of hematopoietic origin. There is a growing body of evidence that implicates the PI3K pathway in B and T cell development and differentiation as well as in numerous functions of the innate immunity such as neutrophil migration, phagocytosis and production of type I IFN by pDCs (52–54).

In vitro studies of human SLE peripheral blood mononuclear cells (PBMC) revealed increased activity of the p110 δ PI3K isoform compared to cells from normal individuals. Interestingly, the p110 δ PI3K isoform appeared to associate with the Fc ϵ RI γ chain, which is the chain that preferentially recruits Syk in the rewired SLE T cell receptor. Inhibition of the p110 δ PI3K with IC87144, resulted in restoration of activation-induced cell death defect in SLE T cells (55).

PI3K signaling is enhanced in Lyn-deficient mice, an animal model of SLE. In order to examine the effects of down-regulating PI3K signaling, these mice were rendered heterozygous for the p110 δ PI3K isoform. As a result, they had milder autoimmune disease characterized by lower levels of autoantibodies, attenuation of glomerulonephritis with decreased IgG and C3 depositions and improved survival. Moreover, plasma cell numbers were reduced, T cell activation was restrained and inflammation and myeloid cell expansion was moderated. This study suggested that pharmacological inhibition of the p110 δ PI3K isoform may be beneficial in lupus (56). Treatment of MRL/lpr mice with AS605240, a PI3K γ inhibitor, was initiated at 2 or 3.5 months of age and continued up to 5.5 months of age. The treatment was compared to treatment with dexamethasone and resulted in prolonging the lifespan of AS605240 treated mice without causing adverse events. Histologic recovery with reduction in the proteinuria, dsDNA titer and immune complex deposition was noted as well as reduction in CD4 $^{+}$ memory T cells (57).

2.8. Calcineurin-NFAT inhibition

Calcineurin is a calcium dependent phosphatase; following TCR ligation, calcium influx leads to the activation of calcineurin, which dephosphorylates many substrates including the nuclear factor of activated T cells (NFAT). The dephosphorylated NFAT translocates to the nucleus and interacts with other transcription factors to regulate gene expression. In SLE, enhanced calcium flux in T cells, leads to increased levels of nuclear NFAT resulting in overexpression of CD154, a key costimulatory molecule for the T cell dependent autoantibody production by B cells (58).

The calcineurin inhibitors cyclosporine and tacrolimus have been used widely as immunosuppressive agents in transplantation and as a second-line therapy in SLE. Their use in SLE patients especially the ones with severe manifestations such as nephritis is limited by significant side-effects, including hypertension, nephrotoxicity and neurotoxicity. More recently, dipyridamole, a widely used anti-platelet agent with a much more benign side-effect profile including vasodilation, bronchospasm in patients with pre-existing asthma and rarely bleeding complications due to the inhibitory effect on platelets, was recognized to inhibit the Calcineurin-NFAT interaction (59).

A recent study showed that *in vitro* treatment of SLE patient-derived lymphocytes with dipyridamole, resulted in decreased expression of surface CD154 on T cells and decreased production of immunoglobulin by B cells. It also inhibited the production of cytokines including IL-6 and IL-17. Furthermore, MRL/lpr mice treated with dipyridamole had delayed onset of clinical disease and decreased levels of IL-6 in their serum compared to controls (60).

3. Chromatin modifiers

Chromatin has a dynamic structure that critically influences transcription of genes in health and disease states. Environmental stimuli modify the structure of chromatin by inducing a series of chromatin modifications including cytosine methylation of DNA, posttranslational modifications of histones and chromatin remodeling. DNA methylation and histone modifications are reversible chromatin marks due to enzymes with opposing actions,

including addition (writers), or removal (erasers) of any given modification. Thus, acetylation of lysine (K-ac) residues is regulated by the actions of histone acetyl-transferases (HATs) and histone de-acetylases (HDACs). The status of K-methylation of histones is regulated by the function of Histone methyl-transferases (HMTs) and histone de-methylases. Finally, DNA methylation results from the effects of DNA methyl-transferases (DNMTs). Chromatin marks recognized by specific proteins or protein-complexes (readers), in turn mediate additional effects on chromatin, such as remodeling of gene regulatory elements (promoters or enhancers). For example, bromodomain (BRD)-bearing proteins recognize and bind to K-ac on histones (61, 62). Extensive evidence derived from *in vitro* studies, animal models and patients, suggests that chromatin modifications play critical roles in human diseases and targeting of chromatin modifiers represents an attractive strategy to treat cancer, inflammation and autoimmunity (63–65). Mutations in chromatin modifiers have been correlated with several types of malignancies and the HDAC inhibitors Vorinostat and Romidepsin have been approved for the treatment of cutaneous T cell lymphoma (63). Moreover, Givinostat another HDAC inhibitor, showed therapeutic benefit in a small clinical trial of patients with systemic-onset juvenile idiopathic arthritis (66). In addition, a small molecule that inhibits BET proteins (I-BET), which are BRD-bearing proteins, leads to repression of a group of inflammatory genes (67).

Accumulating evidence highlights the role of chromatin modifications in the pathogenesis of SLE (68, 69). Monozygotic twins discordant for SLE were reported to have significant differences in DNA methylation in genes related to SLE pathogenesis (70, 71). Furthermore, procainamide and hydralazine that can cause drug-induced lupus in certain individuals, act by inhibiting DNA methylation. The decreased production of IL-2 by SLE T cells is linked to decreased H3K18Ac, increased H3K27me3. Importantly, binding of CREM α to the *IL-2* promoter is accompanied by increased CpG methylation. CREM α recruits both HDACs and DNA Methyltransferases (DNMT) that inhibit *IL-2* transcription. Reciprocal epigenetic changes were observed in the promoter of *IL-17* gene where CREM α mediated increased transcription of the *IL-17* (72, 73).

HDAC inhibitors have been tried as therapies in lupus prone mice. In MRL/*lpr* lupus mouse model, trichostatin (TSA) was given for 5 weeks starting at week 14 and resulted in decreased splenomegaly, proteinuria and improved kidney pathology, but did not affect autoantibody production or the deposition of IgG and C3 in the kidney. *In vitro* experiments highlighted that TSA reduces inflammatory cytokines and increases the acetylation of H3 and H4 (74). Similar results were obtained when a different HDAC inhibitor, suberoylanilide hydroxamic acid (SAHA) that was given to young pre-disease MRL/*lpr* mice for 10 weeks (75). In another study, administration of TSA in NZB/NZW F1 mice, starting at 14–16 weeks for a total period of 20 weeks, decreased anti-dsDNA antibody titers and IgG and C3 deposition in the kidney and increased Tregs with an overall improvement of lupus pathology (76). Although the above studies support the use of HDAC inhibitors in treating SLE, it is now evident that the aforementioned HDAC inhibitors have little specificity, since HDACs deacetylate both histone and non-histone proteins that are ubiquitously distributed within the cell, thus their mechanism of action remains elusive (61).

4. Quinoline-3-Carboxamide Derivatives (Paquinimod, Laquinimod)

Paquinimod and laquinimod are small molecules that belong to the class of quinoline-3-carboxamides. Linomide, the predecessor compound of this class showed efficacy in clinical trials of multiple sclerosis (MS), type I diabetes and in inhibiting disease in experimental models of autoimmune diseases including lupus (77, 78). After linomide was withdrawn from a phase III clinical trial in MS, because of safety concerns, paquinimod and laquinimod

derived from the parent drug and selected for their improved potency and superior safety, gained special interest (79).

For many years, the mode of action of quinoline-3-carboxamide derivatives remained unknown. However, experimental data suggested that this class of drugs exert its effects without suppressing the adaptive immunity. More recently the S100A9 protein was identified as the molecular target of the quinoline-3-carboxamides. Binding of quinoline-3-carboxamides to S100A9 inhibited its interaction with the Toll-like receptor 4 (TLR4) and the receptor of advanced glycation end products (RAGE) (80). The S100 proteins (calgranulins) are calcium-binding proteins mainly expressed in cells of myeloid origin that function as damage-associated molecular pattern molecules (DAMPs) promoting inflammation (81, 82). They are upregulated in various autoimmune diseases including SLE, where serum levels of S100A8/S100A9 correlate with disease activity (83). S100A8 and S100A9 proteins function as TLR4 ligands on CD8+ T cells, upregulating IL-17 expression and inducing autoreactivity in a mouse autoimmune model and human SLE patients (84). In this context, treatment of lupus with paquinimod and laquinimod appears promising.

MRL/*lpr* mice treated with paquinimod did not develop glomerulonephritis (85, 86). In a recent report, its effect in preventing disease was similar to prednisolone and mycophenolate. It also maintained remission after treatment induction with cyclophosphamide or prednisolone and resulted in decreased C3 deposition and a decreased anti-dsDNA levels after induction with cyclophosphamide. The same report included a phase I clinical study in which SLE patients with low disease activity at baseline, received daily oral paquinimod for 12 weeks in addition to standard treatment. The medication was in general well tolerated especially at doses of < 4.5 mg/day. Common adverse events were myalgia, arthralgia and back pain. An interesting observation was the elevation in acute phase reactants, noted at all dose levels, which declined towards the end of the study. No flares of the disease were observed (86). A phase II study of paquinimod in SLE patients with mildly active disease manifested by arthritis, oral ulcers/alopecia or rash has also been completed. Improvement of SLE symptoms was noted in patients with arthritis, oral ulcers and/or alopecia for the subgroup that had stable active disease at baseline and low interferon in the beginning of the study (87).

Laquinimod has also demonstrated therapeutic effect in various experimental autoimmune animal models and more recently a phase III study on patients with MS showed a modest reduction in the annualized rate of disease relapse (88). Administration of laquinimod in NZB/NZW F1 mice resulted in a dose-dependent reduction of anti-dsDNA antibody titers, inhibition of proteinuria and a significant dose-dependent prolongation of survival. Following 12 weeks of treatment, histopathological analysis of kidneys showed that mice treated with the higher dose of 5mg/kg had less immune complex deposits compared with vehicle or cyclophosphamide treated animals. Moreover, combining laquinimod with low dose mycophenolate or dexamethasone enhanced the effect of each compound alone.

In MRL/*lpr* mice treatment with laquinimod was compared to treatment with mycophenolate and again demonstrated a beneficial effect in reduction of proteinuria, dsDNA and reduction in glomerulonephritis and splenomegaly. After these promising results obtained from the animal models, phase I/II studies for lupus nephritis and lupus arthritis are now ongoing (89).

5. Proteasome inhibition

Proteasomes are large protein complexes located in the nucleus and cytoplasm that degrade abnormal and misfolded proteins a function that is crucial for the control of the cell cycle, the regulation of gene expression and the overall cell homeostasis. The immunoproteasome

contains different catalytic subunits compared to proteasome, and is constitutively expressed in immune cells, but its expression in other tissues can be induced by inflammatory cytokines such as IFN- γ . Its specific role is to generate peptides that can be presented by major histocompatibility complex class I (MHC-I) molecules.

Bortezomib, a selective inhibitor of proteasome has been approved for the treatment of multiple myeloma and mantle cell lymphoma (90, 91). Studies have suggested that myeloma cells are particularly sensitive to proteasome inhibition and their sensitivity is directly related to their high degree of immunoglobulin synthesis, inducing the terminal unfolded protein response (92, 93). That was also found to be valid for normal plasma cell in ovalbumin-immunized BALB/c mice (94).

In SLE autoantibodies produced by long-lived plasma cells are major players in disease pathogenesis and are resistant to current treatments (95). Bortezomib administration in NZB/W F1 lupus mice resulted in a significant reduction of both short- and long-lived plasma cells in the spleen and bone marrow (BM) and compared to cyclophosphamide or dexamethasone it was much more efficient in depleting BM total and long-lived plasma cells. Of note, the anti-dsDNA antibody titers were significantly decreased (94). Extending these observations, another report suggested that bortezomib treatment also depletes the short- and long-lived plasma cells that are located within the nephritic kidneys of NZB/W F1 mice (96). When bortezomib was given to NZB/W F1 mice starting either at 18 or 24 week of age and continuing for about 10 months, prevented the formation of anti-dsDNA antibodies, proteinuria, prolonged the survival and abrogated signs of renal pathology including IgG deposition. Similar positive results were obtained even when treatment was started in mice with more established disease and when given to MRL/lpr mice. Importantly the treatment was not associated with overt toxicity or severe immunosuppression (94). Interestingly, a study focusing on morphological changes of renal cells of NZB/W F1 mice treated with bortezomib, indicated that this medication in addition to eliminating anti-dsDNA antibody-secreting plasma cells, it also exerts a specific protective effect on podocyte ultrastructure (97).

Although bortezomib appears effective in preclinical models of SLE, its use may be limited due to unacceptable toxicities such as peripheral neuropathy in a significant number of patients (98). Because of this, great interest has been developed for trying alternative proteasome inhibitors. A recent study using the proteasome inhibitor carfilzomib and the immunoproteasome inhibitor ONX 0914 (formerly known as PR-957) showed efficacy similar to bortezomib in preventing and treating established disease in NZB/W F1 and MRL/lpr mice. Moreover, the study highlighted the potential of proteasome inhibitors to suppress production of IFN α by TLR-activated PDCs in vitro and in vivo (99). Finally, an orally active proteasome inhibitor, delanzomib (CEP-18770) was efficacious in treating both MRL/lpr and NZB/W F1 mice compared to dexamethasone and cyclophosphamide or bortezomib (100). In regards to treating human disease, 13 SLE patients, who had not sufficiently responded or did not tolerate conventional therapy, were treated with bortezomib intravenously (3 or 4 injections per cycle) and treatment cycles were repeated up to 4 times (time interval of 10–14 days between cycles). The treatment in general was well tolerated although 3 out of 7 patients who were treated with 4 bortezomib injections per cycle, developed reversible polyneuropathies. The disease activity as measured by SLEDAI and the anti-dsDNA antibody titers decreased in all 13 patients. Patients with active lupus nephritis experienced a decrease in proteinuria within 6 weeks of treatment and an increase in complements levels (101). The efficacy of bortezomib in the preclinical models of SLE, the positive results from case reports and the aforementioned case series, underlie the need for clinical trials to explore the use of proteasome inhibitors in SLE.

6. Conclusion

The described small molecules for the treatment of SLE have been developed following a deeper understanding of the molecular pathways that underlie the immunopathology of SLE (102, 103). Although, all of them have shown various degrees of efficacy in preclinical models, only a few have been tried in clinical studies of SLE. From those, the mTOR inhibitor rapamycin was efficacious and well tolerated in a small open label study in SLE patients but results from the ongoing phase II study and a study of idiopathic and lupus membranous nephropathy will better define its role in the treatment of SLE. In regards to the new quinolone-3-carboxamide derivatives, paquinimod was safe and tolerated in both phase I and phase II studies and gave positive initial results. In addition, a phase II study on laquinimod for lupus arthritis and lupus nephritis has been initiated. Further information obtained from these studies will determine if these agents represent new therapeutic opportunities in lupus.

The Syk inhibitor fostamatinib, although not yet tried in clinical studies in lupus, has shown efficacy in phase II trials in RA, and despite being non selective for Syk, seems to be very effective. Its effect fosters the view that using compounds that target multiple kinases might be beneficial in diseases such as SLE and RA that are not driven by alterations in only one cytokine. The non-selective Jak inhibitor tryphostin and the Jak2 inhibitor CEP-33779 were efficacious in animal models of lupus. In the light of recent success of tofacitinib in phase III trials in RA a clinical trial with Jak inhibitors in SLE will show its potential to treat the disease. For the other compounds described in this report including the CaMKIV inhibitor KN-93, the ROCK inhibitor fasudil, the Btk inhibitor ibrutinib, the PI3K inhibitor AS605340, the proteasome inhibitors and for dipyrindamole that inhibits the Cn-NFAT interaction further evidence based on clinical studies is warranted, particularly in the view of the encouraging results from the animal models.

Finally, the field of epigenetics and the development of small molecules that interact with chromatin modifiers such as the HDACs or BRD-bearing proteins, although still in early development offer the perspective to treat SLE based on the understanding of the chromatin remodeling state that leads to the expression of disease.

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Highlights

- Inhibition of Syk normalizes the aberrant SLE T cell phenotype
- mTOR inhibition with rapamycin was effective in an open label trial in SLE patients
- Dipyridamole represents a non-toxic alternative to cyclosporine and tacrolimus
- Epigenetic changes in chromatin structure represent a novel target for SLE therapy

Figure 1.

Schematic representation of key signaling pathways that are aberrantly expressed in immune cells from patients with SLE and are targeted by novel small molecules.

Table 1

New small molecules in the treatment of systemic lupus erythematosus

Molecular Target	Treatment	Mode of action	Clinical trials
Syk	fostamatinib	Inhibitor of syk (not selective)	Efficacious in mice. Efficacious in clinical trials of RA with acceptable toxicity profile.
JAK	Tryphostin(AG490) CEP-33779	Non selective JAK inhibitor JAK2 inhibitor	Both AG490 and CEP-33779 were efficacious in mice. Tofacitinib (Jak3>Jak1>Jak2) efficacious in clinical trials of RA and psoriasis. Ruxolitinib (Jak1, Jak2) is FDA approved for MF.
Btk	Ibrutinib (PCI-32765)	Selective Inhibitor of Btk	Successful in mice
CaMKIV	KN-93	CaMK inhibition (not specific for CaMKIV)	Efficacious in mice.
ROCK	fasudil	Non selective ROCK inhibitor	Successful in mice. In Clinical use in Japan since 1995 for treatment of cerebral vasospasm. Well tolerated and safe
mTOR	rapamycin	Inhibits mTOR	Efficacious in mice. Positive results in a small open label study in 9 SLE patients with active disease A Phase II is in progress A phase II for Idiopathic and Lupus membranous nephropathy has been completed.
PI3K	AS605240	Inhibits PI3K γ	Successful in mice
Calcineurin-NFAT signaling pathway	dipyridamole	Inhibits Cn-NFAT interaction without affecting Cn phosphatase activity	Successful in mice
HDACs: enzymes that remove acetyl groups from the lysine residues in histone tails	Trichostatin (TSA) Suberoylanilide Hydroxamic Acid (SAHA)	Block HDACs but exact mechanism of action is unknown.	Successful in mice
S100A9	Quinoline-3-Carboxamides (Paquinimod, Laquinimod)	Binding of quinolone-3-Carboxamides to S100A9 disrupts	Paquinimod: Efficacious in mice. Phase I study in

Molecular Target	Treatment	Mode of action	Clinical trials
		interaction of S100A9 with TLR4 and RAGE	clinically inactive SLE safe and tolerated. Phase II study in mild active SLE safe and tolerated. Positive results for a subgroup of patients. Laquinimod: Successful in mice. Phase II studies for Lupus arthritis and Lupus nephritis have been initiated.
Proteasome	Bortezomib Carfilzomib ONX 0914 Delanzomib	Inhibition of proteasome	All proteasome inhibitors were efficacious in mice Bortezomib: FDA approved for multiple myeloma and mantle cell lymphoma. Positive results in a small case series of 13 SLE patients