Running title: Lymphatic System Targeting in Lupus

Leveraging Lymphatic System Targeting in Systemic Lupus Erythematosus for Improved Clinical Outcomes

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Abstract

The role of advanced drug delivery strategies in drug-repositioning and minimizing drug attrition rates, when applied early in drug discovery, is poised to increase the translational impact of various therapeutic strategies in disease prevention and treatment. In this context, drug delivery to the lymphatic system is gaining prominence not only to improve the systemic bioavailability of various pharmaceutical drugs but also to target certain specific diseases associated with the lymphatic system. While the role of the lymphatic system in lupus is known, very little is done to target drugs to yield improved clinical benefits. In this review, we discuss recent advances in drug delivery strategies to treat lupus, the various routes of drug administration leading to improved lymph node bioavailability, and the available technologies applied in other areas that can be adapted to lupus treatment. Moreover, this review also presents some recent findings that demonstrate the promise of lymphatic targeting in a preclinical setting, offering renewed hope for certain pharmaceutical drugs that are limited by efficacy in their conventional dosage forms. These findings underscore the potential and feasibility of such lymphatic drug targeting approaches, to enhance therapeutic efficacy in lupus and minimize off-target effects of the pharmaceutical drugs.

Significance Statement

The World Health Organization estimates that there are currently 5 million humans living with some form of lupus. With limited success in lupus drug discovery, turning to effective delivery strategies with existing drug molecules, as well as those in the early stage of discovery, could lead to better clinical outcomes. After all, effective delivery strategies have been proven to improve treatment outcomes.
Introduction

Systemic Lupus Erythematosus (SLE) is a heterogeneous autoimmune disease characterized by remissions and recurrent disease flares (Illescas-Montes et al., 2019). Almost 2 million Americans and over 5 million individuals globally are living with SLE, with a higher incidence in women than men and the highest incidence in women of color (Appleton and Major, 2021). SLE is characterized by a diverse range of complications and comorbidities, affecting various organs and systems, including skin, kidneys, lungs, joints, cardiovascular, and nervous systems (Illescas-Montes et al., 2019; Touma and Gladman, 2017) (figure 1). The pathogenesis of SLE centers on the massive imbalance between the production and the clearance of apoptotic material from the systemic circulation, leaving the apoptotic material within reach of the immune system and ultimately resulting in the loss of self-tolerance and sustained production of autoantibody (Tsokos et al., 2016). Management of SLE involves using pharmacological options, including antimalarials, non-steroidal anti-inflammatory drugs, corticosteroids, immunosuppressants, a combination of steroid and immunosuppressants, and biologics targeted at suppressing the immune system, reducing inflammatory responses, and preventing organ damage (Dall'Era, 2017; Illescas-Montes et al., 2019; Thong and Olsen, 2017).

Many therapies (over 30 drugs) in SLE patients have fallen short to meet their clinical endpoint, possibly due to their inability to reach the lymphatic system, the prime site of the disease (Azzi et al., 2016; Dall'Era et al., 2019; Touma and Gladman, 2017). The use of novel delivery strategies such as lymphatic system targeted delivery might enable failed SLE therapies to reach their intended cellular targets, as well as the desired endpoint in clinical trials. SLE is currently being wrapped up from all sides to better understand the condition and increase the chances of remission in more patients while reducing the morbidity and mortality of the disease (Dörner and Furie, 2019). The application of nanomedicine in the management of SLE has shown promise (Azzi et al., 2016; Ganugula et al., 2020; Hu et al., 2020; Nune et al., 2022). However, there is still a need for human clinical trials to explore the efficacy of nanomedicine in treating SLE. This review aims to provide a brief note on the current knowledge available on the pathogenesis of the disease, various pharmacological options available for the management of the disease as well as the preclinical models used in testing them, and a detailed account of novel drug delivery strategies targeting lymphatic system and their therapeutic benefits in SLE.

The pathogenesis of Systemic Lupus Erythematosus

Genetic susceptibilities have been implicated in the pathogenesis of SLE (Tsokos et al., 2016; Wu et al., 2020). Over 60 risk genes related to type I interferons, complement deficiency, production of antibody, and renal damage has currently been identified (Wu et al., 2020). However, the imperfect concordance in the incidence of SLE between monozygotic twins implicates epigenetic regulation and environmental factors in the pathogenesis of SLE (Hedrich et al., 2017; Tsokos et al., 2016; Wu et al., 2020). Environmental factors such as ultraviolet radiation, hormones, and infection have been implicated in SLE (Grimaldi, 2006; Tsokos et al., 2016; Woo et al., 2022). The high prevalence of SLE in females (compared to males, ratio 9:1) has been attributed to the involvement of hormones as well as X chromosome genes in the pathogenesis of lupus (Kim et al., 2022a).
SLE is characterized by the dysregulation of the innate immune system and the adaptive immune system. Although the dysregulated activation of T and B cells plays a major role in the pathogenesis of SLE, other immune cells, including dendritic cells, macrophages, and neutrophils, also contribute to the development of SLE (Chan et al., 2012; Ma et al., 2019; Tzeng and Chyuan, 2021). The accumulation of cellular debris owing to excessive immune cell death coupled with ineffective clearance from circulation represents the main pathogenic event in SLE (Rekvig and Van der Vlag, 2014; Xu et al., 2022).

Programmed cell death has also been implicated in the pathogenesis of SLE. The immune cells undergo accelerated apoptosis, increased autophagy and ferroptosis, aberrant necroptosis, NETosis, and dysregulated pyroptosis, all of which contribute to the pathogenesis of SLE (Xu et al., 2022). These processes result in the formation of chromatin and neutrophilic extracellular traps, a source of major autoantigens seen in SLE (Rekvig and Van der Vlag, 2014). These autoantigens are swiftly phagocytosed in the normal population. However, such a swift clearance is impaired in SLE patients, leading to the accumulation of autoantigens (Licht et al., 2004). This subsequently results in the activation of autoimmune responses, production of autoantibodies such as anti-double-stranded (ds)DNA, and the formation of immune complexes which get deposited in specific tissues (Rekvig and Van der Vlag, 2014; Xu et al., 2022).

Persistent circulation of nucleic acid containing cellular debris alongside the deposition of immune complexes in specific organs leads to inflammation (Lourenço and La Cava, 2009; Theofilopoulos et al., 2011). The deposited immune complexes stimulate the innate immune cells (specifically the plasmacytoid dendritic cell, monocytes, and macrophages), leading to the production of small soluble mediators, including type I interferons, pro-inflammatory cytokines such as interleukins (IL-6 and IL-18) and tumor necrosis factor (TNF), and immunoregulatory cytokines such as IL-10, IL-15, and B lymphocyte stimulator (BLyS) (Aringer, 2020; Aringer and Smolen, 2012; Idborg et al., 2018; Park et al., 1999; Petri et al., 2008; Wong et al., 2000). The defective or excessive production of these soluble mediators, including any abnormal response of the immune cells to the cytokines, favors the development of SLE (Lourenço and La Cava, 2009). The presentation of antigens in major histocompatibility complex molecules to naïve CD4+ T cells coupled with the binding of co-stimulatory agents and cytokines to the cells contribute actively to immune activation and stimulation (Lourenço and La Cava, 2009; Shin et al., 2011; Suárez-Fueyo et al., 2016). Activated naïve CD4+ T cells proliferate and differentiate into effector T helper cells. Overactivation of T-cells results in the amplification of inflammation via secretion of pro-inflammatory cytokines, hyperactivation of B-cells leading to the generation of autoantibodies and further production of immune complexes and immunoglobulins, and maintenance of SLE via the retention of autoreactive memory T cells (Suárez-Fueyo et al., 2016; Xiang et al., 2022) (figure 2). Despite decades of research, the pathogenesis of SLE is yet to be understood entirely. Advances in the understanding of the pathogenesis of SLE are being utilized in the repurposing of existing drugs and the development of targeted therapies for the treatment of SLE.

Management strategy in Systemic lupus erythematosus

According to existing management recommendations, treatment of lupus should focus on maintaining the lowest degree of disease activity by avoiding triggers and use of medications to
prevent organ damage, reduce the complications and comorbidities due to lupus and its management, and optimize the quality of life of lupus patients (Fanouriakis et al., 2019; Fava and Petri, 2019) (figure 3). The heterogeneity of lupus displayed by its various clinical manifestations and severity led to the adoption of individualized treatment strategies (Xiong and Lahita, 2014). The management options available can be broadly classified into pharmacological and non-pharmacological options. The pharmacological options available for the management of lupus include the following:

**Antimalarial:** Hydroxychloroquine, a chloroquine-based antimalarial drug, is recommended for use by all SLE patients at a maximum dose of 5 mg/kg body weight, except in cases with obvious contraindications (Fanouriakis and Bertsias, 2019; Fava and Petri, 2019). Hydroxychloroquine reduces lupus flares (Fava and Petri, 2019; Group*, 1991), protects organs from lupus-induced damage (Fessler et al., 2005) and cardiovascular damage (Fasano et al., 2017), prevents seizure (Hanly et al., 2012), and diminishes the risk of having neuropsychiatric lupus (González et al., 2009). Also, hydroxychloroquine improves arthritis and skin rashes while reducing the risk of diabetes and thrombosis (Fava and Petri, 2019). Hydroxychloroquine’s mechanism of action involves increasing lysosomal pH and altering toll-like receptor (TLR) signaling (via the prevention of TLR7/9 binding to their ligands), thus modulating immune responses, and interfering with the production of autoantigens and the secretion of cytokines (Fava and Petri, 2019; Xiong and Lahita, 2014). Like any other pharmaceutical drug treatment, hydroxychloroquine is associated with mild side effects such as headache, gastrointestinal discomfort, and skin reactions, as well as more serious effects such as muscular toxicity and vision-related problems (Durcan et al., 2019; Thong and Olsen, 2017). The drug’s serious but infrequent side effects include cardiomegaly, kidney toxicity, neuropathy, and myopathy (Fava and Petri, 2019; Xiong and Lahita, 2014).

**Nonsteroidal anti-inflammatory drugs (NSAIDs):** NSAIDs such as ibuprofen, naproxen, diclofenac, aspirin, salicylates, sulindac, and celecoxib are generally effective in managing minor symptoms of lupus, ranging from muscle and joint pain to fever, joint stiffness, serositis, and headache (Lander et al., 2002; Ostensen and Villiger, 2000; Xiong and Lahita, 2014). NSAIDs’ mechanism of action involves blocking the production of prostaglandin, which is achieved by inhibiting both cyclo-oxygenase-1 and cyclo-oxygenase-2 enzymes (Lander et al., 2002). Potential side effects include allergies, gastritis, peptic ulcers, renal toxicity, and cardiovascular complications (Lander et al., 2002; Xiong and Lahita, 2014). Patients should be periodically monitored for signs of gastrointestinal bleeding or renal impairment (Bhatt et al., 2008; Gladman et al., 2013). NSAIDs and antimalarials have been used in combination in the management of SLE (Hill et al., 2021).

**Steroids:** Glucocorticosteroids such as prednisone, which possess anti-inflammatory and immunomodulatory properties, are used in the management of SLE (Basta et al., 2020; Tseng et al., 2006). The dose administered depends on the severity of the disease manifestation. Musculoskeletal and cutaneous symptoms, pleuropericarditis, and hematological impairments which are generally seen in mild SLE, can be treated with the oral administration of prednisone at relatively low doses, while systemic vasculitis, neuropsychiatric, and renal impairment observed in patients with severe SLE can be treated with a high dose of prednisone or by pulse therapy (Gladman et al., 1999; Xiong and Lahita, 2014). Prednisone’s mechanism of action involves the non-selective reduction in the expression of inflammatory cytokines and adhesion
molecules (Durcan et al., 2019). When possible, the use of corticosteroids should be avoided as they produce diverse adverse effects ranging from cardiovascular impairments, osteoporosis, cataracts, fractures, insulin resistance, and others (Durcan et al., 2019; Fava and Petri, 2019). Steroids and immunosuppressants (tacrolimus and mycophenolate) combination have also been used in the management of SLE (Liu et al., 2019; Yap et al., 2022).

**Synthetic immunosuppressants:** Cytotoxic and immunosuppressive drugs such as methotrexate, azathioprine, cyclophosphamide, mycophenolate mofetil (MMF), and calcineurin inhibitors are used in the management of SLE, especially lupus nephritis (Basta et al., 2020; Xiong and Lahita, 2014). Methotrexate, used in the management of SLE in patients with inflammatory arthritis, articular or cutaneous involvement, exerts its effects by inhibiting purine and pyrimidine synthesis and neutrophil function (Chan and Cronstein, 2010; Durcan et al., 2019; Xiong and Lahita, 2014). Methotrexate has steroid-sparing effect. It is well tolerated, and it mildly decreases the overall disease activity in patients. However, it is limited by its teratogenic potential and other adverse effects such as hepatitis, bone marrow suppression, oral stomatitis, alopecia, and pneumonitis (Fava and Petri, 2019; Xiong and Lahita, 2014). Azathioprine, used in the management of lupus nephritis, cutaneous and articular disease, serositis, and other moderate to severe internal organ manifestations, exerts its effects by inhibiting purine metabolism (Durcan et al., 2019; Tiede et al., 2003; Xiong and Lahita, 2014). Azathioprine has steroid-sparing effects, and it is the only cytotoxic immunosuppressive agent safe for use in pregnancy. However, its use is associated with adverse effects, including gastrointestinal intolerance, hepatotoxicity, and bone marrow suppression (Fava and Petri, 2019; Weyand et al., 2001; Xiong and Lahita, 2014).

MMF, which is also used in the management of lupus nephritis, serositis, moderate to severe cutaneous disease, and other moderate to severe internal organ manifestations, exerts its effects by blocking the proliferation of activated T cells and B cells via the inhibition of the rate-limiting enzyme inosine monophosphate dehydrogenase (Fanouriakis et al., 2019; Fava and Petri, 2019; Xiong and Lahita, 2014). MMF is superior to azathioprine in reducing lupus flares and achieving remission. However, it is limited by its teratogenic potential, gastrointestinal side effects, infections, and bone marrow suppression. (Fanouriakis et al., 2019; Xiong and Lahita, 2014). Cyclophosphamide initially used for maintaining lupus nephritis and neuropsychiatric lupus along with life-threatening conditions, exerts its highly toxic effects by depleting T and B cells while suppressing the production of antibodies (Durcan et al., 2019; Xiong and Lahita, 2014). Cyclophosphamide is primarily limited by its severe adverse effects, including infections, bone marrow suppression, nausea, premature ovarian failure, hemorrhagic cystitis, and malignancies (Fava and Petri, 2019; Mok, 2016).

Calcineurin inhibitors such as cyclosporine A (CsA) and tacrolimus, which are used to prevent the rejection of transplant organs, have proven to be effective in the treatment of lupus nephritis (Alamilla-Sanchez et al., 2021; Moroni et al., 2006). They exert their effect by blocking the activation of T-cells via the inhibition of the calcium/calmodulin-dependent phosphatase calcineurin (Alamilla-Sanchez et al., 2021). However, complications such as nephrotoxicity, infection, dyspepsia, and hepatotoxicity limit their application (Alamilla-Sanchez et al., 2021). Voclosporin, a CsA analog with an improved pharmacokinetic-pharmacodynamic profile, was recently approved in the United States for treating lupus nephritis in adult patients (Fava and
Petri, 2019; Saxena et al., 2022) with an enhanced immunosuppressive effects compared with cyclosporine (Kuglstatter et al., 2011).

**Hormone therapy**

Steroid hormones ranging from estrogen to testosterone and dehydroepiandrosterone (DHEA) have been studied for their safety and efficacy in the management of SLE (Grygiel-Górniak and Puszczewicz, 2014). The use of hormone therapy including oral contraceptives (OC) and hormone replacement therapy (HRT), has been explored in postmenopausal women with lupus. Estrogen is suspected to modulate SLE by influencing cytokine production by the immune cells (Kassi and Moutsatsou, 2010). Literature evidence suggests that estrogen replacement therapy (transdermal) prevents bone loss in osteopenic postmenopausal women with SLE (Bhattoa et al., 2004). Similarly, other studies have shown that HRT (oral conjugated estradiol, 0.625 mg daily) prevented bone loss in young hypogonadal women with SLE who are on chronic steroid therapy (Kung et al., 1999). In another study, treatment with DHEA (200 mg per day) over 24-week, stabilized the overall lupus activity in adult Chinese women without resulting in any serious adverse effects (Chang et al., 2002). Similarly, treatment with DHEA (200 mg per day, orally) led to a reduction in disease activity despite reducing the dosage of corticosteroids in steroid-dependent female SLE patients (Petri et al., 2002). However, some other studies reported no positive effects of hormonal therapy in SLE as the hormones only help in managing menopausal symptoms (Gompel and Piette, 2007; Soares-Jr et al., 2022). In a particular study, estrogen (even at a low dose) exacerbated lupus nephritis and modulated the production of TLR7/9 cytokine in lupus-prone female mice (Edwards et al., 2020). Similarly, estrogen influences the expression of interferon-stimulated genes in SLE in another study (Singh et al., 2021). More studies are however required to know the other effects of hormones in SLE and the mechanisms by which these hormones exact their effects.

**Targeted Immunotherapies:** Biologics including belimumab, rituximab, anifrolumab, atacicept, baricitinib, and ustekinumab have been studied for their efficacy as targeted immunotherapies in the management of SLE (Fava and Petri, 2019; Xiong and Lahita, 2014). Starting from the involvement of B-cells in the presentation of T-cell antigens to the formation of cytokines and production of autoantibodies, aberrant B-cell pathways have been implicated in the pathogenesis of SLE (Durcan et al., 2019). Belimumab, the first targeted biologic agent approved by the FDA for the management of lupus, is a humanized monoclonal antibody that targets the B-cell activating factor (BAFF), a protein that plays a crucial role in autoimmunity, thus decreasing the number of activated B-cells (Basta et al., 2020; Durcan et al., 2019). However, a recent phase 3 clinical study (Belimumab International Study in Lupus Nephritis) reported an improvement in renal outcomes in patients with active lupus nephritis treated with belimumab in addition to mycophenolate or cyclophosphamide (Furie et al., 2022; Rovin et al., 2022). Rituximab, an anti-CD20 monoclonal antibody, is sometimes used in the management of refractory disease in SLE patients (Basta et al., 2020; Wise and Stohl, 2020). It exerts its effects by targeting the CD20 on B cells, thus depleting mature B cells and their precursors (Durcan et al., 2019; Xiong and Lahita, 2014). Despite the high efficacy of rituximab in depleting B cells when compared with belimumab, rituximab failed to reach its primary clinical endpoints in two different randomized controlled trials (EXPLORER and LUNAR studies) (Basta et al., 2020; Wise and Stohl, 2020), therefore the use of rituximab in lupus is off-label (McCarthy et al., 2018; Wise and Stohl, 2020; Witt et al., 2013). On the contrary, the European League Against Rheumatism (EULAR) recently
recommended the use of rituximab for the treatment of refractory lupus with severe organ involvement (Fanouriakis et al., 2019). Anifrolumab is a first-in-class medication approved for the treatment of non-renal SLE by both the United States and the European Union ((Report). 2022). As a type 1 interferon receptor antagonist, anifrolumab achieved an overall reduction in disease activity in a phase three clinical trial named TULIP (Burki, 2021). Other biologics, such as atacicept, baricitinib, and ustekinumab, are still under evaluation for their pros and cons in the efficient management of SLE (Fava and Petri, 2019).

Preclinical Models of Systemic Lupus Erythematosus

Understanding the pathogenesis of SLE and developing therapeutics for SLE involves the use of in vitro, ex-vivo, and in vivo assessments using preclinical animal models before proceeding to the clinical assessment of therapeutics in human patients. Preclinical animal models for SLE serve as an irreplaceable tool in understanding the cellular mechanism implicated in the onset and progression of the disease in conjugation with their genetic and immunological illustration (Li et al., 2017b; Moore et al., 2021a; Perry et al., 2011). Preclinical murine models of SLE range from classic spontaneous models to genetically modified models, inducible models, and humanized models (Richard and Gilkeson, 2018; Xin et al., 2022). Given the prominent level of heterogeneity observed clinically in SLE patients, no single mouse model fully represents the comprehensive clinical spectrum seen in SLE patients. However, each model displays an overlapping subset of lupus phenotypes as seen in humans, thus offering specific features of interest needed to explore different preclinical purposes (Table 1). Despite the substantial difference between the immune systems of mice and humans coupled with the low translatability of efficacious lupus treatments from mice to humans, mouse models of lupus currently play an irreplaceable role in modeling autoimmunity. These mouse models help to understand pathogenesis and assess candidate treatments for lupus (Moore et al., 2021a; Richard and Gilkeson, 2018). Experimental manipulation in various mouse models of lupus has tremendously accelerated research avenues, including the identification of internal and external triggers of lupus, organ-specific mechanisms, establishing of druggable targets for lupus, and developing new targeted therapies for the disease (Moore et al., 2021b).

The lymphatic system in Systemic lupus erythematosus

The lymphatic system includes a strong network of vessels and nodes that transport lymph containing immune cells, lipoproteins, clear liquids, infectious materials, macromolecules, small molecules, and clear fluid from the intestine and other peripheral tissues into the bloodstream (Kataru et al., 2019; Trevaskis et al., 2015). The lymphatic system directly participates in immune activation and modulation by facilitating the transport of antigens into the lymph node and subsequently to the site of inflammation (Schwartz et al., 2019). Aside from immunity, the lymphatic system plays a vital role in several physiological processes, including the maintenance of fluid homeostasis (Schwartz et al., 2019) and cholesterol metabolism (Escobedo and Oliver, 2017). A dysfunction in the fluid transport function or immune response regulation by the lymphatic system has been implicated in various pathological conditions, including inflammatory disorders (Zhang et al., 2007), infectious diseases (Pantaleo et al., 1991), metabolic syndrome (Escobedo and Oliver, 2017), cardiovascular disorders (Jones and Min, 2011), and
metastasis (Padera et al., 2016). All these pathological conditions have been implicated in SLE, and thus, it is reasonable to conclude that a dysfunctional lymphatic system plays a significant role in the progression of SLE.

SLE is characterized by the release of pro-inflammatory mediators and lymphangiogenic factors (Baluk et al., 2009; Proulx et al., 2013; Zhang et al., 2007). These factors promote lymphatic hyperplasia, alter the flow of lymphatics, and thus modulate inflammation and immunity. If inflammation persists, the adipose tissue surrounding the lymph node expands (Baluk et al., 2009; Proulx et al., 2013; Zhang et al., 2007). A recent study identified dysfunctional lymphatic flow as one of the factors that contribute to the pathophysiology of lupus (Ambler et al., 2022). The authors reported evidence of dysfunctional lymphatic flow in both murine and human models of SLE and concluded that this contributes to cutaneous photosensitivity and the activation of B cells in the lymph node in SLE (Ambler et al., 2022). This correlates with previous studies that reported a correlation between a reduction in lymphatic flow and skin inflammation (Kajiya and Detmar, 2006; Thomas et al., 2012). There have been limited studies conducted on lymphatic function in SLE. However, there have been reports of lymphatic obstruction in the mesentery leading to cases of pleural effusions and chylous ascites in SLE patients (Dalvi et al., 2012; Daniel et al., 2017; Lee et al., 2002; Manzella et al., 2013). Also, studies have shown that disease progression can lead to lymphatic remodeling as demonstrated by the enlargement of the intracellular gap between the lymphatic endothelial cells (300-500nm) during inflammatory diseases and cancer (Cueni and Detmar, 2006; Zhang and Lu, 2014). All these changes affect the transport of substances via the lymphatic system. Taken together, it is safe to say that a dysfunctional lymphatic system contributes to the pathophysiology of SLE. Having a comprehensive understanding of the structural and functional changes in the lymphatic system of SLE patients will enhance the adoption of lymphatic targeting in designing novel diagnostics, and therapeutic and possible vaccination options for SLE patients. Hence, more studies are needed to better understand the structural and functional changes in the lymphatic system of SLE patients.

**Lymphatic Drug Targeting in SLE**

Based on recent advancements in understanding the role played by lymphatics in pathological manifestations and immunity, lymphatic drug targeting has been recognized as a transformational tool in the treatment of various diseases (Trevaskis et al., 2015). Being an immunologically driven disease (Ganugula et al., 2020; Tsokos et al., 2016), delivering potent immunosuppressive agents into the systemic lymphatic circulation holds significant therapeutic potential in the management of SLE (Ganugula et al., 2020). Orally and parenterally administered drugs end up in the interstitial space, from where they are selectively delivered into the lymphatic capillaries or the blood capillaries. Most small particles (with size <10 nm) are primarily delivered into the blood capillaries owing to the 100-500 folds higher flow rates in the blood capillaries as compared to the flow rate in the lymphatic capillaries (Ryan et al., 2014; Trevaskis et al., 2015) amidst other key physiologic factors controlling the in vivo behavior of small particles (Glassman and Muzykantov, 2019). The larger particles precluded entry into the blood capillaries owing to their size, end up in the more permeable lymphatic capillaries (Trevaskis et al., 2015). Hence, lymphatic drug targeting centers on delivering macromolecular therapies such as large peptides or constructing desired microparticles into larger particles.
Hitchhiking microparticles on endogenous macromolecular carriers in situ or associating a microparticle with macromolecular carriers increases the size of the desired particle and ensures the selective transport of the microparticle into the lymphatic capillaries (Trevaskis et al., 2015). Particles can enter the lymphatics via different routes based on their sizes. Particles between 10–100 nm in size can move easily into the lymphatics either by passive transport involving lymphatic interendothelial cell junctions or active transcytosis. Once inside the lymphatic capillaries, particles flow along with the lymph in a unidirectional way through progressively wider pre-collecting and collecting afferent vessels to the lymph node and then flow into the efferent lymphatic (post-nodal) vessels and finally into the thoracic lymph duct where the majority of the lymph is emptied into the venous system (Trevaskis et al., 2015). Table 2 presents non-exhaustive ongoing clinical trials in lupus, of which some of the molecules can significantly benefit from lymphatic targeting approaches.

**Lymphatic Drug Delivery System**

There are multiple physicochemical technologies (Table 3) that determine the rate and extent of delivery and release of therapeutic agents into various immune cells and lymphoid tissues/organs involved in the pathogenesis of SLE (Schudel et al., 2019). The efficiency of these technologies is directly proportional to the efficacy of the therapeutic agents and route of administration. Altering the route of administration of drugs or leveraging advanced formulation procedures enables better-targeted delivery, a significant reduction in systemic dosing and off-target toxicities, and increases the bioavailability of drugs in the lymphatic system, thus resulting in enhanced efficacy of immune therapies (Azzi et al., 2016; Ganugula et al., 2020; Hu et al., 2020; Nune et al., 2022; Schudel et al., 2019). The following section discusses various routes of administration and the materials and technologies available for lymphatic targeting of pharmacologically active agents (figure 4).

**Evaluation of routes of administration of lymphatic targeting**

1. **Oral**

The suitability of oral drug administration for long-term use coupled with its dosage flexibility, convenience of usage, safety, and high chances of patient compliance makes it the most compelling route of drug administration, especially in the treatment/management of chronic diseases such as SLE. However, oral delivery of drugs such as immunosuppressants used in the management of SLE has been hampered by low permeability owing to the formidable barrier presented by the enteric epithelia, low efficacy, and off-target toxicity, and poor bioavailability due to first-pass metabolism, amidst other limitations (Ganugula et al., 2020; McHugh, 2020; Zhang et al., 2021). Orally administered drugs end up in the intestine, where they are either absorbed into the portal circulation via the portal vein or the lymphatic circulation via the gut-associated lymphatic tissue (Ganugula et al., 2020; Yáñez et al., 2011). Increasing the molecular weight and lipophilicity of drugs facilitates the lymphatic absorption of drugs from the intestine (Yáñez et al., 2011). Studies have shown that lymphatic targeting enhances the oral bioavailability of therapeutics in various disease conditions (Shackleford et al., 2003; Trevaskis et al., 2006; White et al., 2009; Zhang et al., 2013) as the intestinal lymphatic system enables
orally administered drugs to avoid the first pass hepatic metabolism, thus enhancing their bioavailability (Zhang et al., 2021) (figure 5). In a recent study conducted by our group, harnessing the gut-associated lymphatic tissue (GALT) transport process, a highly potent nanoparticle cyclosporine targeted lymphocytes and reverted all analyzed cytokines (including interleukin 2, 4, 6, 10, 1β and CXCL12, CXCL13) to their baseline levels and prevented glomerulonephritis in murine lupus model following oral drug administration (Ganugula et al., 2020).

2. Subcutaneous

The subcutaneous route of drug administration has been used extensively as an alternative route of administering drugs with poor oral bioavailability. This route of drug delivery is also adopted when prolonged release and systemic exposure of a therapeutic agent is desired (McLennan et al., 2005). Diverse formulations and delivery strategies have been designed for lymphatic targeting of therapeutics following subcutaneous administration (McLennan et al., 2005). Subcutaneously administered therapeutics go into the interstitial fluid at the site of administration and are drained either into the systemic circulation or the lymphatics based on the molecular size of the therapeutics (Porter et al., 2007). Nano-formulating of small molecules increases their size and facilitates preferential drainage into the lymphatics after subcutaneous administration (Trevaskis et al., 2015). In a particular study, the lymphatic targeting of anti-TNF-α oligonucleotide was achieved after subcutaneous administration (Huang et al., 2012). The accumulation of anti-TNF-α oligonucleotide in the CD169+ macrophages led to specific inhibition of TNF-α in these cells, thus significantly inhibiting the excessive proliferation of immune cells and abnormal cytokine production, consequently ameliorating SLE in animals (Huang et al., 2012). Similarly, some researchers achieved lymphatic targeting via the subcutaneous administration of DNA nanoflowers which are specific antagonists for TLR7 and TLR9 (Wang and Gan, 2022). They observed a significant reduction in the levels of cytokines and autoantibodies and an alleviation of lupus nephritis in a lupus mouse model.

3. Intravenous

The intravenous route is a favored route of drug administration, including delivery of immunosuppressants to the lymphatic system in SLE (Azzi et al., 2016; Hu et al., 2020) as it avoids the first-pass metabolism, resulting in direct entry of therapeutics into the systemic circulation (Bolger, 2018). Lymphatic targeting has been observed after the intravenous administration of nanoparticles, PEGylated proteins, and liposomes used in drug delivery (Trevaskis et al., 2015). The lymphatic targeting of intravenously administered macromolecules is achieved by the extravasation of the macromolecules into the interstitium. These macromolecules are then drained from the interstitium in a manner similar to subcutaneously administered drugs (Porter et al., 2007; Trevaskis et al., 2015). Abdi and co-workers (Azzi et al., 2016) developed antibody (MECA79) coated microparticles for target immunomodulators. In their proof-of-concept study, they observed the accumulation of tacrolimus loaded microparticles in the draining lymph nodes and achieved immunomodulation in an SLE mouse model (Azzi et al., 2016). Other investigators have tested azathioprine-laden polyhydroxyalkanoate
nanoparticles that exhibited better therapeutic efficacy and reduced toxicity compared to conventional PLA nanoparticles in the murine model of lupus (Hu et al., 2020).

4. Transcutaneous

The transcutaneous or transdermal route is a non-invasive route of drug administration which is very attractive owing to its ease of administration, safety, patient compliance, and low rejection rate (Jeong et al., 2021). The transcutaneous route of administration protects therapeutic agents from first-pass metabolism, pH, enzymes, intestinal bacteria, and other gastrointestinal barriers experienced by orally delivered drugs (Jeong et al., 2021). Transdermally administered drugs end up in the dermal layer and are subsequently drained into the skin-associated lymphatic tissue. The epidermal layer of the skin is known for its accessibility and competent immune environment, thus making the site attractive for lymphatic targeting via transcutaneous delivery (Denis et al., 2007; Kabashima et al., 2019). Hence, the administration of therapeutics via the skin-associated lymphatic system seems promising in the treatment of lymph-associated diseases such as SLE. Lymphatic delivery via the transcutaneous route of administration has been utilized in promoting adaptive immune response following vaccination (Denis et al., 2007). It has also been used in facilitating the efficacy of chemotherapy (Yang et al., 2019). A recent study conducted in mice reported induction of immune responses in skin-associated lymphatics following transcutaneous administration (Bialojan et al., 2019).

5. Nasal

The recent surge in the targeted delivery of drugs to the non-gastrointestinal mucosa lymphatic system has focused on delivering vaccines to mucosa surfaces such as the nasal mucosa (Trevaskis et al., 2015). The nasal mucosa shares similarities with the structure of gut-associated lymphatic systems. The mucosa-associated lymphatic system (MALT) is lined by M cells that readily transport antigens and particulate matter, including therapeutic macromolecules (Trevaskis et al., 2015). The nasal mucosa is a target for rapid local and systemic delivery of therapeutics owing to its rich blood vasculature (Bolger, 2018). Among various non-parenteral routes of drug administration, the intranasal route is unique for its ease of administration, large surface area for drug absorption, and ability to directly target different compartments in the body (Holmgren and Czerkinsky, 2005). After nasal administration, drugs cross the nasal epithelium and are drained into the mucosal lymph nodes via MALT. In a particular study, lymphatic targeting was achieved following nasal administration of ovalbumin conjugated to polypropylene sulfide-based nanoparticles of various sizes, with the biggest nanoparticle (200nm) producing the most effective immunomodulation (Stano et al., 2011). Immunomodulation via induction of mucosal tolerance seems promising in the treatment of various autoimmune diseases, however, this is yet to be fully explored in the management of SLE (Holmgren and Czerkinsky, 2005).

6. Pulmonary

Pulmonary administration is achieved clinically via nasal or oral inhalation of therapeutics and preclinically via intratracheal instillation (Bolger, 2018). The thin alveolar epithelium, large alveolar surface area, high solute permeability, less proteolytic activity, and extensive vasculature are some of the attributes that make the pulmonary pathway an exceptional route of
drug administration (Trevaskis et al., 2015). Different drug delivery systems, including nanoparticle, dry powder, and aerosolized liquids, can be administered via the pulmonary route (Bolger, 2018). The lung’s dense vascular supply, coupled with its epithelial permeability, enables the rapid absorption of small molecules directly into the systemic circulation following pulmonary administration. However, macromolecules excluded from entering the systemic capillaries are drained into the lymphatics (Trevaskis et al., 2015). Reineke and co-workers (Mohammad et al., 2013) investigated the role of particle size using model polystyrene particles in lymph accumulation following pulmonary administration. They observed a significantly higher lymph deposition of nanoparticles compared to other major tissues, and the deposition was size-dependent with the highest for 250 and 900 nm particles compared to 50 and 90 nm (Mohammad et al., 2013). While it is often difficult to extrapolate polystyrene distribution data for particles used in drug delivery, further studies are required to explore the potential of lymphatic targeting following pulmonary administration in the management and treatment of lymph-related disorders such as SLE.

**Choices of materials and technologies available for lymphatic targeting**

The physiological structures of biomaterials have been leveraged in targeting tissues and specific cells. These materials can potentially prolong the circulation of intravenously administered drugs and the retention of drugs in peripheral tissues. They can alter the pharmacodynamics and pharmacokinetics of any associated small molecules (Schudel et al., 2019). Generally, biomaterials enhance lymphatic targeting by increasing the molecular weight of small molecules, thus facilitating lymphatic uptake (Schudel et al., 2019). Several materials ranging from nanoparticles, polymer micelles, silicon, liposomes, and nano-gel, among others, have been utilized in the targeted delivery of drugs to the lymphatic system (Schudel et al., 2019). Nanomedicine has evolved in preventing, diagnosing, and treating various rheumatology diseases, including SLE. Nanoparticle-based delivery systems enable the targeted delivery of small-molecule immunosuppressants to immune cells, which are the key players in SLE (Ganugula et al., 2020; Look et al., 2013) and modulate the hyperactivity of the immune system (Rostamzadeh et al., 2016).

1. **Polymeric Nanoparticles**

Polymers are indispensable to mankind and offer flexibility for customization based on end-use applications, e.g., gels, implants, microparticles, and nanoparticles. Polymeric nanoparticles are capable of delivering a variety of payloads, ranging from hydrophobic to hydrophilic compounds and small molecules, which can either be encapsulated in the nanoparticle’s core or entrapped in its matrix or chemically conjugated or physically bound to the surface of the nanoparticle as conjugates (Mitchell et al., 2021). With easily modifiable surfaces, polymeric nanoparticles are a perfect candidate for precise targeting of therapeutics. In a recent study, the immunosuppressive agent azathioprine (AZA) was loaded into two different biocompatible polymeric nanocarriers, polyhydroxyalkanoate (AZA-PHA) and polylactic acid (AZA-PLA) (Hu et al., 2020). The AZA-PHA nanoparticles led to a higher concentration of the therapeutic in the lymphatics and had significantly better therapeutic effects when compared with the plain AZA and AZA-PLA nanoparticles (Hu et al., 2020). The researchers achieved a significant improvement in the
treatment response and a remarkable reduction in the side effects of azathioprine by delivering the drugs directly to the immune cells using a biodegradable and biocompatible nano-carrier. Our group recently came up with a versatile synthetic process based on polycondensation of PLA-PEG-PLA triblock leading to periodic functional polyesters that enable ligand-receptor stoichiometry and better targeting efficiency (Ganugula et al., 2017; Ganugula et al., 2020). These polymers allowed flexibility in drug loading, release kinetics, and efficient lymph node bioavailability, thereby preclinical efficacy in mouse models of lupus.

2. Liposomes

Liposomes are lipid bilayers containing vesicles with hydrophilic heads and hydrophobic tails used in the targeted delivery of drugs to the lymphatics (Cai et al., 2011). The encapsulation of drug-encapsulated liposomes enables their targeted delivery into the lymphatics following intestinal, pulmonary, and subcutaneous routes of administration, thus enhancing the lymphatic penetration and retention of drugs while increasing their stability and bioavailability (Cai et al., 2011). In a proof-of-concept study, liposomes were used to co-deliver anti-high-mobility group box 1 small interfering RNAs (anti-HMGB1 siRNA) and dihydroartemisinin (DHA) in a mouse model of SLE (Diao et al., 2022; Diao et al., 2019). This liposome delivery system successfully achieved the lymphatic targeting of the therapeutics to the lymph node and suppressed the proliferation and activation of immune cells, especially B cells (Diao et al., 2022). To treat murine lupus, Fahmy and co-workers (Look et al., 2013) fabricated nano gels via remote loading of liposomes with the immunosuppressive agent, mycophenolic acid, and cross-linkable poly oligomers. The internalization of the MMF nanogels by the dendritic cells present in the lymph draining the intraperitoneal injection site attenuated the inflammatory and stimulatory activities of the dendritic cells while reducing the adverse effects of MMF (Look et al., 2014; Look et al., 2013).

3. Solid lipid nanoparticles

Solid lipid nanoparticles are promising candidates for lymphatic delivery owing to their controlled release properties, targetability, and provision of better chemical stability for therapeutics (Ali Khan et al., 2013; Paliwal et al., 2020). As solid core lipid nanocarriers made from biocompatible physiological lipids (which are solid at room temperature) and surfactants, solid lipid nanoparticles are capable of delivering diverse hydrophilic and hydrophobic therapeutics ranging small molecules to macromolecules, vaccine antigens, and genetic materials (Ali Khan et al., 2013; Paliwal et al., 2020). The incorporation of cytotoxic drugs such as etoposide, idarubicin, and methotrexate in solid lipid nanoparticles enables their targeted delivery into the lymphatics following duodenal, pulmonary, and subcutaneous routes of administration, thus overcoming the severe toxicity, lack of specificity, poor bioavailability, and drug resistance limitations of these cytotoxic drugs (Ali Khan et al., 2013). In a study conducted to investigate the effect of surface charge on the efficacy and lymphatic penetration of solid lipid nanoparticle, rapamycin, an oral drug that exert its effect by inhibiting the mTOR pathway (but is limited by low bioavailability) was encapsulated in solid lipid nanoparticles and delivered via the pulmonary route (Landh et al., 2020). The encapsulation of rapamycin in negatively charged solid lipid nanoparticles led to efficient lymphatic access to the drug and inhibition of lymphangiogenesis (Landh et al., 2020).
4. Microneedles

Microneedle was conceptualized out of the desire to create a painless drug delivery system and improve the intradermal and transdermal delivery of drugs (Sabri et al., 2020). As a microscale method of physically enhancing the transdermal permeation of therapeutics, microneedle physically breaches the stratum corneum, thus enabling the easy diffusion of drugs into the interstitium (Aldawood et al., 2021; Sabri et al., 2020). Various types of microneedles, including solid microneedles, hollow microneedles, coated microneedles, and dissolving microneedles, have been created from materials such as silicon, metals, and polymer (Aldawood et al., 2021). In a study conducted to compare the pharmacokinetics of protein uptake following different routes of administration, an increase in lymphatic uptake was observed following microneedle-based intradermal delivery of proteins (Harvey et al., 2011).

5. Microparticles

Microparticles are small-sized particles with a reasonable surface area to volume ratio that can easily be functionalized, making them good materials for targeted delivery of drugs to specific cells and organs (Suri et al., 2013). They can be administered via various routes and encapsulate both hydrophobic and hydrophilic drugs. Microparticles have been used in research that focused on the controlled release of drugs, targeted drug delivery, and reducing drug toxicity (Suri et al., 2013). Both natural materials (such as chitosan, alginate, gelatin, and dextran) and synthetic materials (such as polymers, and ceramic silicone) have been utilized in synthesizing microparticles (Suri et al., 2013). Microparticles have also been used in lymphatic drug targeting. In a bid to achieve targeted immune suppression in the field of organ transplantation, Abdi and co-workers (Azzi et al., 2016) developed tacrolimus loaded microparticles coated with MECA79. Treatment with this particle led to an improvement in heart allograft survival with negligible changes in tacrolimus level in peripheral blood (Azzi et al., 2016).

The therapeutic benefits of lymphatic drug delivery in SLE

Targeted delivery of therapeutics to the lymphatic system in SLE patients holds great potential in improving lymph node bioavailability, enhancing targeted immunosuppression, reducing the dosage administered, and preventing off target toxicity.

**Lymph node bioavailability**

Lymph node bioavailability is a measure of the fraction of the dose of therapeutics that successfully reach the lymph node following different routes of administration (Price and Patel, 2023). The lymphatic system, which includes the lymph nodes, is a crucial target for diagnostic and therapeutic agents owing to the system’s role in detecting immune related diseases and the response of the immune system to these diseases (Ye et al., 2018). Lymphatic targeting improves the lymph node bioavailability of unstable and poorly soluble drugs as well as orally administered drugs that undergo first-pass metabolism in the liver (Ye et al., 2018). Both active and passive lymphatic targeting have been utilized to improve the lymph node bioavailability of therapeutics (Permama et al., 2021). Passive lymphatic targeting leverages the structure of the lymphatic system in delivering drugs to the lymph node (Permama et al., 2021). Active targeting,
which is achieved by attaching a targeting ligand to a molecule, involves ligand receptor interactions. Our lab explored active lymphatic targeting in the oral delivery of the immunosuppressant ‘cyclosporine’ to the lymphatic system by encapsulating cyclosporine in nanoparticles and attaching a ligand- gambogic acid (P2Ns-CsA and P2Ns-GA-CsA) (Ganugula et al., 2020). Gambogic acid targets and binds CD71, a receptor expressed by the intestinal epithelia, thus facilitating the endocytosis of the nanoparticle into the GALT (Ganugula et al., 2020). The results from the study indicated a 4-fold increase in the amount of drug deposited in the lymph nodes by the P2Ns-GA-CsA as compared to P2Ns-CsA while 18-fold increase in comparison to conventional CsA confirming that gambogic acid facilitates the transport of the drug into the gut-associated lymphatic tissue (GALT). We also observed a decrease in the deposition of the drug in the liver and spleen when using P2Ns-GA-CsA, further confirming that the transport of the drug from the intestine was via the GALT, a lymphatic transport system that circumvents the liver’s first-pass metabolism (Trevaskis et al., 2015).

**Targeted immunosuppression**

The lymphatic system houses over 90% of the entire lymphocyte pool, making lymphatic drug targeting a potent therapeutic option in the management of disease with targets located in the lymphatic system such as SLE (Hu et al., 2016; Trevaskis et al., 2015). Several immunosuppressive drugs have been used to treat SLE, but the drugs are limited by their adverse effects coupled with their high rate of non-response (McHugh, 2020). The targeted delivery of immunosuppressive drugs to the lymphatic system might alter the drugs’ efficacy and toxicity profiles by changing their systemic distribution pattern (Porter et al., 2007). The various lymphatic based immunosuppressant delivery studies described in this review article show that delivering immunosuppressive drugs to targets such as immune cells (the key players in SLE), lymph nodes, and lymphatic tissues which are within the lymphatic system holds great potential in enhancing immune modulation and treating SLE.

**Minimize undesired effects.**

All drugs used in the treatment/management of SLE, irrespective of the route of administration, are expected to end up in systemic circulation. The systemic distribution of these drugs, especially the ones that act on ubiquitous targets, is bound to cause adverse side effects (Yasuda, 2019). The non-specific systemic distribution of cytotoxic agents such as cyclosporine and azathioprine resulted in high doses of off-target toxicity. Lowering the dose of these drugs minimized undesired effects and alleviated their toxicity without producing any therapeutic effects (Ganugula et al., 2020; Hu et al., 2020). However, the targeted delivery of these drugs at very low doses to the immune cells via the lymphatic system significantly enhances their efficacy (Ganugula et al., 2020; Hu et al., 2020). Despite the ubiquitous nature of Calcium/calmodulin-dependent protein kinase IV, the lymphatic system targeted delivery of KN-93 (an inhibitor of CaMK4) to CD4+ cells enabled a ten-fold reduction in the dosage required to effectively block the differentiation of Th17 cells and suppress the production of interleukin-17 (Otomo et al., 2015). Hence, the targeted delivery of the drugs to the lymphatic tissues enables a significant reduction in the effective dose and thus minimizes the undesired effects of the drugs.

**Leveraging lymphatic targeting technologies used in other disease areas in SLE.**
Lymphatic targeting following oral and parenteral administration of therapeutics and imaging agents has been studied in various conditions, including diverse types of cancers as well as infectious and inflammatory diseases (Perazzolo et al., 2018; Ravizzini et al., 2009; Xie et al., 2009; Zhang and Lu, 2014) (Table 4). The targeted delivery of chemotherapeutics in cancer facilitates lymphatic exposure and retention of chemotherapeutics in the lymph nodes (the primary site of metastasis) (Ryan et al., 2014). The lymphatic system has been identified as the primary route for the distribution of metastasis in diverse types of cancer owing to the unique nature of the lymphatic vessels (incomplete underlying basement membrane and large intercellular gap between lymphatic endothelial cells) (Kawada and Taketo, 2011), hence, getting rid of metastasis in the lymphatic system has become the most important goal in the treatment of cancer. There are various treatment options available for eradicating lymph node metastasis. However, these options are limited by various factors, including, the associated side effects of invasive procedures such as lymphadenectomy and radiotherapy (Ashikaga et al., 2010), poor correlation between lymphadenectomy and improved survival (Fife and Thompson, 2001), poor bioavailability of chemotherapeutic in the lymphatic system (Ryan et al., 2013), and off target (systemic) toxicity of the chemotherapeutic (Ryan et al., 2014). Recent studies have documented that the lymphatic targeting of chemotherapeutics via oral or parenteral routes improves lymphatic exposure, reduces systemic toxicity, and reduces the growth of lymphatic metastases, thus improving prognosis and survival in cancer patients (Kaminskas et al., 2013; Khullar et al., 2012; Liu et al., 2014; Rao et al., 2010).

Bae and co-workers (Kim et al., 2022b) designed a nucleic acid-based adjuvant and an antigen encapsulated in a cationic liposome that prevented melanoma development/recurrence via the exhibition of favorable immune-triggering effects following oral administration. The liposome was coated with glycocholic acid conjugated chondroitin sulfate to increase bioavailability and mannose-linked chondroitin sulfate to facilitate uptake by dendrites in the lymph node. The repeated oral administration of the particle effectively triggered immune responses via the small intestinal lymphatic system prevented tumor metastasis and reoccurrence (Kim et al., 2022b). The study described the potential of using oral delivery system for the treatment of cancer, an approach which can also be adopted in the treatment of autoimmune diseases such as SLE.

Lymphatic targeting has also been explored in prophylactic and therapeutic vaccination. Studies have shown that lymphatic targeting increases the lymphatic uptake of antigens and adjuvants from vaccines and thus enhances the efficacy of vaccines (Andorko et al., 2015; De Titta et al., 2013). Lymphatic targeting in cancer vaccination facilitates immune system activation while enhancing antitumor immunity (Du and Sun, 2020). Some authors reported a long-term antitumor efficacy of the lymph node targeted delivery system for a subcutaneously administered mRNA cancer vaccine (Chen et al., 2022b).

The use of monoclonal antibodies as ligands for targeting nanoparticles to specialized cell populations has been adopted in cancer (Jin et al., 2022; Wathoni et al., 2022). In a particular study, docetaxel loaded chitosan nanocapsules were functionalized with a monoclonal antibody (Castro et al., 2021). Functionalizing the nanocapsules enhanced the active targeting of docetaxel to carcinomas expressing the monoclonal antibody. Similarly, Li and co-workers (Wang et al., 2017) achieved active targeting of nano-theranostic agents to carcinomas overexpressing epidermal growth factor receptor (EGFR) by decorating nanoparticles with a monoclonal antibody (anti-EGFR). These approaches tailored appropriately can be adopted in SLE.
Immunosuppressive and cytotoxic agents can be encapsulated in nanoparticles and attached to a monoclonal antibody. This will enhance the active targeting of cytotoxic agents to immune cells overexpressing the monoclonal antibodies. For example, the therapeutic efficacy of anti-CD20 monoclonal antibody rituximab (which failed to reach its primary clinical endpoints (Basta et al., 2020; Wise and Stohl, 2020)) might be enhanced by attaching rituximab as a ligand to nanoparticle containing cytotoxic agent.

The lymphatic targeting of long-acting, antiretroviral (ARV) is one of the significant steps forward in the treatment of HIV (Flexner et al., 2021; Mc Crudden et al., 2018). In a proof of concept study conducted by some researchers, the intradermal delivery of long-acting rilpivirine nanosuspension led to a significant uptake of the drug by the lymphoid tissues and inhibition of HIV replication (Mc Crudden et al., 2018). The study described the potential benefits of intradermal administration of long-acting drugs (1: reduced frequency of dosing, thus eradicating pill fatigue associated with daily oral medication. 2: needle-free, thus eliminating the risks and discomfort of injections). Lymphatic targeting of long-acting immunosuppressants can also be explored in the treatment of autoimmune diseases such as SLE.

Additionally, there are few products in the market for autoimmune disorders targeting lymphatic drug delivery routes, including Sofusa® Lymphatic Delivery System (S-LDS) (Sorrento Therapeutics), by hitchhiking amphiphiles immunogens on albumin from the site of injections to Lymph nodes. The Pharmaporum, along with UK biotech Evox therapeutics, prepared milk-derived exosomes to deliver antisense oligonucleotides. MaryRuth Organics prepared an herbal blend for the healthy lymphatic system, although not approved by FDA.

**Future outlook**

Starting from the use of antimalarial, steroids, NSAIDs, and cytotoxic immunosuppressants to the adoption of target-based therapies, we have come a long way in managing SLE. While each of these classes of therapeutics has its benefits, they have all proven to be insufficient in managing SLE. Benlysta (belimumab), the first drug that was approved by the FDA for the treatment of lupus nephritis was available as an intravenous infusion. The intravenous route of administration poses a significant limitation owing to the stress, discomfort, and lack of physicians’ time to administer such intravenous dosage. The improvised version of the new self-injectable formulation of Benlysta (belimumab), which allows once a week subcutaneous injection, is a significant step forward in treating chronic diseases like lupus, but this is not optimal. The approval of lupkynis (voclosporin) signifies a breakthrough in lupus research as this is the 1st FDA-approved oral treatment for lupus nephritis. Aside the many serious side effects which can be caused by voclosporin (infection, kidney problems, cancer), the frequency of the recommended dosage administration (twice daily) might become uncomfortable and can result in non-compliance and pill-fatigue. Developing the next generation of long-acting dosage forms (with a recommended dosage of once a week or once a month), such as those developed for the treatment of alcohol abuse (Hua et al., 2021) and malaria (Bellinger et al., 2016) which are capable of extended release, will be a game changer in the treatment of SLE. Combining the principle of active lymphatic targeting with long-acting dosage formulation technologies applied to subcutaneous and oral routes could be the ultimate solution to the treatment of SLE and associated complications.
Notes

M.A., R.G. and M.N.V.R.K. are inventors on patent applications related to the technology described in the manuscript (owned and managed by Texas A&M University, exclusively licensed to Peroral Biosciences Inc.). M.N.V.R.K. is a non-paid scientific advisory board member of Peroral Biosciences, Inc., and M.A. is a shareholder of Peroral Biosciences, Inc.

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Data Availability Statement

This review article contains no datasets generated or analyzed during the current study.

Authorship contribution

Wrote or contributed to the writing of the manuscript: Babalola, Arora, Ganugula, Agarwal, Mohan, and Kumar. All authors have read, edited, and approved the final version.

Non-Standard Abbreviations

B lymphocyte stimulator (BLyS)
B-cell activating factor (BAFF),
Cyclosporine A (CsA)
Epidermal Growth Factor Receptor (EGFR)
Gut-Associated Lymphatic Tissue (GALT)
Mucosa-Associated Lymphatic System (MALT)
Mycophenolate Mofetil (MMF)
Nonsteroidal Anti-Inflammatory Drugs (NSAIDs)
Systemic Lupus Erythematosus (SLE)
Toll-Like Receptor (TLR)
Tumor Necrosis Factor (TNF)
Table 1: Preclinical Models of Systemic lupus erythematosus

<table>
<thead>
<tr>
<th>Example</th>
<th>Generation</th>
<th>Clinical manifestations of lupus present</th>
<th>Clinical manifestations of lupus absent</th>
<th>Disease progression</th>
<th>Setbacks</th>
<th>Use for:</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous model</td>
<td></td>
<td>Autoantibodies (ANA and anti-dsDNA), immune complex, glomerulonephritis, mild vasculitis, Splenomegaly, hypergammaglobulinemia, elevated serum IgG levels, strong female bias</td>
<td>Rash Arthritis Cerebritis Serositis</td>
<td>Onset: 5-6 months of age</td>
<td>Mortality: 10-12 months of age</td>
<td>Long disease incubation period single-organ involvement</td>
<td>Studying genetic underpinning Assessing therapeutic responses in preclinical studies</td>
</tr>
<tr>
<td>NZB/NZW F1 (BW) mouse</td>
<td>F1 hybrid of New Zealand Black and New Zealand White mouse</td>
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<tr>
<td>NZM strains (NZM2328 and NZM2410)</td>
<td>Accidental backcross between F1 hybrid of NZB/W and NZW followed by sibling mating</td>
<td>Autoantibodies (ANA and anti-dsDNA), immune complex, glomerulonephritis, splenomegaly, hypergammaglobulinemia, elevated serum IgG levels, female bias</td>
<td>Vasculitis Rash Arthritis Cerebritis</td>
<td>Faster onset Mortality: 50% at 6 months</td>
<td>Generation takes a long time as it requires 10–12 generations of backcrosses.</td>
<td>Defining the genetic components involved in the pathogenesis of lupus</td>
<td>(Richard and Gilkeson, 2018; Rudofsky and Lawrence, 1999; Waters et al., 2001)</td>
</tr>
<tr>
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<td>Intercrosses of four inbred strains ( LG/J, AKR/J, C3H/Di, and C57BL/6 strains)</td>
<td>Full panel of autoantibodies (ANA, anti-dsDNA, anti-Ro, anti-La, anti-Sm) Skin rash, Arthritis, Splenomegaly Lymphadenopathy Vasculitis, Cerebritis, elevated serum IgG levels, immune complex, glomerulonephritis</td>
<td></td>
<td>Faster onset Mortality: 50% at 6 months</td>
<td>The disease differs from human lupus in that: It is primarily driven by IFNγ. lpr mutation accelerates the disease.</td>
<td>Assessing candidate treatments for lupus</td>
<td>(Nicoletti et al., 1992; Perry et al., 2011; Rauch et al., 1982; Richard and Gilkeson, 2018)</td>
</tr>
<tr>
<td>BXSB/Yaa Strain</td>
<td>Backcrossing SB/Le to F1</td>
<td>Autoantibodies Secondary lymphoid tissue hyperplasia,</td>
<td>Rash Arthritis</td>
<td>Faster onset Mortality:</td>
<td>Dependent on Y chromosome, hence it</td>
<td>Understanding the pathogenesis of lupus</td>
<td>(Izui et al., 1988; Izui et al., 1988;...)</td>
</tr>
<tr>
<td>Models</td>
<td>Induced models</td>
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<td>Cerebritis Onset: 4-6 months after pristane injection</td>
<td>Not an ideal model for figuring out the genetic abnormalities implicated in spontaneous lupus</td>
<td>Assessing the link between dysregulated production of IFN-α and the pathogenesis of SLE in humans (Li et al., 2017a; Reeves et al., 2009; Satoh et al., 1995)</td>
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<td></td>
<td>Imiquimod-induced model</td>
<td>Epicutaneous application of imiquimod on both ears</td>
<td>Splenomegaly, Autoantibodies, immune complex deposition, renal inflammation, vascular impairment, proteinuria, liver, and hematopoietic system involvement.</td>
<td>Nervous complications 3 weeks</td>
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<td>Investigating the role of a TLR7/TLR9 imbalance in the pathogenesis of SLE. (Liu et al., 2018)</td>
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<td></td>
<td>Graft-versus-host disease</td>
<td>Injections of donor lymphocytes into a semi-allogenic recipient</td>
<td>Autoantibodies, Proteinuria, Immune complex-mediated glomerulonephritis, polyclonal B-cell and T-cell activation Rash</td>
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<tr>
<td>Genetically modified models</td>
<td>Transgenic models</td>
<td>Microinjection of the desired transgene into the oocyte</td>
<td>Varies with the gene or protein expressed or overexpressed.</td>
<td>Varies with the gene or protein expressed or overexpressed.</td>
<td>Faster onset Mortality: 50% at 5-6 months</td>
<td>The disease differs from human lupus. None of the transgenic models covers all the major aspects of lupus</td>
<td>Studying/identifying the roles of a gene product or protein in the pathogenesis of lupus (Richard and Gilkeson, 2018)</td>
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</table>
### Knockout models

<table>
<thead>
<tr>
<th>Genetic inactivation or removal of specific gene(s) or cell(s) performed on MRL/lpr background</th>
<th>Varies with the gene/cell knocked out</th>
<th>Faster onset mortality: 50% at 6 months</th>
<th>The disease differs from human lupus. None of the knockout models covers all the major aspects of lupus</th>
</tr>
</thead>
</table>

### Humanized models

| PBMCs humanized mouse model | Intraperitoneal/intravenous injection of PBMCs from SLE patients into immunodeficient mice | Human IgG deposition, autoantibodies (anti-dsDNA, ANA, ACL IgG), Proinflammatory cytokines, Proteinuria, Rashes, Glomerulonephritis | Rashes | Faster onset mortality: 50% at 4-5 weeks | Short lifespan and low survival rate. Differences in the clinical manifestation of lupus in SLE patients whose PBMCs were used often lead to inconsistency in the model generated. |

| HSCs-pristane humanized mouse model | Intravenous injection of HSC from SLE patients into immunodeficient mice followed by intraperitoneal injection of pristine | Human IgG deposition, autoantibodies (anti-dsDNA, ANA, ACL IgG), Proinflammatory cytokines, Proteinuria, Rashes, Glomerulonephritis, Pulmonary serositis, Lymphopenia, Lymphocytes hyperactivation | Rashes | Onset: four weeks post pristine injection | Impairment in the maturation of B cells and production of antibodies. Further studies are required to confirm the stability of this model as well as its clinical value. |

Studying the effects of specific genes and cells in the pathogenesis of lupus, identifying genes/cells critical to the development of lupus (Reilly and Gilkeson, 2002; Richard and Gilkeson, 2018) | Reconstructing the severe complicated clinical manifestations observed in lupus patients. Assessing candidate treatments for lupus (Andrade et al., 2011; Chen et al., 2022a; Kerekov et al., 2011; Richard and Gilkeson, 2018) | Reconstructing the severe complicated clinical manifestations observed in lupus patients. Assessing candidate treatments for lupus (Chen et al., 2022a; Gunawan et al., 2017; Richard and Gilkeson, 2018) |
Table 2: List of ongoing clinical trial studies on SLE (extracted from Clinicaltrials.gov in March 2023)

<table>
<thead>
<tr>
<th>Identifier:</th>
<th>Study-phase</th>
<th>Intervention</th>
<th>Class of drug</th>
<th>Mechanism of Pharmacological Action</th>
<th>Mode of administration</th>
<th>Aim of the study</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT03920267</td>
<td>Long-term safety and efficacy study-phase 2</td>
<td>Drug: Deucravacitinib</td>
<td>Tyrosine kinase 2 inhibitor</td>
<td>Blocks the cytokine-signaling pathways in SLE</td>
<td>oral</td>
<td>Characterize the long-term safety and tolerability of deucravacitinib in SLE patients</td>
</tr>
<tr>
<td>NCT04179032</td>
<td>Pharmacokinetics, safety, and pharmacodynamics study-phase 2</td>
<td>Biologics: Belimumab</td>
<td>Recombinant Human monoclonal antibody</td>
<td>Inhibits the binding of B-cell activating factor (BAFF) to B cells</td>
<td>Subcutaneous injection</td>
<td>Support the subcutaneous administration of Belimumab in pediatric SLE patients</td>
</tr>
<tr>
<td>NCT04433585</td>
<td>Safety and efficacy study-phase 2</td>
<td>Drug: LY3471851</td>
<td>Regulatory T cell stimulator</td>
<td>Targets IL-2 receptor complex and stimulates the proliferation of Treg cells</td>
<td>Subcutaneous injection</td>
<td>Assess the safety and efficacy of LY3471851 in restoring the balance of the immune system</td>
</tr>
<tr>
<td>NCT04082416</td>
<td>Combinational safety and efficacy study-phase 3</td>
<td>Biologics: RC18 plus standard therapy</td>
<td>Recombinant Human B Lymphocyte Stimulating Factor Receptor-Antibody Fusion Protein</td>
<td>Blocks the activities of B Lymphocyte Stimulator and A proliferation-inducing ligand</td>
<td>Subcutaneous injection</td>
<td>Assess the safety and efficacy of combining RC18 with standard treatment</td>
</tr>
<tr>
<td>NCT03845517</td>
<td>Safety and efficacy study-phase 2</td>
<td>Drug: PF-06700841</td>
<td>Dual Tyrosine kinase 2/ Janus kinase 1 inhibitor</td>
<td>Blocks the cytokine-signaling and Janus kinase 1-dependent signaling pathways in immune cells</td>
<td>Oral</td>
<td>Assess the safety and efficacy of PF-06700841 in patients with active SLE</td>
</tr>
<tr>
<td>NCT01729455</td>
<td>Safety and efficacy study-observational cohort study</td>
<td>Biologics: BENLYSTA (Belimumab)</td>
<td>Recombinant Human monoclonal antibody</td>
<td>Inhibits the binding of B-cell activating factor (BAFF) to B cells</td>
<td>Subcutaneous injection</td>
<td>Evaluate Adverse Events of Special Interest and Efficacy of Belimumab in active, antibody-present adult SLE patients</td>
</tr>
<tr>
<td>NCT04058028</td>
<td>Safety and efficacy study-phase 2</td>
<td>Drug: Rozibafusp Alfa</td>
<td>Bispecific antibody-peptide conjugate</td>
<td>Simultaneously block maturation of B cell, activation of T cell, and aberrant T cell and B cell communication by binding both BAFF and inducible costimulator</td>
<td>Subcutaneous injection</td>
<td>Evaluate the safety and efficacy of Rozibafusp Alfa subjects with active SLE and inadequate response to standard-of-care therapies</td>
</tr>
<tr>
<td>NCT Number</td>
<td>Study Type</td>
<td>Drug(s)</td>
<td>Biomarker(s)</td>
<td>Description</td>
<td>Outcomes</td>
<td></td>
</tr>
<tr>
<td>------------</td>
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<td></td>
</tr>
<tr>
<td>NCT04451772</td>
<td>Safety and efficacy study - phase 2</td>
<td>Drug: ABBV-599 (elselbrutinib and upadacitinib)</td>
<td>combination of Bruton's tyrosine kinase inhibitor and Janus kinase inhibitor</td>
<td>Blocks the B-lymphocyte development, differentiation, cytokine-signaling, and Janus kinase 1-dependent signaling pathways in immune cells</td>
<td>To assess the safety and efficacy of the combination therapy in patients with active SLE</td>
<td></td>
</tr>
<tr>
<td>NCT05287581</td>
<td>a pilot randomized controlled trial</td>
<td>Behavioral: moderate aerobic activity and strength training</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>NCT04983784</td>
<td>Efficacy study</td>
<td>Behavioral: Walking</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>NCT01649765</td>
<td>Safety and efficacy study - phase 2</td>
<td>Biologics: Belimumab</td>
<td>Recombinant Human monoclonal antibody</td>
<td>Inhibits the binding of B-cell activating factor (BAFF) to B cells</td>
<td>Intraavenous injection</td>
<td></td>
</tr>
<tr>
<td>NCT03656562</td>
<td>Safety and efficacy study - phase 2</td>
<td>Drug: VAY736 Drug: CFZ533</td>
<td>A human monoclonal antibody Anti-CD40 monoclonal antibody</td>
<td>Inhibits the binding of B-cell activating factor (BAFF) to B cells. Blocks the activation of B cells by blocking CD40</td>
<td>Subcutaneous injection Intravenous injection</td>
<td></td>
</tr>
<tr>
<td>NCT04461158</td>
<td>Disparity study</td>
<td>Behavioral: Patient Navigator Services</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>NCT05203692</td>
<td>Safety study: Phase 1</td>
<td>Drug: DS-7011a</td>
<td>A monoclonal antibody Toll-like receptor 7 antagonists</td>
<td>Intravenous injection and</td>
<td>Assess the safety, PK, and PD properties of single ascending doses of DS-7011a in healthy</td>
<td></td>
</tr>
<tr>
<td>NCT</td>
<td>Study Type</td>
<td>Drug/Condition</td>
<td>Methodology</td>
<td>Subcutaneous injection</td>
<td>Oral</td>
<td>Assess the safety, PK, and PD properties of CUG252</td>
</tr>
<tr>
<td>-----------------</td>
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<td>-------------------------------------------------------------------------------</td>
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</tr>
<tr>
<td>NCT03030118</td>
<td>Safety and efficacy study-phase 2</td>
<td>Drug: Hydroxychloroquine Anti-Malarial</td>
<td>Suppresses the immune system</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT05328557</td>
<td>Safety study: Phase 1</td>
<td>Drug: CUG252 A Treg-selective IL-2 mutein</td>
<td>Stimulates and expands immune-suppressive Treg cells and lowers undesired IL-2 activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT03915652</td>
<td>Disparity study</td>
<td>Behavioral: Integrated Care Management Program (Rheum-ICMP)</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT04233164</td>
<td>Safety study: Early Phase 1</td>
<td>Drug: SOLU-MEDROL An anti-inflammatory glucocorticoid</td>
<td>Inhibits the expression of several inflammatory genes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT03734055</td>
<td>randomized controlled trial</td>
<td>Behavioral: Peer Mentoring</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT02281513</td>
<td>Activity and Nutrition Trial</td>
<td>Smartphone application for self-monitoring changes in sleep, physical activities, and nutritional intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT02988661</td>
<td>Disparity study</td>
<td>Behavioral: Chronic Disease Self-Management Program</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT04925934</td>
<td>Safety and efficacy study-phase 2</td>
<td>Drug: VIB7734 A monoclonal antibody dendritic cells</td>
<td>Depletes plasmacytoid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT05704088</td>
<td>Efficacy study-Phase 4</td>
<td>Drug: Dapagliflozin A sodium-glucose co-</td>
<td>inhibit SGLT-2 proteins in the renal tubules,</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The article has not been copyedited and formatted. The final version may differ from this version.
| NCT04493541 | BMS-986256 | An oral novel Toll-like Receptor 7 and 8 Inhibitor | Inhibits both the Toll-like Receptor 7 and 8 | Oral | Assess the safety and pharmacokinetics of BMS-986256 in patients with active cutaneous lupus

| NCT04754698 | Efficacy study-Phase 4 | Biological: CoronaVac | A whole inactivated virus COVID-19 vaccine | Teaches the immune system to produce antibodies against the SARS-CoV-2 virus | Intramuscular injection | Evaluate the safety and immunogenicity of the COVID-19 vaccine in SLE patients

| NCT05247203 | Safety and Efficacy Study-Phase 1 | Biological: Telitacicept | A TACI-Fc fusion protein |suppresses the growth and survival of B cells and plasma cells by neutralizing the activities of B-lymphocyte stimulator and a proliferation-inducing ligand. | Subcutaneous injection | Assess the safety and pharmacokinetics of Telitacicept in SLE patients who are Chinese

| NCT03791827 | Observational | Current treatment options | - | - | Evaluate the safety, efficacy, and outcome of the current treatment option available for Chinese pediatric lupus nephritis patients

| tablet transporter-2 (SGLT-2) inhibitor | preventing the reabsorbing of glucose back into the blood and thus facilitating the loss of glucose in the urine | on bone and mineral metabolism in SLE patients |
Table 3: Recent advances in lymphatic targeting for immune disorders

<table>
<thead>
<tr>
<th>Drug approach</th>
<th>Drug</th>
<th>Purpose</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD71 targeted precision-polyester nanoparticles functioning independent of</td>
<td>Cyclosporine</td>
<td>Prevention of glomerulonephritis</td>
<td>(Ganugula et al., 2020)</td>
</tr>
<tr>
<td>transferrin binding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST2+ Treg cell population targeted by the fusion of cytokines in the presence</td>
<td>IL233, an IL-2-IL-33 Hybrid</td>
<td>Treatment of Lupus glomerulonephritis</td>
<td>(Stremska et al., 2019)</td>
</tr>
<tr>
<td>of a polypeptide linker.</td>
<td>Cytokine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Self-assembling of amphiphilic polyethylene glycol-based macromolecular prodrug</td>
<td>Treatment of Lupus glomerulone</td>
<td></td>
<td>(Jia et al., 2018)</td>
</tr>
<tr>
<td>with glutamate linker into micelles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transdermal delivery of fabricated microneedles</td>
<td>Melittin</td>
<td>Inhibition of Rheumatoid Arthritis</td>
<td>(Du et al., 2021)</td>
</tr>
<tr>
<td>Targeted delivery of drug-coupled inflammation-responsive shape transformable</td>
<td>Dexamethasone</td>
<td>Treatment of Rheumatoid Arthritis</td>
<td>(Qin et al., 2022)</td>
</tr>
<tr>
<td>nanoparticle to inflamed synovium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Targeted delivery of drug-loaded nano lipid carrier following oral route</td>
<td>Leflunomide</td>
<td>Treatment of Rheumatoid Arthritis</td>
<td>(Krishnan et al., 2018)</td>
</tr>
<tr>
<td>Targeted delivery of immunosuppressant loaded in a novel human serum albumin</td>
<td>Tacrolimus</td>
<td>Prevention of organ rejection after</td>
<td>(Zhang et al., 2019)</td>
</tr>
<tr>
<td>(HSA) nanoformulation</td>
<td></td>
<td>transplantation</td>
<td></td>
</tr>
<tr>
<td>The use of solid lipid nanoparticles and dissolving microneedles in the targeted</td>
<td>Doxycycline, diethylcarbamazine</td>
<td>Treatment of lymphatic filariasis</td>
<td>(Permama et al., 2019)</td>
</tr>
<tr>
<td>delivery of anti-filariasis drugs</td>
<td>and albendazole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Targeted delivery of engineered exosome proteins to lymphatic endothelial</td>
<td>CD63-VEGFC fusion protein</td>
<td>Treatment of Lymphedema</td>
<td>(Li et al., 2020)</td>
</tr>
<tr>
<td>cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Targeted delivery of long-chain fatty acid-conjugated mesoporous silica</td>
<td>Laquinimod</td>
<td>Treatment of Crohn’s Colitis</td>
<td>(Yin et al., 2020)</td>
</tr>
<tr>
<td>nanoparticles to the mesenteric lymphatic system</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Targeted delivery of antibody-peptide conjugate toward dendritic cells in the</td>
<td>hybrid insulin peptide (HIP)</td>
<td>Prevention of Autoimmune Diabetes</td>
<td>(Lin et al., 2022)</td>
</tr>
<tr>
<td>thymus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Targeted Delivery of drug-loaded micelles to Schlemm’s Canal Cells</td>
<td>latrunculin A</td>
<td>Reduction of intraocular pressure in glaucoma</td>
<td>(Stack et al., 2020)</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Targeted delivery of drugs to the HIV reservoirs in the mesenteric lymphatic system using a combination of lipid-based formulation and the lipophilic prodrug approach</td>
<td>lopinavir</td>
<td>Reduction of HIV reservoirs in the mesenteric lymphatic system</td>
<td>(Qin et al., 2021)</td>
</tr>
<tr>
<td>Targeted delivery of Macromolecular prodrug formed by the conjugation of the drug to poly (l-lysine succinylated) to scavenger receptor Al</td>
<td>Emtricitabine</td>
<td>Treatment of infectious diseases</td>
<td>(Stevens et al., 2020)</td>
</tr>
</tbody>
</table>
Table 4: Non-exhaustive list of selected patents issued in the last 20 years on lymphatic delivery.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Patent Number/publication</th>
<th>Delivery method/technology</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>US20050209345A1</td>
<td>Lipid-based formulation improving lymphatic transport of enhancing systemic bioavailability of encapsulated lipophilic drug when administered in the fasted state</td>
<td>Abandoned</td>
</tr>
<tr>
<td>2</td>
<td>US8905999B2</td>
<td>Introduction of instrumentation into the lymphatic system that can be useful for physiological monitoring</td>
<td>Expired</td>
</tr>
<tr>
<td>3</td>
<td>US2007008841A1</td>
<td>Liposomal delivery of biologically active agents, diagnostic or therapeutic agents intradermally for lymphatic targeting drug delivery</td>
<td>Active</td>
</tr>
<tr>
<td>4</td>
<td>US7390506B2</td>
<td>This invention is about encapsulating or covalently attaching the biologically active drug to the lipids for oral liposomal delivery to enhance lymphatic uptake.</td>
<td>Expired</td>
</tr>
<tr>
<td>5</td>
<td>US9919005B2</td>
<td>Use of negatively charged PLGA nanoparticles encapsulating proteolipid epitope in the treatment of various autoimmune disorders.</td>
<td>Active</td>
</tr>
<tr>
<td>6</td>
<td>US20200338329A1</td>
<td>Method of administrating the biologically active agent via epidermis to lymphatic vasculature. The model drug, fluorescently tagged Etanercept, was injected and was followed through Optical coherence tomography (OCT) imaging.</td>
<td>Withdrawn</td>
</tr>
<tr>
<td>7</td>
<td>US10806913B2</td>
<td>Targeted delivery of bioactive agents via the skin in cancer therapy</td>
<td>Active</td>
</tr>
<tr>
<td>8</td>
<td>US-11642416-B2</td>
<td>Enhancing the antigenicity of immunomodulatory molecules via the conjugation of albumin binding peptide to the immunomodulatory molecule</td>
<td>Active</td>
</tr>
<tr>
<td>9</td>
<td>US-11638762-B2</td>
<td>Ligand-drug conjugation for the targeted delivery of compounds that inhibit the production of Nicotinamide Adenine Dinucleotide within abnormal cells in cancer and autoimmune diseases.</td>
<td>Active</td>
</tr>
<tr>
<td>10</td>
<td>US11628208B2</td>
<td>Use of microneedles for the transdermal delivery of vaccine and bioactive proteins to skin-associated lymphatic tissue.</td>
<td>Active</td>
</tr>
<tr>
<td>11</td>
<td>US2023011159A1</td>
<td>Genetic modification of lymphocytes</td>
<td>Pending</td>
</tr>
<tr>
<td>12</td>
<td>US20220396556 A1</td>
<td>Use of lipid nanoparticle formulations for the targeted delivery of nucleic acid molecules and other therapeutic agents</td>
<td>Pending</td>
</tr>
<tr>
<td>13</td>
<td>US20220387686 A1</td>
<td>Assessment of the lymphatic system for diagnosis and treatment</td>
<td>Pending</td>
</tr>
<tr>
<td>14</td>
<td>US20220218674 A1</td>
<td>Prevention, treatment, and management of disease conditions involving the lymphatic vasculature via the reduction of the activity of p53</td>
<td>Pending</td>
</tr>
<tr>
<td>15</td>
<td>US20220162336 A1</td>
<td>Treatment of diseases involving the lymphatic system using therapeutic payloads that can cross the blood-brain barrier</td>
<td>Pending</td>
</tr>
</tbody>
</table>
REFERENCES

Andorko JI, Hess KL and Jewell CM (2015) Harnessing biomaterials to engineer the lymph node microenvironment for immunity or tolerance. The AAPS journal 17:323-338.


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Theofilopoulos AN and Dixon FJ (1985) Murine Models of Systemic Lupus Erythematosus11This is Publication No. 3665IMM from the Department of Immunology, Research Institute of Scripps Clinic, 10666 North Torrey Pines Road, La Jolla, California 92037. Our work cited herein was supported, in part, by National Institutes of Health Grants AI-07007, AM-31023, AM-33826, National Cancer Institute Grants CA-27489 and AG-01743 and the Cecil H. and Ida M. Green Endowment Fund, in *Advances in Immunology* (Dixon FJ ed) pp 269-390, Academic Press.


LIST OF FIGURES

Figure 1: Complications and Comorbidities of Systemic lupus erythematosus. SLE is characterized by a diverse range of complications and comorbidities affecting various organs and systems.

Figure 2: Pathogenesis of SLE. The impaired clearance of apoptotic cells leads to the formation and accumulation of secondary necrotic cells (SNECs). This facilitates the presentation of autoantigen to the immune cells, consequently compromising the immunological tolerance and initiating autoimmune responses and organ damage seen in SLE.

Figure 3: Life-threatening involvement of organs in SLE and current treatment options. Rx$^1$ - Complement activation (Eculizumab), Rx$^2$ - T cell targeted therapies (Hydrochloroquine, Voclosporin, Cyclosporine), Rx$^3$ - B cell targeted therapies (Belimumab, rituximab, Mycophenolate mofetil), Rx$^4$ - Cytokine targeted therapies (Ustekinumab, Baricitinib, Immunomodulatory imide drugs)

Figure 4: Routes and Materials for lymphatic targeting. This figure summarizes the various routes of administration and the materials and technologies available for the lymphatic targeting of pharmacologically active agents.

Figure 5: Oral drug delivery in SLE. Orally administered drugs (microparticles and macroparticles) are absorbed in the small intestine (1). The drugs are transported across the enterocytes (2) passively or actively. Small molecules end up in the portal vein and are transported into the liver (3) where they undergo first-pass metabolism before they are returned to the systemic circulation (5). Macromolecules excluded from entry into the portal vein owing to their size end up in the lymphatic capillary and are transported into the lymph nodes (4) before they are returned into the systemic circulation (5). Lymphatic drug delivery increases the oral bioavailability of drugs by avoiding first-pass hepatic metabolism.
Figure 2
Figure 3

Life-threatening involvement of organs in SLE and current treatment options
Figure 4

Routes of administration

- Nasal
- Oral
- Pulmonary
- Subcutaneous
- Intravenous
- Transcutaneous

Materials and technologies available

- Polymeric Nanoparticle
- Solid-Lipid Nanoparticle
- Liposome
- Microparticles
- Microneedles
Figure 5