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REVIEW ARTICLE

Artemisinin derivative SM934 in the treatment of autoimmune and inflammatory diseases: therapeutic effects and molecular mechanisms

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Artemisinin and its derivatives are the well-known anti-malarial drugs derived from a traditional Chinese medicine. In addition to antimalarial, artemisinin and its derivatives possess distinguished anti-cancer, anti-oxidant, anti-inflammatory and anti-viral activities, but the poor solubility and low bioavailability hinder their clinical application. In the last decades a series of new water-soluble and oil-soluble derivatives were synthesized. Among them, we have found a water-soluble derivative β-aminoarteether maleate (SM934) that exhibits outstanding suppression on lymphocytes proliferation in immunosuppressive capacity and cytotoxicity screening assays with 35-fold higher potency than dihydroartemisinin. SM934 displays significant therapeutic effects on various autoimmune and inflammatory diseases, including systemic lupus erythematosus, antiphospholipid syndrome nephropathy, membranous nephropathy, rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, and dry eye disease. Here, we summarize the immunomodulatory effects, anti-inflammatory, anti-oxidative and anti-fibrosis activities of SM934 in disease-relevant animal models and present the probable pharmacological mechanisms involved in its therapeutic efficacy. This review also delineates a typical example of natural product-based drug discovery, which might further vitalize natural product exploration and development in pharmacotherapy.

Keywords: artemisinin derivative; β-aminoarteether maleate (SM934); autoimmune and inflammatory diseases; T helper cells; macrophages; inflammatory cytokines

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INTRODUCTION

Artemisinin, derived from an ancient Chinese herbal remedy, is the natural product for malaria treatment [1], and artemisinin-based combination therapy is now a globally recognized standard treatment for malaria [2]. Cumulative researches indicated that artemisinin and its derivatives possess immunoregulatory effects in addition to the anti-malarial, anti-cancer and anti-inflammatory activities [2–5]. Recently, Thomas Efferth et al. reviewed that artemisinin-type compounds exhibited therapeutic effects on rheumatic diseases, lung diseases, skin diseases, neurological diseases, inflammatory bowel disease, and other inflammatory and autoimmune diseases [6]. They also concluded that artemisinin and its derivatives inhibit numerous receptor-coupled signaling pathways and ultimately result in the suppression of transcription factor nuclear factor κ B (NF-κB), whereby regulate the cytokines, chemokines, and immune receptors [6]. Besides, mechanic investigations on artemisinin-type drugs provided additional interpretation of their functions. Artesunate down-regulated mammalian target of rapamycin (mTOR) expression and hypoxia-inducible factor-1α (HIF-1α) translocation in rheumatoid arthritis models; promotion of nuclear factor erythroid-2-related factor 2 (Nrf2) transcription was correlated with the improvement of asthma, acute lung injury and neuroinflammation after artesunate treatment [7–12]. Dihydroartemisinin ameliorated systemic lupus erythematosus (SLE), asthma and autoimmune encephalitis by activating Nrf2 signaling pathway and inhibiting mTOR signaling pathway [13–15]. Artemether exerted anti-inflammatory and neuroprotective activities via inducing Nrf2 expression [16]. Artemisinin derivatives are available for clinical use successfully. Since the last century, dihydroartemisinin, artemether and artesunate were used to fight malaria, and a growing number of studies are expanding their clinical indications. With development, they are used in combination therapy with pyronaridine phosphate or lumefantrine in market [17, 18], (https://data.pharmacodia.com/drug#/main/druglnfo).

Nevertheless, poor solubility and bioavailability limited a broader clinical application of artemisinin and its derivatives. For decades, dozens of artemisinin derivatives have been synthesized in order to optimize its solubility and bioavailability while enhancing its potency. Among a series of novel immunologically active synthetic artemisinin derivatives, the water-soluble β-aminoarteether maleate (SM934) exhibited distinguished biological property in the immunosuppression and cytotoxicity screening, with 35-fold higher potency than dihydroartemisinin (DHA) on suppressing lymphocytes proliferation [19, 20]. Due to the prominent immunosuppressive and anti-inflammatory effects, SM934 has been tested for treating

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various experimental autoimmune and inflammation models. In this review, we summarize the pharmacological effects of SM934 on disease-relevant animal models of SLE, antiphospholipid syndrome nephropathy associated lupus nephritis (APSN-LN), membranous nephropathy (MN), rheumatoid arthritis (RA), multiple sclerosis (MS), inflammatory bowel disease (IBD), and dry eye disease (DED). In addition, we interpreted the mechanisms of action of SM934 both in a systemic and local perspective, highlight the synergistic immune suppressive effects benefit from its multi-targeted property that impact multiple functional aspects of immune system.

TOXICITY AND PHARMACOKINETIC CHARACTERISTICS OF SM934

The drug tolerance and safety are satisfied in healthy people according to the results of Phase I clinical trial of SM934. The pharmacokinetics investigation in healthy people showed that SM934 is rapidly absorbed and reaches its peak of the plasma concentration in 0.5–1 h after oral administration, and the total drug exposure (the area under the curve) is proportional to the dose ranging 5–60 mg/day. Moreover, the oral bioavailability of SM934 is 11%–14% and 43%–71% in rats and dogs, respectively. After being orally administered, SM934 quickly distributes to tissues (mainly to small intestine, lung, kidney, stomach, liver and muscles) and reaches the peak concentration at 30 min.

The major elimination route of SM934 is metabolism, mainly including oxidation, reduction, isomerization, and glucuronidation. The eliminating half-life of SM934 is 0.5–1 h in rats and dogs. All these data are the preclinical studies, without references.

IMMUNOSUPPRESSIVE EFFECTS OF SM934 ON IMMUNE CELLS AND RESPONSE

SM934, namely β-aminoarteether maleate, is synthesized from β-hydroxyarteether based on the sesquiterpene trioxane skeleton of artemisinin by breaking the carbon-oxygen bond of ester and connecting the primary amine, and is finally salted with butene diacid. It has been defined as a compound with higher water-solubility because of the induction of polar groups.

SM934 manifested prominent immunosuppressive activity in vitro and in vivo [21]. The initial discovery study of SM934 elucidated it has low cytotoxicity on murine splenocytes with the CC_{50} (the cytotoxic concentration of the compound that reduces cell viability by 50%) value of 67.3 ± 32.7 μM, relative to its anti-proliferation activity with the IC_{50} (the inhibitory concentration of the compound that reduces cell proliferation by 50%) value of 1.2 ± 0.5 μM and 2.6 ± 1.4 μM on concanavalin A (ConA)- and lipopolysaccharides (LPS)-induced splenocyte proliferation, respectively. In addition, SM934 significantly inhibited splenocytes proliferation induced by IL-2, alloantigen, and TCR cross-linking (anti-CD3/CD28). Particularly, SM934 preferentially promoted apoptosis of the subsets with activated phenotype in $CD4^+$ T cell. Oral administration of SM934 to ovalbumin (OVA)-immunized mice abated the OVA-specific recall T cell responses characterized by cell proliferation and IFN-γ production. A similar in vivo immunosuppressive effect of SM934 has been observed in the sheep red blood cell (SRBC)-induced delayed-type hypersensitivity (DTH) reactions in mice.

Subsequent researches reported that SM934 balanced pro-inflammatory and anti-inflammatory cytokines especially enhanced macrophages to secret anti-inflammatory cytokine IL-10 [22]. SM934 impeded Th1, Th17 cells, and T follicular helper cells differentiation in vitro and induced the accumulation of regulatory T cells in vivo in the autoimmune context [23–25]. Similarly, SM934 also suppressed the spontaneous B cell activation and prevented their development into plasma cells [26].

THERAPEUTIC EFFECTS AND PHARMACOLOGICAL MECHANISMS OF SM934 IN AUTOIMMUNE AND INFLAMMATORY DISEASES

Based on the preliminary in vitro and in vivo evidence that indicated a potent immunosuppressive effect of SM934, scientists from Zuo's group set out to explore the therapeutic potential of SM934 in autoimmune and inflammatory diseases from 20 years ago, endeavored to expound the underlying mechanism of action, and made continuous effort to broaden the indications of SM934 (Fig. 1).

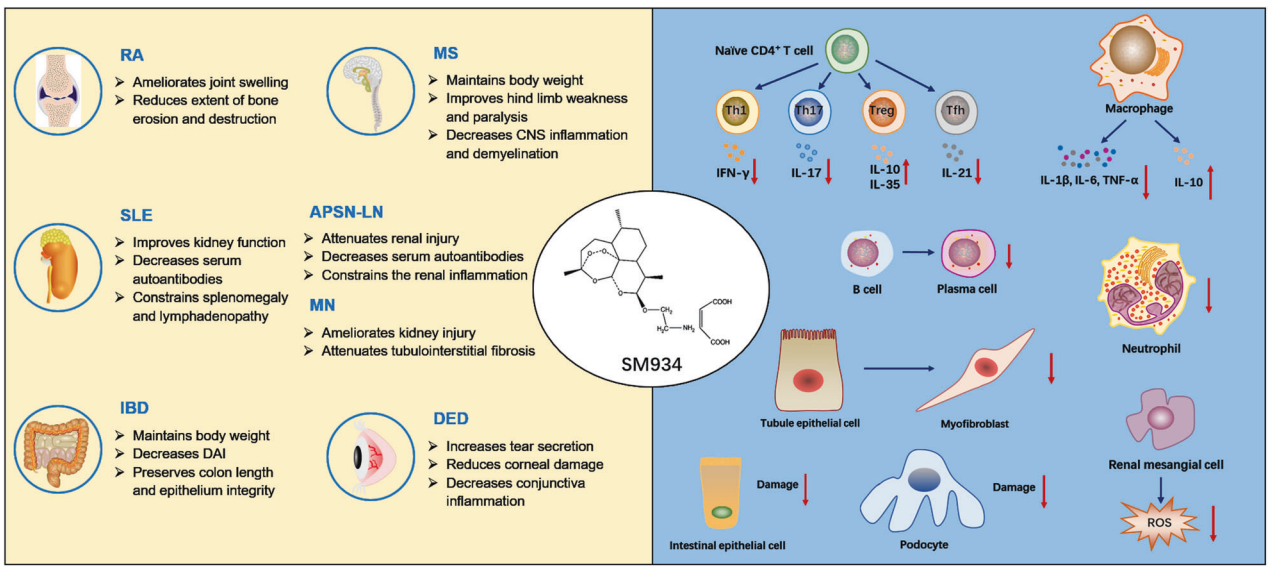

Fig. 1 The therapeutic efficacy and affected cells of SM934 in inflammatory and autoimmune diseases. SM934 ameliorates disease progression of systemic lupus erythematosus, antiphospholipid syndrome nephropathy associated lupus nephritis, membranous nephropathy, rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, and dry eye disease. SM934 reduces infiltration of macrophages and neutrophils, inhibits abnormal differentiation of Th1, Th17, Tfh, and PCs, restores Treg cells, and balances the secretion of cytokines to maintain immune homeostasis. SM934 also reduced ROS in H_2O_2-elicited SV40 MES 13 renal mesangial cells, decreases fibrosis in C3a-induced proximal tubular epithelial cell line HK-2 cells, and reduces the injury in visceral glomerular epithelial cell podocyte.

Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease characterized by the abnormal accumulation of autoreactive T lymphocytes and the production of autoantibodies against self-antigens [27]. The recent two decades witnessed remarkable progress in the development of clinical drugs for SLE treatment including glucocorticoids, antimalarial agents, nonsteroidal anti-inflammatory drugs, immunosuppressive agents, and B cell-targeting biologics. However, their clinical applications are limited due to nephrotoxicity, osteoporosis, skin hyperpigmentation, hepatotoxicity, and so on [28]. In recent decades, artemisinin and its derivatives have attracted widespread attention due to their potential efficacy in the treatment of lupus. Clinical studies have demonstrated that artemisinin alleviated renal lesions, prevented the recurrence of lupus nephritis, and improved the quality of life of patients with SLE [29]. In lupus mice, it separately or combined with hydroxychloroquine exerted ideal therapeutic effect by inhibiting the serum levels of inflammatory factors or down-regulating NF-κB pathway [30, 31]. Dihydroartemisinin was proved to inhibit production of TNF-α and type I interferon, restore the Treg/Th17 balance, impede activation of TLR4 pathway and obstruct the translocation of NF-κB to ameliorate SLE [32–34]. Besides, artesunate improved SLE by inhibiting activation of B cells, restraining Tfh cells differentiation, and down-regulating pro-inflammatory factors production [31, 35].

We initially evaluated the therapeutic potential of SM934 in SLE by using the classic spontaneous lupus model MRL/*lpr* and NZB×NZW F_1 mice. Comparison of SLE symptoms and immunological correlates was made between SM934 and clinical applied SLE drugs like rapamycin or prednisolone. SM934 exerted protective effect on renal dysfunction, reduced circulating autoantibodies and immune complex deposition in kidney, improved survival rate. Anti-dsDNA antibody has high specificity for the diagnosis of SLE, it binds to DNA to form immune complexes deposited in glomerular basement membrane, or directly acts on glomerular antigen causing renal damage in SLE patients [36]. SM934 significantly decreased serum total anti-dsDNA IgG antibody, IgG2a isotype, and IgG3 isotype, as well as increasing the protective IgG1 isotype level.

In MRL/*lpr* mice, SM934 inhibited the production of IL-17 and IFN-γ and correspondently inhibited the expression of T-bet and RORγ and potentiated FoxP3 expression. Likewise, the polarization of naïve $CD4^+$ T cell to Th1 and Th17 cell was markedly impeded but regulatory T (Treg) cells accumulation was enhanced by SM934 treatment, accompanied by the inhibition of the phosphorylation of STAT-1, STAT-3, and STAT-5, which play key roles in the proliferation and differentiation of Th cells [23]. Another study in MRL/*lpr* mice showed SM934 treatment lowered the serum level of pathogenic cytokines and autoantibodies, and regulated the splenic B cell compartment by increasing quiescent B cell numbers, maintaining germinal center B-cell numbers, decreasing activated B cell numbers, and reducing plasma cell (PC) numbers. Mechanically, SM934 inhibited B-cell activation and PC differentiation by interfering with the MyD88-dependent TLR signaling pathways [26].

In NZB×NZW F_1 mice, similar to MRL/*lpr* mice, SM934 also promoted the apoptosis of activated $CD4^+$ T cells, while enhancing the accumulation of Treg cells. Furthermore, SM934 treatment promoted the IL-10 production of macrophages from NZB×NZW F_1 mice. Similar results were obtained in macrophages isolated from OVA-immunized C57BL/6 mice and IFN-γ-elicited C57BL/6 mice [22].

In conclusion, SM934 ameliorated SLE by inhibiting Th1 and Th17 cell response, enhancing IL-10 secretion, and inhibiting TLR-triggered B cell activation and PC formation. It is gratifying to see that after years of constant effort, SM934 has been authorized by the National Medical Products Administration in China for phase I/II/III clinical trials as a Class 1 chemical drug candidate to treat SLE in 2015, and the phase II clinical trial is now in progress.

Albeit multiple new drugs for SLE are on the horizon that target different elements of the innate immune systems, research remains challenging due to the heterogeneous clinical presentation and animal models that inadequately recapitulate human disease. As we demonstrated, the artemisinin derivative SM934, with multi-target properties, has advantages in the treatment of complex diseases and health conditions linked to drug resistance issues.

Lupus-associated antiphospholipid syndrome

Antiphospholipid syndrome (APS) is a kind of autoimmune disease characterized by the presence of antiphospholipid antibodies (APLAs), and usually occurs with other immune diseases especially SLE. Previous studies have shown the efficacy of SM934 on SLE, accordingly, we used NZW×BXSB M mice, which suffered from both SLE and APS, to expand more indications of SM934.

SM934 (10 mg/kg) prevented the weakening of renal function, protected renal pathology, impeded the development of antiphospholipid syndrome nephropathy associated lupus nephritis (APSN-LN), and significantly improved the survival rate in NZW×BXSB M mice. The serum levels of lupus nephritis hallmark antibodies including anti-CL, anti-PI, anti-Ps, anti-β2GPI, anti-dsDNA IgG, and antinuclear antibodies (ANA), and the deposition of glomerular IgG immune complex were decreased after SM934 treatment and consistent with plasma cell ratio. In addition to the formation of autoantibodies-autoantigen complexes in the kidney, the abnormal distribution of cytokines is also a prominent feature of histopathology. SM934 significantly inhibited IL-1β, IL-6, IL-17, IL-23, TNF-α, and GM-CSF, which indicated SM934 might intervene antiphospholipid antibodies (aPLs)-induced innate inflammatory reaction to improve the tissue injury. Excessive oxidative stress plays a fundamental role in pathophysiology of renal damage in APS and SLE [37]. Mechanically, Nrf2 as the main regulator of oxidative stress, can regulate the expression of multiple genes which coding antioxidant enzymes and regulating different processes such as immune and inflammatory responses as well as fibrosis and tissue remodeling [38, 39]. Our studies demonstrated that SM934 reduced reactive oxygen species (ROS) accumulation, improved antioxidant enzyme activity, and inhibited myeloperoxidase (MPO) activity by activating Nrf2 pathway in renal tissue and H_2O_2-conditioned murine RAW264.7 macrophages. Furthermore, SM934 also dose-dependently prohibited ROS generation in SV40 MES 13 renal mesangial cells stressed by H_2O_2. Enhanced Nrf2 may be a potential therapeutic target for APSN-LN treated with SM934 [40].

Clinical antiphospholipid syndrome treatment includes prevention strategies (low-dose aspirin, hydroxychloroquine) and long-term anticoagulation after thrombosis [41]. SM934 impeded the pathogenic autoimmune response other than generating systemic extensive immunosuppression. Therefore, SM934 is a potential supplement for standard therapy to meet the requirement of minimizing the side effects of existing drugs.

Membranous nephropathy

Membranous nephropathy (MN) is one of the main causes of adult nephrotic syndrome, and a high proportion of patients progress to end-stage renal failure [42]. There is no research of artemisinin or its derivatives on MN at present.

Passive Heymann nephritis (PHN) rat model had been used and SM934 significantly attenuated the progression of glomerulonephritis and renal fibrosis. The levels of proteinuria and circulating antibodies were reduced, and the symptoms of podocyte injuries and tubulointerstitial fibrosis were also improved. Fibrosis is the main performance at the end stage of MN, which is improved by SM934 treatment according to the reduced extracellular matrix (ECM) deposition and reduced interstitial myofibroblasts hallmark

Table 1. The therapeutic benefits and pharmacological mechanisms of SM934 in autoimmune and inflammatory diseases.

Disease	Experimental models	Indication	Pharmacological mechanisms	Reference
SLE	MRL/*lpr* mice; NZB×NZW F_1 mice	Improves kidney function; Decreases serum autoantibodies; Constrains splenomegaly and lymphadenopathy	Inhibits Th1 and Th17 cells differentiation, promotes Treg accumulation, enhances IL-10 production from macrophages, impedes TLR ligands-induced B cell activation	[22, 23, 26]
APSN-LN	NZW×BXSB F_1 mice	Attenuates renal injury; Decreases serum autoantibodies; Constrains the renal inflammation	Enhances Nrf2 activity and downregulates ROS-dependent inflammatory pathway	[40]
MN	Anti-Fx1A antiserum-induced rat passive Heymann nephritis	Ameliorates kidney injury; Attenuates tubulointerstitial fibrosis	Down-regulates TGF-β1/Smad signaling pathway, reduces C3a-induced EMT of proximal tubule epithelial cell	[43]
RA	Collagen-induced arthritic mice	Ameliorates joint swelling; Reduces extent of bone erosion and destruction	Inhibits Tfh and Th17 cells differentiation and impedes STAT3 activation	[25]
MS	MOG_{35-55}-induced EAE mice	Maintains body weight; Improves hind limb weakness and paralysis; Decreases CNS inflammation and demyelination	Induces Treg differentiation and expansion	[24]
IBD	DSS-induced colitic mice	Maintains body weight; Decreases DAI; Preserves colon length and epithelium integrity	Suppresses macrophages and neutrophils, inhibits NF-κB signaling	[60]
DED	SCOP-induced DED mice; BAC-induced DED rat	Increases tear secretion; Reduces corneal damage; Decreases conjunctiva inflammation	Decreases TLR4 expression and abates inflammatory signaling in conjunctiva macrophages	[68]

SLE Systemic lupus erythematosus, *TLRs* Toll-like receptors, *Treg* Regulatory T cell, *APSN-LN* Antiphospholipid syndrome nephropathy associated lupus nephritis, *Nrf2* Nuclear factor erythroid-2 factor 2, *ROS* Reactive oxygen species, *MN* Membranous nephropathy, *TGF-β* Transforming growth factor β, *EMT* Epithelial-mesenchymal transition, *RA* Rheumatoid arthritis, *STAT3* Signal transducer and activator of transcription 3, *MS* Multiple sclerosis, *MOG* Myelin oligodendrocyte glycoprotein, *EAE* Experimental autoimmune encephalomyelitis, *CNS* Central nervous system, *IBD* Inflammatory bowel disease, *DSS* Dextran sulfate sodium, *DAI* Disease activity index, *NF-κB* Nuclear factor kappa-B, *DED* Dry eye disease, *SCOP* Scopolamine, *BAC* Benzalkonium chloride.

α-SMA expression. Mechanically, TGF-β1/Smad pathway is one of the crucial pro-fibrosis pathways in renal fibrosis and SM934 significantly decreased the expression of TGF-β1 and the phosphorylation of Smad2/3. Moreover, SM934 blocked the C3a-induced epithelial-mesenchymal transition in proximal tubular epithelial cell line HK-2 cells in vitro [43].

For decades, clinicians have treated MN mainly with glucocorticoids, immunosuppressive therapy, or cytotoxic agents. Recently, complement inhibitors attracted attention with the developing understanding of autoantibody IgG and complement acted in MN [44]. The therapeutic effects of SM934 in lupus nephritis have been already demonstrated previously. SM934 exerted ideal therapeutic effect on PHN model, and possessed great anti renal fibrosis potential, suggests that SM934 is expected to become a potential clinical drug for MN.

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic inflammatory arthropathy, distinguished by synovitis and irreversible joint injury as clinical manifestations [45]. Studies have shown that artesunate suppressed the production of ROS via activating p62/Nrf2 signaling [46], thus inhibited the migration and invasion of fibroblast-like synoviocytes from RA patients [47]. Dihydroartemisinin and dimeric artesunate phospholipid conjugate significantly suppressed ankle joint swelling of CIA model and also decreased secretion of anti-inflammatory cytokines [48, 49].

Previous researches from our team demonstrated that SM934 (10 mg/kg) significantly improved erythema and joint swelling as well as improved bone erosion and destruction of collagen-induced arthritis (CIA) in DBA/1 mice. The levels of serum antibodies were predominantly decreased after SM934 treatment especially IgG, IgG1, and IgG2a. Since autoreactive T cells play an important role in CIA mice [50], SM934 treatment diminished the development of T follicular helper (Tfh) cells and Th17 cells. In vitro, differentiation of Tfh cells was significantly impeded when treated with SM934, decreased expression of Bcl-6 also confirmed this. IL-21, one of the main cytokines derived from Tfh and Th17, exerts pleiotropic actions on the immune system [50]. SM934 treatment inhibited the generation of IL-21-producing T helper cells and abolished the downstream signaling of IL-21 through STAT3 [25].

Arthritis and arthralgias have been noted in up to 95% of patients with SLE. Our findings on the therapeutic effects of SM934 in SLE and RA models, potentiated the prospective application potential of SM934 to treat comorbid condition of a complex and multifactor disorder like SLE.

Multiple sclerosis

Multiple sclerosis (MS) is an inflammatory disease of demyelination caused by myelin damage around the axons of the brain and spinal cord [51]. Experimental autoimmune encephalomyelitis (EAE) was usually used for the study of MS. Increasing evidence has demonstrated artemisinin and its derivatives have potential therapeutic effect on MS. Artemisinin derivative TPN10466 and 9,10-anhydrodehydroartemisinin ameliorated EAE by suppressing immune cell migration and Th1/Th17 differentiation [52, 53]. Artesunate ameliorated EAE by inhibiting leukocyte migration to the central nervous system [54].

In our research, SM934 (10 mg/kg) improved the severity of EAE both at the initial stage and after the onset, presented as maintaining body weight, improving hind limb weakness and paralysis, decreasing CNS inflammation and demyelination. Mechanism study found that the inflammatory cytokines including IL-2, IFN-γ, IL-17, and IL-6 were decreased, while the production of IL-10 and TGF-β production was increased from the splenocytes isolated from SM934-treated mice, which was consistent with the decrease of Th17 and Th1 and the increase of Treg cells both in the peripheral immune system and the CNS lesion. Further study demonstrated that SM934 treatment directly enhanced Treg cells expansion and differentiation by up-regulating anti-inflammatory cytokines, enhancing the inhibitory receptors CTLA-4 and PD-1, and increasing the expressions of apoptosis-promoting proteins galectin-1 and granzyme B in Treg

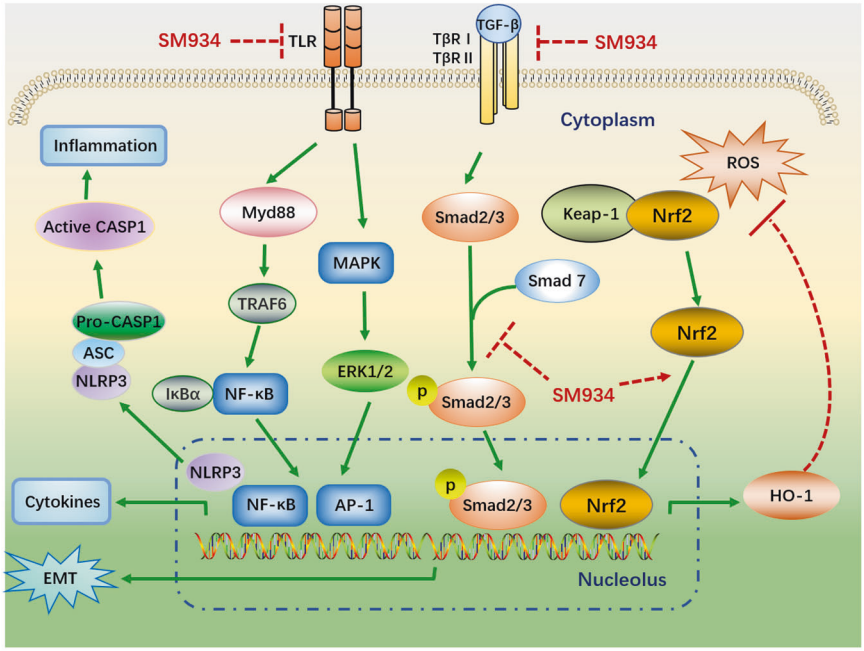

Fig. 2 The potential mechanisms of SM934 in treating inflammatory and autoimmune diseases. SM934 regulates inflammation by regulating NF-κB or MAPK signal messengers in toll-like receptor pathway, abates epithelial-mesenchymal transition through TGF-β/Smad signal pathway and enhances Nrf2 activity, and downregulates ROS-dependent inflammatory pathway.

cells. In conclusion, SM934 treatment improved the severity of EAE by promoting the expansion and function of Treg cells [24].

Currently, most therapeutics for MS are systemic immunosuppressive or immunomodulatory drugs, which still are unable to halt or reverse the disease, and also have the potential to cause serious adverse events [55]. Hence, there is an urgent need for the development of novel treatments that could stop the undesired autoimmune response and contribute to the restoration of homeostasis. Our study of SM934 on EAE models painted a hopeful picture for its application on MS as a therapy proposed to restore immune homeostasis.

Inflammatory bowel disease

Inflammatory bowel disease (IBD) is a chronic inflammatory autoimmune disease idiopathic which is characterized by idiopathic and relapsing inflammation and includes ulcerative colitis (UC) and Crohn's disease (CD) [56]. Studies have shown that artemisinin and its analogues have therapeutic effects on IBD via a variety of mechanisms. For example, artemisinin ameliorated dextran sulfate sodium (DSS)-induced intestinal inflammation by inducing macrophage polarization to M2 phenotype and inhibiting the process of epithelial-mesenchymal transition (EMT) [57]. Dihydroartemisinin protected against mouse models of colitis induced by oxazolone (OXA) and 2,4,6-trinitro-benzene sulfonic acid (TNBS), which is associated with the regulation the Th/Treg balance by inducing activated $CD4^+$ T cell apoptosis [58]. Dihydroartemisinin (DHA) also ameliorated DSS-induced intestinal inflammation by regulation of the expression of cell junction-associated genes and gut microbiota [59].

Previous studies from our team showed that SM934 effectively improved clinical symptoms in DSS-induced UC mice [60]. SM934 (10 mg/kg) treatment reversed body weight loss and colon shortening, along with a lower disease activity index (DAI) score and reduced splenomegaly, which was equivalent to the effect of cyclosporine A (CsA, 20 mg/kg). The severity of histology disruption was analyzed by H&E staining. SM934 ameliorated the colon damage and inflammation, including mucosal ulcers, loss of crypts, epithelial barrier disorders, and infiltration of inflammatory cells. Consistently, the expression levels of IL-1β, IL-6, TNF-α, iNOS and MIP-1α in colon tissues were decreased. In addition, SM934 decreased the secretion of inflammatory factors in RAW264.7 cells and THP-1-derived macrophages induced by LPS in a dose-dependent manner in vitro. Excessive activation of NF-κB plays an important role during the progression of experimental colitis [61]. Mechanistically, SM934 attenuated DSS-induced colitis by suppressing the infiltration of neutrophils and macrophages and the activation the NF-κB signaling pathway.

Despite the availability of current biologics, such as anti-TNFα, anti-interleukins, and small molecules such as tofacitinib, these therapies are limited in their effectiveness across the spectrum of IBD patients [62], thereby necessitating additional treatment options. Recently, studies have correlated the success of mucosal healing with improved prognosis in IBD patients [63, 64], thus further exploration should be conducted to evaluate the impacts of SM934 on intestinal epithelial repair and to clarify its effectiveness in the individuals who are resistant to regular therapies.

Dry eye disease

Dry eye disease (DED) is a multifactorial disease characterized by unstable tear film, high osmotic pressure, ocular surface inflammation and injury, as well as abnormal nerve sensation [65]. At present, the main clinical treatment for DED is to increase tears fluid and anti-inflammatory therapy.

The stability and solubility of SM934 in aqueous solution and its good anti-inflammatory and immunomodulatory activity provide the basis for the treatment of DED. Scopolamine (SCOP)-induced [66] and benzalkonium chloride (BAC)-induced [67] DED model have been used to investigate the curative effects of SM934. Topical application of SM934 (0.1%, 0.5%) significantly increased tear secretion, maintained the number of conjunctival goblet cells, and reduced corneal damage. The secretion of inflammatory mediators, such as IL-1β, IL-6, IL-10, and TNF-α were also decreased. In the conjunctival epithelium of DED mice, the TLR4/NF-κB/NLRP3 signaling pathway was activated. SM934 treatment decreased TLR4 expression and abated inflammatory

signaling, accompanied by the inhibition of inflammasome components, including NLRP3, ASC, and cleaved caspase 1. In LPS-induced RAW264.7 cells, SM934 impeded the upregulation of TLR4 and inhibited the activation of downstream pathways [68].

In addition to traditional methods, cyclosporine A, intense pulsed light, and scleral lenses are used in clinical application. However, they are still accompanied with many side effects, such as eye burns, conjunctival congestion, and visual disturbance [69]. Compared with the existing therapeutic approaches which have intense irritation and only short-term alleviation, SM934 has fundamentally anti-inflammatory effect and stability in aqueous solution, which makes it a potential therapeutic drug for DED.

CONCLUSION AND PERSPECTIVE

SM934 has been proved to be a potent immunosuppressive agent with high efficacy and low toxicity, systemic administration and topical application of SM934 exhibit therapeutic benefits in autoimmune diseases including SLE, APSN-LN, RA, MS as well as inflammatory diseases including IBD and DED. Rather than impacting single cellular population or target protein, SM934 acts on multiple components of immune system approaching a synergistic immune suppressive effect in autoimmune and inflammatory diseases (Table 1), which makes SM934 a suitable agent to treat refractory autoimmune diseases with complicated pathogenesis.

Functional abnormality of the immune system involved but not limited to excessive proliferation, misled differentiation, improper responses, and cytokine dysregulation, all of which could take part in particular stage of disease progression. The multi-targeted property of SM934 could adapt to the alteration of principal pathogenic immune component or cellular event during the development of diseases, thus potentiate the recovery of immune homeostasis (Fig. 2).

In the meantime, advanced chemical biology strategy and probe technology like drug affinity responsive target stability (DARTS) and biological target fishing combined with bioinformatics could provide fresh insight into the cellular and molecular mechanisms of SM934, which might facilitate identifying potential markers in predicting clinical response to SM934 treatment.

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ADDITIONAL INFORMATION

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