

Spleen Tyrosine Kinase: A Crucial Player and Potential Therapeutic Target in Renal Disease

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Key Words

Antibodies · Interstitial fibrosis · Glomerulonephritides · Acute renal rejection · Immunoglobulin A nephropathy

Abstract

Spleen tyrosine kinase (Syk), a 72 kDa cytoplasmic non-receptor protein-tyrosine kinase, plays an important role in signal transduction in a variety of cell types. Ever since its discovery in the early 1990s, there has been accumulating evidence to suggest a pathogenic role of Syk in various allergic disorders, autoimmune diseases and malignancies. Additionally, there is emerging data from both pre-clinical and clinical studies that Syk is implicated in the pathogenesis of proliferative glomerulonephritis (GN), including anti-glomerular basement membrane disease, anti-neutrophil cytoplasmic antibody-associated GN, lupus nephritis and immunoglobulin A nephropathy (IgAN). Moreover, recent animal studies have shed light on the importance of Syk in mediating acute renal allograft rejection, Epstein Barr virus-associated post-transplant lymphoproliferative disease and kidney fibrosis. Fostamatinib, an oral Syk inhibitor, has undergone clinical testing in rheumatoid arthritis, refractory immune thrombocytopenic purpura, leukemia and lymphoma. The recent STOP-IgAN trial showed that the addition of non-selective immunosuppressive therapy to intensive sup-

portive care did not improve clinical outcomes in high-risk IgAN patients. A Syk-targeted approach may be beneficial and is currently being evaluated in a phase II randomized controlled trial. In this review, we will discuss the pathogenic role of Syk and potential use of Syk inhibitor in a variety of renal diseases.

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Introduction

Protein tyrosine kinases (PTKs) play a crucial role in the regulation of cellular growth and transformation by catalyzing the transfer of γ -phosphate of ATP to the hydroxyl group of tyrosine in a protein substrate [1]. There are 2 classes of PTKs in cells, including the transmembrane receptor PTKs and non-receptor PTKs. Receptor PTKs typically have an extracellular domain responsible for binding of ligands, a transmembrane domain for anchorage and an intracellular domain for signal transduction [2]. After binding of a ligand to a receptor PTK, it triggers dimerization and autophosphorylation of the receptor, followed by activation of downstream signaling pathways [2]. Non-receptor PTKs are subdivided into 9 main families. They interact with receptor PTKs and are involved in signaling pathways which regulate

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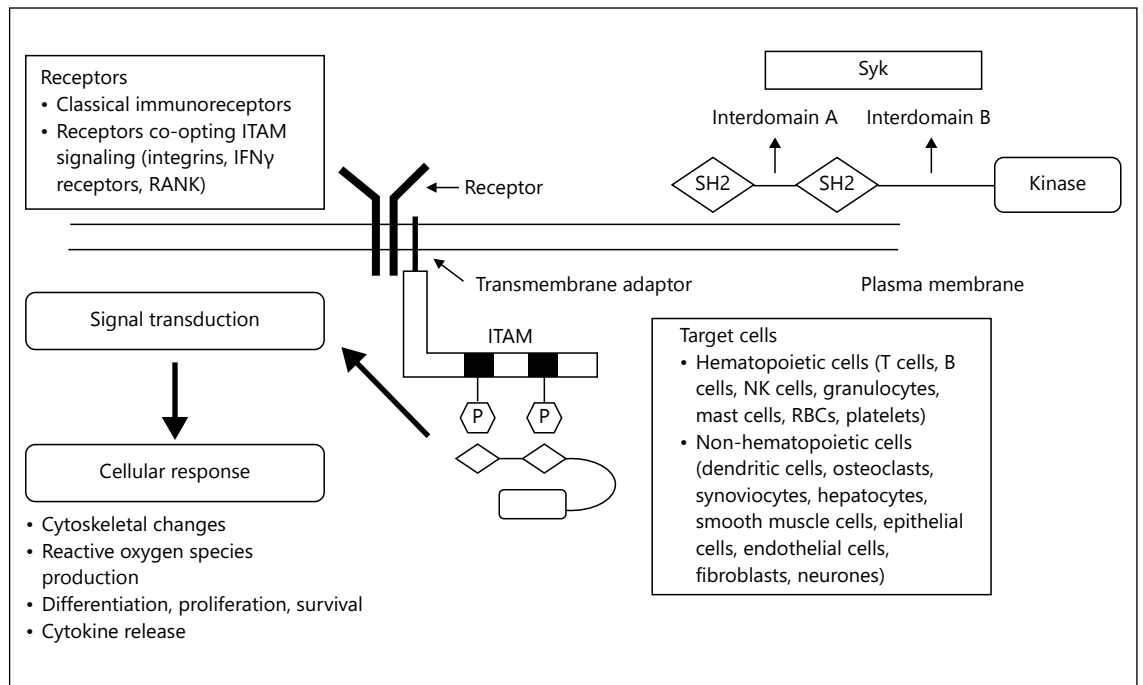


Fig. 1. Summary of the schematic structure, general mechanism of Syk activation, receptors involved and cellular responses in different cell types after activation of Syk. Recruitment of Syk to plasma membrane receptors occurs through binding of the SH2 domains of Syk to 2 phosphorylated tyrosine residues (P) in the immunoreceptor tyrosine-based activation motif (ITAM) present in the receptor-associated transmembrane adaptor. Recruitment of Syk to dually phosphorylated ITAM triggers the activation of Syk

and activates downstream signal transduction, which eventually triggers various cellular responses. Classical immunoreceptors refer to those signaling molecules that are directly coupled to ITAM adapters FcR γ or DAP12 through transmembrane charged residues. These immunoreceptors consist of immunoglobulin superfamily-containing proteins (such as the Fc receptors, PIR-A or the TREMs) or the C-type lectin receptors (Dectin-2, Mincle, or MDL-1).

cellular migration, proliferative, differentiation and survival [3].

In 1990, Kobayashi et al. [4] purified a 40 kDa cytosolic PTK which had autophosphorylation activity from porcine spleen. In 1991, Taniguchi et al. [5] showed that this 40 kDa kinase was a proteolytic breakdown product of a 72 kDa non-receptor PTK and cloned the spleen tyrosine kinase (Syk) gene. In 1994, the Syk gene was mapped to chromosome 9q22 in humans [6]. The Syk protein (629 amino acids) contains a pair of Src homology 2 (SH2) domains at the N-terminus and a carboxy-terminal tyrosine kinase domain [7]. These domains are linked by 2 linker regions: interdomain A between the 2 SH2 domains and interdomain B between the C-terminal SH2 domain and the kinase domain. In inactive form, Syk assumes a closed, autoinhibited structure where the aromatic residues in interdomain A, interdomain B and the kinase domain interact to form a 'linker-kinase sandwich' [7]. Activation of Syk occurs via engagement of the SH2 domains or phosphorylation of ty-

rosine residues involved in the 'linker-kinase sandwich'. Syk activation results in a conformational change that allows the exposed catalytic kinase domain to interact with downstream targets [7]. Syk is widely expressed in hematopoietic cells and involved in coupling activated immunoreceptors to downstream signaling events that mediate cellular proliferation, differentiation and phagocytosis [8]. The schematic structure, general mechanism of Syk activation, receptors involved and cellular responses in different cell types after activation of Syk is summarized in figure 1. The role of Syk in various biological functions has been comprehensively reviewed elsewhere [8].

Ever since its discovery, there has been accumulating evidence to suggest a pathogenic role of Syk in various allergic disorders, autoimmune diseases [9] and malignancies [10]. There is also emerging data from both pre-clinical and clinical studies that Syk is implicated in the pathogenesis of various renal disorders, including proliferative glomerulonephritis (GN), acute renal allograft re-

Table 1. Summary of intervention studies of Syk inhibitor in animal models

Animal model, reference	Resemblance of human disease	Intervention	Salient findings
EAG [16]	Anti-GBM disease	Syk inhibitor (R788)	Prevention of EAG (given before immunization) Reduced GN severity and prevention of pulmonary hemorrhage (given after established disease)
NTN [17, 18]	Anti-GBM disease	Syk inhibitor (R788) Conditional Syk gene deletion in myeloid cells	Reduced GN severity when treatment started after established disease Reduced GN severity
EAV [24]	ANCA-associated vasculitis/AAGN	Syk inhibitor (R788)	Reduced GN severity and pulmonary hemorrhage
Lupus prone NZB/NZW mice [31]	SLE and LN	Syk inhibitor (R788)	Delayed onset and reduced GN severity (given before disease onset) Reduced GN severity (given after disease onset)
MRL/lpr and BAK/BAX double-knockout mice [32]	SLE and skin disease	Syk inhibitor (R788)	Delayed onset and reduced severity of skin disease (given before disease onset) Reduced severity of skin disease (given after disease onset)
MRL/lpr mice [32]	SLE and lupus nephritis	Syk inhibitor (R788)	Prevention of GN (given before immunization) Reduced GN severity (given after established disease)
Experimental acute renal allograft rejection (Brown Norway to Lewis) [35, 36]	Acute renal allograft rejection	Syk inhibitor (R788)	Prevention of allograft infarction and reduced interstitial infiltrates Decreased donor-specific antibody
Experimental acute renal allograft rejection (Wistar to Dawley) [37]	Acute renal allograft rejection	Syk inhibitor (CC0482417)	Improved allograft function, reduced infiltration of macrophages and neutrophils, attenuated acute tubular injury and peritubular capillary thrombosis
UO [41]	Renal fibrosis	Syk inhibitor (CC0417)	Reduced macrophage infiltration
NTN [40]	Renal fibrosis	Syk inhibitor (R788)	Late treatment (days 14–28) using Syk inhibitor reduced deposition of interstitial collagen, glomerular expression of α -smooth muscle actin and glomerular synthesis of transforming growth factor- β Reduced renal fibrosis Improved renal function

jection, Epstein Barr virus (EBV)-associated post-transplant lymphoproliferative disease (PTLD) and kidney fibrosis. Given the role of Syk in a wide range of diseases, various small molecular Syk inhibitors are being developed [11]. Fostamatinib is an oral Syk inhibitor that has been tested in clinical trials on patients with rheumatoid arthritis (RA), refractory immune thrombocytopenic purpura, leukemia and lymphoma [12, 13]. There is an ongoing phase II randomized controlled trial to assess the safety and efficacy of fostamatinib in immunoglobulin A nephropathy (IgAN). In this review, we will discuss the pathogenic role of Syk and potential use of Syk inhibitor in different renal diseases.

Pathogenic Role of Syk in Renal Disease (Table 1)

Glomerulonephritis

GN is an important cause of acute kidney injury (AKI) and chronic kidney disease. Rapidly progressive GN (RPGN), or crescentic GN, is an aggressive disease. Anti-glomerular basement membrane (anti-GBM) disease and anti-neutrophil cytoplasmic antibody (ANCA)-associated GN (AAGN) are important causes of RPGN, and Syk has been shown to play a crucial role in both conditions. Syk has also been implicated in the pathogenesis of immune complex-mediated GN, including lupus nephritis (LN) and IgAN.

Anti-GBM Disease

Experimental autoimmune GN (EAG) and nephrotoxic nephritis (NTN) are 2 widely used animal models that resemble human anti-GBM disease. In EAG, animals are immunized with heterologous or homologous GBM or NC1 domain of the α -3 chain of type IV collagen. Immunized animals subsequently mount an autoimmune response and develop anti-GBM antibodies that attack their own kidneys [14]. On the contrary, there are 2 phases of injury in NTN. In the heterologous phase, animals are injected with a heterologous anti-GBM antibody, which deposits in the kidney and causes transient injury. In the autologous phase, the foreign antibody acts as a planted antigen on the GBM which triggers an autoimmune response in the animal [15]. Although these models have been developed in various species, the most consistent model is that induced in Wistar-Kyoto (WKY) rats. Immunohistochemistry (IHC) study has demonstrated increased glomerular total Syk (T-Syk) and phosphorylated Syk (P-Syk) expression in EAG [16]. T-Syk includes both phosphorylated (active) and unphosphorylated (inactive) forms of Syk. Syk plays a crucial role in the activation of stress-activated protein kinases like c-Jun N-terminal kinase and p38 mitogen-activated protein kinase, which are responsible for leukocyte-mediated renal injury [17, 18]. When NTN was induced in mice with conditional Syk gene deletion in myeloid cells, there were significantly less leukocytic infiltration, crescent formation, inflammation and fibrosis when compared with controls [19]. Administration of fostamatinib (R788), an oral prodrug of the selective Syk inhibitor taminib (R406), completely prevented the induction of EAG when it was given 1 h before immunization. When given from day 18 to 36 after induction of EAG, fostamatinib led to cessation of autoantibody production, reversal of renal injury, preservation of renal function and complete protection from pulmonary hemorrhage [16]. Additionally, fostamatinib inhibited production of proinflammatory cytokines by nephritic glomeruli *ex vivo* and cultured bone marrow-derived macrophages *in vitro*, suggesting additional therapeutic effects related to inhibited Fc receptor signaling within macrophages in diseased glomeruli which were independent of suppression of autoantibody production [16]. Similar beneficial effects of fostamatinib have also been observed in NTN models [18, 20]. In human anti-GBM disease, previous IHC study showed increased glomerular T-Syk and P-Syk expression [21]. Syk appeared to localize to infiltrating macrophages and neutrophils. A strong correlation between

glomerular T-Syk expression and serum creatinine at presentation was observed (fig. 2a). Dialysis-dependent patients had significantly higher levels of glomerular T-Syk expression [21].

ANCA-Associated GN

ANCA-associated vasculitis and AAGN are characterized by the presence of antibodies directed against proteinase-3 or myeloperoxidase (MPO). *In vitro* study showed that ANCA-IgG induced phosphorylation of Syk in tumor necrosis factor-primed neutrophils from healthy donors [22]. Piceatannol (a Syk inhibitor) inhibited Syk phosphorylation as well as ANCA-stimulated respiratory burst [22]. Several animal models have been developed to demonstrate the pathogenicity of ANCA *in vivo*, as recently summarized elsewhere [23]. In experimental autoimmune vasculitis, where WKY rats are immunized with MPO, renal disease can be induced after 4 weeks [23]. Fostamatinib, given for established renal disease, significantly reduced hematuria, proteinuria, macrophage infiltration and pulmonary hemorrhage [24]. In patients with AAGN, glomerular T-Syk expression was upregulated and correlated with serum creatinine at the time of biopsy as well as the histological class of disease [21]. Four histopathological classes of lesions have been proposed for prognostication of AAGN patients, namely focal, crescentic, mixed and sclerotic [25]. T-Syk staining was highest in patients with crescentic (acute) disease but minimal in sclerotic (chronic) disease (fig. 2b) [21].

Lupus Nephritis

Systemic lupus erythematosus (SLE) is a complex, multi-system, autoimmune disease characterized by abnormalities in both T and B cells [26]. Compared with normal T cells, T cells obtained from SLE patients demonstrated substantially higher Syk expression and activity [27]. *In vitro* study showed that Syk was responsible for upregulation of various cytokines, receptors and enzymes involved in the immunopathogenesis of SLE, including CD44 (involved in T cell migration), interleukin (IL)-21 (involved in antibody production), OAS2 (2'-5'-oligoadenylate synthetase; involved in type I interferon response) and protein phosphatase 2A (involved in regulation of IL-2 production) [28]. The cardinal abnormalities of B cells in SLE include loss of B cell tolerance resulting in autoantibody production and hyperactive B cell receptor (BCR)-triggered signaling [29]. Murine models of SLE and LN are widely used and considered to be the best tool to study human SLE [30]. Fostamatinib, no matter given before or after disease onset, delayed the

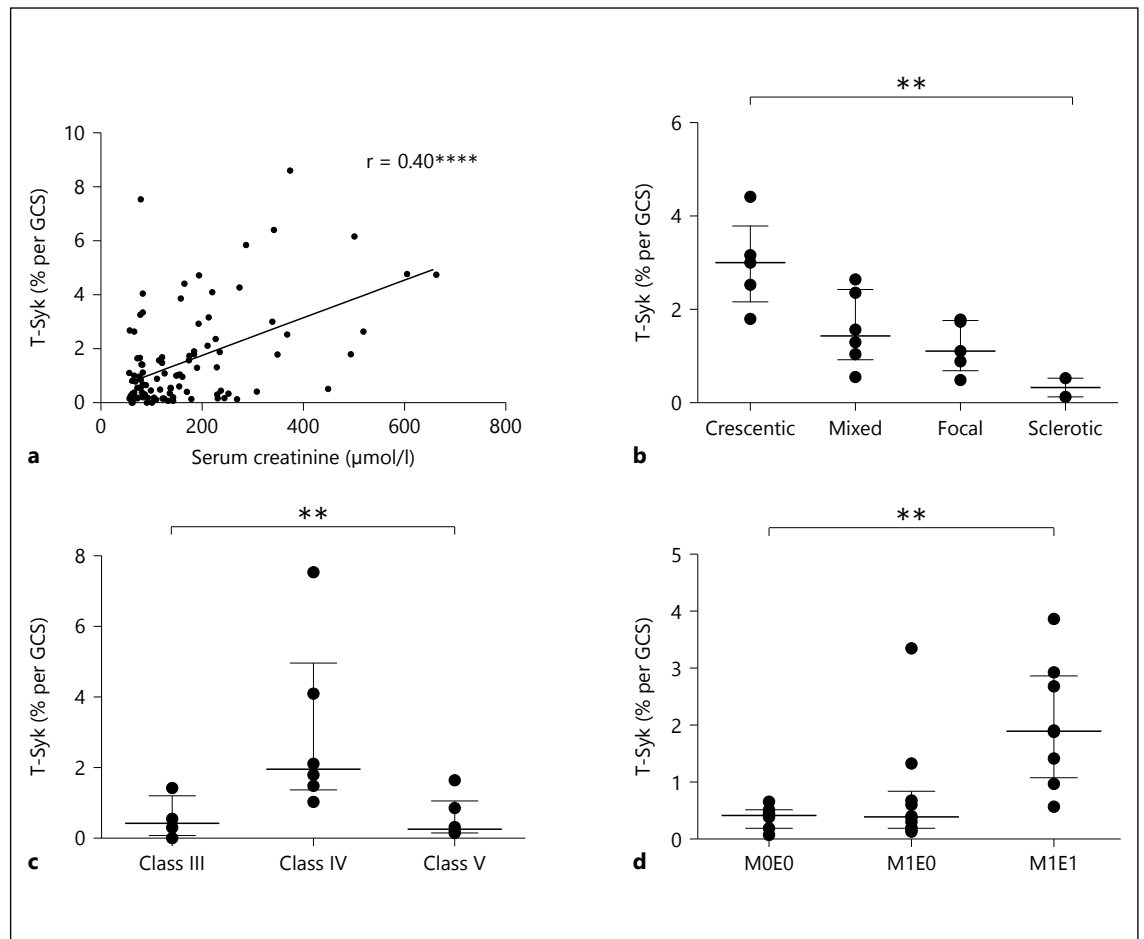


Fig. 2. Correlation between Syk expression and disease activity in proliferative GN (reproduced with permission from [21]). **a** Correlation between T-Syk expression and serum creatinine in human anti-GBM disease. **b** Correlation between T-Syk expression and histopathological classes in human AAGN. **c** Correlation between

T-Syk expression and histopathological classes in human LN. **d** Correlation between T-Syk expression and Oxford classification scores in human IgA nephropathy. ** $p < 0.01$; **** $p < 0.0001$. E = Endocapillary proliferation; GCS = glomerular cross section; M = mesangial hypercellularity.

onset of proteinuria and azotemia, reduced renal pathology and kidney infiltrates and significantly prolonged survival in lupus prone NZB/NZW mice [31]. In MRL/lpr and BAK/BAX double-knockout mice, fostamatinib prevented development of skin disease when given before disease onset and significantly reduced severity of skin disease when given after disease onset [32]. In MRL/lpr mice, fostamatinib could prevent the development of renal disease and reduce proteinuria in established disease [32]. In LN patients, those with class IV (diffuse proliferative) LN had the highest T-Syk and P-Syk expressions [21]. Minimal T-Syk staining was seen in class V disease (fig. 2c). There was also a trend of higher T-Syk expression in patients who failed to achieve complete remission (CR) by 6 months compared with patients who

achieved CR. However, there was no association between T-Syk expression and serum creatinine or proteinuria [21].

IgA Nephropathy

IgAN is the most common primary GN worldwide. A multi-hit pathogenesis model has been proposed [33]. Initially, there is increased production of galactose-deficient IgA1 (hit 1), followed by formation of autoantibodies directed against galactose-deficient IgA1 (hit 2). These autoantibodies subsequently bind with galactose-deficient IgA1 to form pathogenic immune complexes (hit 3), which deposit in the mesangium, activate mesangial cells and induce glomerular injury (hit 4). Previous in vitro study demonstrated that Syk was involved in activation of

mesangial cell proliferation [34]. Inhibition of Syk by pharmacological antagonist (R406) and knockdown of Syk by small interfering RNA downregulated synthesis and release of a number of proinflammatory mediators and platelet-derived growth factor, and reduced cellular proliferation in mesangial cells stimulated with IgA1 isolated from IgAN patients [34]. In IgAN patients, those with endocapillary proliferation demonstrated highest levels of T-Syk staining, whereas those without proliferative features or mesangial proliferation only had negligible staining within the glomerular tuft (fig. 2d) [21]. In this study, there was no association between T-Syk expression and serum creatinine or proteinuria. However, a recent study found a significant correlation between the number of P-Syk positive glomerular cells and the degree of proteinuria and renal function in patients with IgAN, further supporting the role of Syk activation in the pathogenesis of IgAN [19].

Other Renal Diseases

In recent years, various animal studies have shed light on the pathogenic role of Syk in acute renal allograft nephropathy, EBV-associated PTLD, renal fibrosis and possibly diabetic nephropathy.

Acute Renal Allograft Rejection

In experimental model of early severe renal allograft rejection (Brown Norway to Lewis rats), IHC study showed that all rats with acute rejection had Syk-positive tubulointerstitial infiltrates [35]. Increased Syk expression was also detected in kidney transplant biopsies from patients with antibody-mediated rejection or T-cell mediated rejection [35]. Fostamatinib treatment has been shown to completely prevent allograft infarction and reduce interstitial infiltrates in experimental renal allograft rejection [36]. In another experimental model of renal allograft rejection, where Sprague-Dawley rats underwent bilateral nephrectomy and orthotopic renal transplant from Wistar rats, Ramessur Chandran et al. [37] showed dense leukocytic infiltration with strong Syk expression in the allograft. Administration of Syk inhibitor (CC0482417) significantly improved allograft function, reduced leukocytic infiltration and attenuated acute tubular injury and peritubular capillary thrombosis [37].

Post-Transplant Lymphoproliferative Disease

EBV, a B-cell lymphotropic virus, is associated with a wide variety of B cell-derived lymphoid neoplasms, including PTLD. The EBV protein latent membrane protein 2a, which is expressed in the membrane of infected B

cells, mimics BCR and provides survival signals to EBV-infected cells through Syk [38]. In vitro study showed that Syk-activated PI3K/Akt signaling was required for the survival of EBV-positive B-cell lymphomas and R406 induced apoptosis and cell cycle arrest in EBV-positive PTLD cell lines [39].

Renal Fibrosis

Renal fibrosis is the hallmark of progressive renal disease. In WKY rats induced with NTN, fostamatinib treatment from day 14 to 28 (i.e., after established renal disease and entering into fibrotic phase) was shown to be effective in reducing glomerular expression of α -smooth muscle actin (a profibrotic marker) and deposition of interstitial collagen, as well as improving renal function [40]. Ex vivo culture of nephritic glomeruli showed that Syk inhibition reduced glomerular synthesis of transforming growth factor (a pro-fibrotic cytokine) [40].

The unilateral ureteral obstruction (UUO) model in rodents generates progressive renal fibrosis. There is rapid and robust induction of renal fibrosis in obstructed kidney; hence the day 7 time point is the most commonly used end point for analysis of renal fibrosis in the UUO model. When UUO mice were treated with a selective Syk inhibitor (CC0417) from the time of surgery till day 7, there was significantly reduced macrophage infiltration (~50%) compared with vehicle and no treatment groups [41]. It will also be interesting to know if administration of Syk inhibitor at a later stage of UUO (e.g., day 14) will have the same inhibitory effect on the fibrotic process. It should be noted, however, that the effect of Syk inhibition on kidney function is difficult to assess in UUO.

Diabetic Nephropathy

Previous in vitro studies showed that Syk was involved in high-glucose-induced activation of nuclear factor (NF)- κ B in human glomerular endothelial cells [42] and proximal tubular cells [43]. NF-B activation plays an important role in the signaling pathway of fibrosis. Preliminary animal study also showed a beneficial role of Syk inhibitor in autoimmune diabetes [44].

Syk as a Therapeutic Target

A search of the patent literature has revealed more than 70 filings describing the development of small molecular Syk inhibitors [11]. Fostamatinib is the first clinically available oral Syk inhibitor. After oral administration, it is rapidly converted to R406 by human intestinal

microsomes, and only low levels of fostamatinib appear in the plasma [45]. Fostamatinib is metabolized primarily by cytochrome P450 3A4 [46]. On an average, fecal and urine excretions account for 80 and 19% of drug excretion, respectively [45]. The terminal half-life was estimated to be 12–21 h and steady state is achieved after 3–4 days following twice daily administration. Co-administration with food results in delay in peak time and lower peak concentrations of R406, but without any change in overall exposure. Renal or hepatic impairment did not affect R406 exposure to a clinically relevant extent [47]. Fostamatinib is a P-glycoprotein inhibitor both in vitro and in vivo. Reported drug–drug interactions include ketoconazole, verapamil, rifampicin [46], oral contraceptives, simvastatin, rosuvastatin [48] and digoxin [49], but not warfarin [48]. The inhibitory effect of R406 is relatively specific for Syk. Although previous in vitro kinase assays showed that R406 did have inhibitory effects on other kinases (e.g., Flt3, Lyn, Lck), cell-based assays showed that the inhibitor effects were much less potent compared with Syk [50].

Fostamatinib has been investigated in multiple phase II and phase III clinical trials on RA patients [51–54]. Hypertension, diarrhea and headache were the most common adverse effects observed in these trials. The effect of fostamatinib on blood pressure elevation (mean increase of ~3 mm Hg in both systolic and diastolic) appeared to be dose dependent, and a concentration-dependent increase of blood pressure was observed with increasing R406 concentrations. Fostamatinib-induced hypertension may be attributed to increased vascular resistance, secondary to reduced vascular endothelial growth factor-induced nitric oxide release from endothelium [55]. Blood pressure usually returns to normal upon decrease in fostamatinib dose or its withdrawal. Alternatively, addition or modification of anti-hypertensive drugs can be considered.

The latest KDIGO guidelines suggest that IgAN patients with persistent proteinuria ≥ 1 g/day despite 3–6 months of optimized supportive therapy and estimated glomerular filtration rate (eGFR) > 50 ml/min/1.73 m² to receive a 6-month course of corticosteroid therapy [56]. However, this recommendation was recently challenged by results of the STOP-IgAN trial [57]. In this multicenter, open-label, randomized controlled trial, IgAN patients with persistent proteinuria ≥ 0.75 g/day after a 6-month run-in period of supportive care were randomized to receive supportive care alone or supportive care plus immunosuppressive therapy for 3 years. In the immunosuppression group, patients with eGFR ≥ 60 ml/

min/1.73 m² received glucocorticoid monotherapy for 6 months, whereas those with eGFR 30–59 ml/min/1.73 m² received cyclophosphamide for 3 months and followed by azathioprine, plus a tapering course of steroid. A total of 80 and 82 patients were randomized to the supportive care and immunosuppression group, respectively. After 3 years, clinical remission (defined as proteinuria with a protein-to-creatinine ratio of < 0.2 and stable renal function with a decrease in the eGFR of < 5 ml/min/1.73 m²) was observed in 5% of the supportive care group, as compared with 17% in the immunosuppression group. However, there was no significant difference in the annual decline in eGFR between the 2 groups. More importantly, more patients in the immunosuppression group had severe infections, impaired glucose tolerance and weight gain. Recently, another double-blind, randomized controlled trial comparing mycophenolate mofetil (MMF) and placebo was prematurely terminated 6 months after it was observed that MMF did not significantly reduce proteinuria compared with supportive treatment alone [58]. Results of these recent studies challenged the efficacy and safety of non-selective immunosuppressive therapy in IgAN patients. It should be noted that renal biopsy data were not reported in these studies. Previous study showed that T-Syk expression was observed predominantly in biopsies with endocapillary proliferation, whereas biopsies with no proliferative features or mesangial proliferation only had negligible staining within the glomerular tuft [21]. The use of fostamatinib in IgAN patients demonstrating proliferative lesions on renal biopsies is currently being investigated in a phase II randomized controlled trial (NCT02112838). The recruitment phase is ongoing and the result is eagerly awaited. A previous phase II trial on treatment of SLE by Syk inhibitor (NCT00752999) was withdrawn prior to enrollment for business reasons.

Conclusion

Since its discovery in 1991, there have been more than 2,000 publications related to Syk. In particular, the last 10 years have witnessed an intense flowering of interest in this unique PTK. Based on the current evidence from both pre-clinical and clinical studies, we see a great potential in the development of Syk-targeted therapy for treatment of various renal diseases. More studies are required to delineate the role of Syk in other renal diseases, including other types of GN, diabetic nephropathy, tubulointerstitial nephritis and AKI. A clinical trial of fosta-

matinib in IgAN is ongoing, and future clinical studies on the efficacy and safety of fostamatinib for the treatment of proliferative and immune complex GN are warranted.

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Disclosure Statement

F.W.K.-T. is the chief investigator of the randomized controlled trial of Syk inhibitor in IgA nephropathy. He has received research project grants from AstraZeneca Limited, and has a consultancy agreement with Rigel Pharmaceuticals.

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