Targeting the tyrosine kinase signalling pathways for treatment of immune-mediated glomerulonephritis: from bench to bedside and beyond

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Abstract

Glomerulonephritis (GN) affects patients of all ages and is an important cause of morbidity and mortality. Non-selective immunosuppressive drugs have been used in immune-mediated GN but often result in systemic side effects and occasionally fatal infective complications. There is increasing evidence from both preclinical and clinical studies that abnormal activation of receptor and non-receptor tyrosine kinase signalling pathways are implicated in the pathogenesis of immune-mediated GN. Activation of spleen tyrosine kinase (SYK), Bruton’s tyrosine kinase (BTK), platelet-derived growth factor receptor (PDGFR), epidermal growth factor receptor (EGFR) and discoidin domain receptor 1 (DDR1) have been demonstrated in anti-GBM disease. SYK is implicated in the pathogenesis of ANCA-associated GN. SYK, BTK, PDGFR, EGFR, DDR1 and Janus kinase are implicated in the pathogenesis of lupus nephritis. A representative animal model of IgA nephropathy (IgAN) is lacking. Based on the results from in vitro and human renal biopsy study results, a phase II clinical trial is ongoing to evaluate the efficacy and safety of fostamatinib (an oral SYK inhibitor) in high-risk IgAN patient. Various tyrosine kinase inhibitors (TKIs) have been approved for cancer treatment. Clinical trials of TKIs in GN may be justified given their long-term safety data. In this review we will discuss the current unmet medical needs in GN treatment and research as well as the current stage of development of TKIs in GN treatment and propose an accelerated translational research approach to investigate whether selective inhibition of tyrosine kinase provides a safer and more efficacious option for GN treatment.

Keywords: crescentic glomerulonephritis, glomerulonephritis, IgA nephropathy, immunosuppression, lupus nephritis, tyrosine kinase

Introduction

Glomerulonephritis (GN) affects patients of all ages and is an important cause of morbidity and mortality. It is estimated that there were >100 million prevalent cases of chronic kidney disease (CKD) secondary to GN globally in 2013, the number of which had increased by >30% since 1990 [1]. Immune-mediated glomerular injury plays an important role in the pathogenesis of anti-glomerular basement membrane (anti-GBM) disease, anti-neutrophil cytoplasmic antibody (ANCA)–associated glomerulonephritis (AAGN), lupus nephritis (LN) and immunoglobulin A nephropathy (IgAN). In recent years, advances in understanding the immunopathogenesis of these entities have provided translational opportunities for the development of novel therapeutic interventions [2].

Protein tyrosine kinases (PTKs) catalyze phosphorylation of tyrosine residues on protein substrates. They play a crucial role in the modulation of enzymatic activity and recruitment of downstream signaling molecules, which in turn regulate cellular growth and transformation [3]. PTKs can be classified into receptor tyrosine kinases (RTKs) and non-receptor tyrosine kinases (NRTKs). RTKs are transmembrane receptors that have...
intrinsic tyrosine kinase activity, whereas NRTKs are involved in different intracellular signalling pathways [4]. RTKs typically have an extracellular domain (for binding of different ligands), a transmembrane domain (for anchorage) and an intracellular domain (for signal transduction). Upon ligand binding to an RTK, it triggers dimerization and autophosphorylation of the receptor, followed by activation of various downstream signalling pathways [5]. NRTKs are subdivided into nine main families based on their similarities in domain structure. They interact with RTKs and mediate important signalling pathways that regulate cellular proliferative, differentiation, survival and apoptosis [6]. Dysregulation of PTK activity (e.g. overexpression) has been implicated in tumourigenesis, and the development of tyrosine kinase inhibitors (TKIs) has been one of the most important recent advances in oncology [7–9].

Recently there is increasing evidence from both preclinical and clinical studies that targeting tyrosine kinase signalling pathways is a potential therapeutic strategy for immunemediated GN [10–13]. In this review we will focus our discussion on anti-GBM disease, AAGN, LN and IgAN. The potential clinical applications of TKIs in these conditions, their stage of development and preliminary results from clinical studies will be emphasized.

CURRENT UNMET MEDICAL NEEDS

Rapidly progressive glomerulonephritis (RPGN) is an aggressive disease and the renal prognosis is often poor despite intensive treatment. A recent study from China showed that the 5-year the renal survival of anti-GBM disease and AAGN was 17.6 and 44.3%, respectively [14]. In another UK study of 43 patients (81% dialysis dependent at presentation), the 1-year renal survival of anti-GBM disease was just 16% [15]. AAGN usually affects elderly patients, and the use of non-selective immunosuppressive therapy can result in significant systemic side effects and sometimes fatal infectious complications. Rituximab (an anti-CD20 monoclonal antibody) is increasingly used in AAGN, but a recent study showed that there was no difference in clinical outcome of AAGN patients who were treated before and after the introduction of rituximab as an induction agent [16]. More importantly, the toxicity of rituximab was comparable to cyclophosphamide in the RAVE [17] and RITUXVAS [18] studies.

LN usually affects young female patients of child-bearing age. Some patients experience frequent relapses and require long-term immunosuppressive drugs. Corticosteroid-related systemic side effects and cyclophosphamide-related gonadal toxicity are important safety concerns. Multiple randomized controlled trials (RCTs) in ANCA-associated vasculitis (AAV) and LN have compared cyclophosphamide-based regimens with newer agents such as rituximab and mycophenolate mofetil. Disappointingly, their adverse event profiles were similar to those of cyclophosphamide-based protocols [19]. In high-risk IgAN patients with persistent proteinuria despite maximal supportive therapy and preserved renal function, the latest Kidney Disease: Improving Global Outcomes (KDIGO) guideline recommended immunosuppressive therapy using 6 months of corticosteroid [20]. However, the efficacy and safety of non-selective immunosuppressive treatment were recently challenged by the STOP-IgAN trial [21]. Compared with patients receiving supportive treatment alone, patients in the immunosuppression group had no significant improvement in the annual estimated glomerular filtration rate (eGFR) decline after 3 years but experienced significantly higher rates of severe infections, impaired glucose tolerance and weight gain.

With the current limitations of non-selective immunosuppressive therapy, a targeted approach using selective immunosuppressive drugs is more desirable and warrants further investigation.

CURRENT LIMITATIONS OF TRANSLATIONAL RESEARCH IN GN

The essence of translational research is to make use of biomedical advances in basic science to address unmet medical needs of patients so as to improve patient outcomes [22]. In GN research, although various useful animal (mostly rodent) models of anti-GBM disease (e.g. experimental autoimmune GN, nephrotic nephritis), AAGN (e.g. experimental autoimmune vasculitis) and LN (e.g. lupus-prone mice) have been developed, none of them is perfect (Table 1) [23–25]. Development of an animal model of IgAN has been attempted, but none was sufficiently representative of human IgAN, partly attributed to the complex pathophysiology of IgAN [26]. This underscores the uncertainty of the predictive value of data from animal studies in human diseases. In the absence of a perfect animal model, immunohistochemistry (IHC) study of human renal biopsy becomes a valuable tool to provide additional evidence on the pathogenic role of a certain therapeutic target, assuming that the target protein is expressed in the kidney and not in circulating cells that regulate the autoimmune response. Using combined results from in vitro studies and IHC study of human renal biopsy may be a reasonable approach to provide a scientific basis for future clinical studies [27]. Various TKIs have been approved for the treatment of malignancy and have long-term efficacy and safety data in oncology patients. As a result, targeting the tyrosine kinase signalling pathways provides an attractive opportunity for accelerated translation research in GN treatment.

EVIDENCE FROM PRECLINICAL STUDIES TO JUSTIFY FURTHER CLINICAL TRIALS OF TKIs IN IMMUNE-MEDIATED GN

Anti-GBM disease

Compared with other types of immune-mediated GN, anti-GBM disease has been more extensively studied due to the availability of more robust animal models and it is considered a ‘prototypic’ autoimmune disease, such that findings may translate to other forms of GN.

Spleen tyrosine kinase (SYK) is an NRTK that plays a crucial role in a variety of biological functions, including intracellular signalling cascade for classic immunoreceptors like activatory
<table>
<thead>
<tr>
<th>Model</th>
<th>Resemblance of human disease</th>
<th>Animal</th>
<th>Method of induction</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental autoimmune GN (EAG)</td>
<td>Anti-GBM disease</td>
<td>Wistar Kyoto rat</td>
<td>Single intramuscular injection of collagenase-solubilized GBM (e.g. from Sprague-Dawley rat or sheep) in FCA or single intramuscular injection of recombinant rat α3(IV)NC1 in FCA</td>
<td>Invariable progression to chronic phase of injury which resembles human disease</td>
<td>Technically more demanding Some strains (e.g. Lewis rats) are resistant to EAG More gradual onset of disease compared with nephrotoxic nephritis</td>
</tr>
<tr>
<td>NTN</td>
<td>Anti-GBM disease</td>
<td>rat</td>
<td>Single intravenous injection of rabbit anti-GBM antiseran</td>
<td>Relatively simple Rapid onset of renal injury</td>
<td>Rabbit antiseras may contain antibodies towards other components apart from GBM Variable progression to chronic phase of injury</td>
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<td>Accelerated nephrotoxic nephritis</td>
<td></td>
<td>Sprague-Dawley rat</td>
<td>Subcutaneous injection of sheep IgG in FCA followed by intravenous injection of sheep anti-rat/mouse GBM serum 5–10 days later</td>
<td>Rapid onset of renal injury</td>
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<tr>
<td>Attenuated passive model of anti-GBM disease</td>
<td>Anti-GBM disease</td>
<td>C57BL/6 mouse</td>
<td>Intravenous injection of rabbit anti-mouse GBM antibody followed by intraperitoneal injection of purified mouse anti-rabbit IgG monoclonal antibody</td>
<td>Rapid onset of renal injury Degree of proteinuria is dependent on the amount of antibody used</td>
<td>Attenuated form of anti-GBM disease Only ~50% of wild-type mice progressed to chronic phase Technically demanding Mild disease severity (reported crescent fraction 5–15%)</td>
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<td>Passive anti-MPO transfer</td>
<td>ANCA-associated vasculitis</td>
<td>C57BL/6/SJL wild-type or RAG2-deficient mice, with or without LPS priming</td>
<td>Anti-MPO antibody induced in MPO-deficient mice and transfected into recipients</td>
<td>Pauci-immune GN resembling human disease</td>
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<td>Experimental autoimmune vasculitis</td>
<td>ANCA-associated vasculitis</td>
<td>Wistar Kyoto rat</td>
<td>Immunization with human MPO in CFA</td>
<td>Dose-dependent effect of MPO on disease severity</td>
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<td>Spontaneous mouse models of lupus nephritis</td>
<td>Lupus nephritis</td>
<td>MRL/lpr mouse</td>
<td>Spontaneous disease</td>
<td>A broad spectrum of SLE features including arthritis, inflammatory skin lesions and GN are seen</td>
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<td>Anti-Thy 1.1 GN</td>
<td>Mesangial proliferative/IgAN</td>
<td>rat</td>
<td>Single intravenous injection of a mouse monoclon antibody TCR 1.1 antibody</td>
<td>Closest approximation of human lupus nephritis in terms of characteristics of disease development and the underlying genetics driving autoimmunity Mesangial cell proliferation and mesangial matrix expansion, histologically similar to human IgAN</td>
<td>Slow onset of disease Progressive proteinuria beginning ~5 months and azotemia ~7 months onward No evidence of IgA deposition in glomeruli Lesions do not fully mimic the wide range of lesions seen in human IgAN Only a variable proportion of mice develop the disease model No haematuria and mild proteinuria Issues with reproducibility Human IgA1 may not be representative of the pathogenetic IgA1 in patients</td>
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<td>Spontaneous animal model for IgAN</td>
<td>IgAN</td>
<td>ddY strain mouse</td>
<td>Spontaneous disease</td>
<td>Elevated levels of circulating IgA and mouse IgA mesangial deposits, similar to human IgAN</td>
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<td>IgAN</td>
<td>IgA1-expressing mouse</td>
<td>sCD89 injection</td>
<td></td>
<td>Mouse expressing both human IgA1 and CD89 have circulating and mesangial deposition of IgA1-sCD89 complexes resulting in kidney inflammation, haematuria and proteinuria</td>
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FCA, Freund’s complete adjuvant; GBM, glomerular basement membrane; MPO, myeloperoxidase.
Fc receptors (FcRs) and B-cell receptors (BCRs) [28]. IHC study showed increased SYK expression in both experimental [29–31] and human anti-GBM disease [32]. Increased SYK expression seemed to localize predominantly to areas of crescent formation and proliferating cells within the glomeruli [29, 32]. Administration of fostamatinib (an oral SYK inhibitor) completely aborted the development of nephritis when given before induction [29] and significantly reduced disease severity when given after established disease [29, 33]. In experimental autoimmune GN (EAG), fostamatinib treatment starting from Day 18 (where there were severe segmental necrotizing injury and crescent formation in ~26% of glomeruli) to Day 36 led to a rapid and complete resolution of urinary abnormalities (100% reduction of both haematuria and proteinuria) that was sustained until Day 36 [29]. Fostamatinib-treated animals also had preserved levels of serum urea compared with a 103% increase in the vehicle group [29]. In nephrotoxic nephritis (NTN), high-dose fostamatinib treatment starting from Day 7 (where cellular crescents were present in ~90% of glomeruli) to Day 14 significantly reduced proteinuria (23%), glomerular crescents (21%), infiltration of glomerular macrophages (93%) and CD8+ cells (74%) and serum creatinine (28%) [33]. SYK appeared to mediate glomerular injury by upregulation of pro-inflammatory cytokines, glomerular leukocyte recruitment and activation of c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK) pathways [30]. JNK inhibitor (CC-401) suppressed glomerular and tubulointerstitial damage when given before induction of experimental anti-GBM disease [34]. When given from Day 7 (where there was significant proteinuria, focal glomerular lesions, marked glomerular macrophage and T-cell accumulation and upregulation of pro-inflammatory mediators) to Day 14, CC-401 prevented renal impairment, suppressed proteinuria and prevented the development of severe glomerular and tubulointerstitial lesions, including crescent formation [35]. Pharmacological inhibition of p38 MAPK/β, both early (1 h before induction) and late (starting from Day 4), have also been shown to be effective in reducing GN severity in NTN [36].

Bruno’s tyrosine kinase (BTK) is an NRTK that plays an important role in signal transduction pathways that regulate B-cell survival, activation, proliferation and differentiation [37]. Activated SYK can induce phosphorylation of BTK, which cooperatively activates phospholipase C (PLC)-γ. PLC-γ catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) into diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3). IP3 induces calcium mobilization from the endoplasmic reticulum. DAG and calcium promote the activation of protein kinase C (PKC) and MAPK family downstream signalling cascades [38]. In experimental anti-GBM disease, administration of PF-06250112 (an oral BTK inhibitor) at the time of induction reduced proteinuria in a dose-dependent manner [39]. Interestingly, PF-06250112 inhibited disease development even in the presence of glomerular deposition of antibody and C3, indicating that the antiproteinuric effect was secondary to inhibition of the BTK signalling pathway instead of the effect on deposition or clearance of anti-GBM antibody. The effect of late treatment was not assessed in this study. Platelet-derived growth factor receptors (PDGFRs) are RTKs that are expressed constitutively or inducibly in most renal cells. PDGFRs regulate cellular proliferation and migration, extracellular matrix accumulation, production of pro-inflammatory cytokines, tissue permeability and intrarenal haemodynamics [40]. PDGFR-β and PDGFB-BB are overexpressed in the crescents of experimental and human anti-GBM disease [41]. An early study showed that intraperitoneal rapidip (a PDGFR antagonist) administration was associated with worse outcome in vivo [42]. However, recent studies using intraperitoneal imatinib (a multitargeted RTK inhibitor that can block PDGFR) showed significant renoprotective effects in vivo. In NTN, late imatinib treatment from Day 7 (where there was endocapillary proliferation, severe fibrinoid necrosis, cellular crescent formation and prominent glomerular fibrin deposition) to Day 20 led to less crescent formation and fibrinoid necrosis, reduced proteinuria and preserved renal function [43]. Using a similar NTN model, longer-term imatinib treatment from Day 7 to Day 49 significantly suppressed proteinuria, improved renal function and attenuated the development of glomerulosclerosis and tubulointerstitial injury [44]. In these in vivo studies, however, it was uncertain to what extent the beneficial effects were mediated specifically via inhibition of PDGFR signalling.

Epidermal growth factor receptor (EGFR) is an RTK that plays an important role in many cellular functions, including proliferation, migration and differentiation [45]. Heparin-binding epidermal growth factor-like growth factor (HB-EGF), a member of the EGFR family, is a potent inducer of cellular proliferation and migration (e.g. macrophages, T-lymphocytes). Uregulation of HB-EGF was found in both experimental and human anti-GBM disease [46]. HB-EGF deficiency status and pharmacological EGFR blockade (before induction) in vivo prevented renal leukocytic infiltration before the appearance of crescents and interstitial fibrosis, suggesting that the HB-EGF/EGFR pathway was involved in the very early stage of renal damage [46]. Pharmacological blockade of EGFR using erlotinib from Day 4 to Day 14 after induction of NTN was shown to reduce the expression of EGFR in the renal cortex, the proportion of crescent glomeruli and blood urea nitrogen [46].

Discoidin domain receptor 1 (DDR1) is a collagen receptor with tyrosine kinase activity. As with most RTKs, MAPK and PI3 pathways are the downstream effectors of DDR1 [47]. DDR1 expression was increased in experimental and human anti-GBM disease [48]. DDR1-deficient mice had less severe renal disease and lower mortality than their wild-type littermates after induction of anti-GBM disease [49]. Administration of DDR1-specific antisense oligodeoxynucleotides at the time of induction decreased DDR1 expression and reduced disease severity. DDR1 antisense administration given on Day 4 (presence of proteinuria) and Day 8 both prevented progression of NTN, although the protective effect of the antisense treatment started at Day 8 was less efficient compared with antisense treatment started at Day 4 [49].

ANCA-associated GN. In vitro activation of neutrophil respiratory burst by ANCA from patients with systemic vasculitis required PTK and PKC activation. Blocking both kinases using pharmacological inhibitors abrogated ANCA-induced
superoxide generation [50]. However, the specific tyrosine kinases involved were not investigated in this study. A previous study showed that p38 MAPK inhibition markedly reduced ANCA-induced neutrophil activation in vitro and partly reduced crescent formation in vivo [51].

SYK phosphorylation is induced during ANCA-triggered neutrophil activation [52]. In a study using the experimental autoimmune vasculitis model, where WKT rats developed haematuria and proteinuria at 4 weeks, fostamatinib treatment from Week 4 to Week 6 significantly reduced proteinuria, haematuria, glomerular histological abnormalities, glomerular macrophage infiltration, pulmonary haemorrhage severity and haemosiderin deposition in lung tissue [53]. Since SYK is involved in upstream signalling pathways of MAPK, the beneficial effect of SYK inhibition may be explained by its inhibitory effect on downstream MAPK signalling pathways. In patients with AAGN, glomerular SYK expression was increased and correlated with serum creatinine. SYK expression was highest in patients with crescentic GN (active disease) and minimal in those with sclerotic GN (chronic disease) [32].

In the kidney, vascular endothelial growth factor (VEGF) plays a crucial role in maintaining the integrity of the glomerular filtration barrier. Soluble fms-like tyrosine kinase 1 (sFlt-1) acts as an antagonist of VEGF. An imbalance of VEGF/sFlt-1 has been observed in many diseases with endothelial dysfunction, including diabetic nephropathy [54]. An in vitro study showed that ANCA antibodies increased sFlt1 during acute AAV, leading to an anti-angiogenic state that hinders endothelial repair [55].

**Lupus nephritis.** In prediseased lupus-prone NZB/NZW mice 6–7 months of age, fostamatinib treatment (up to Day 240) significantly delayed the onset of proteinuria and azotemia, reduced renal inflammatory infiltrates and significantly prolonged animal survival [56]. In mice with established disease and proteinuria, fostamatinib treatment reduced proteinuria and preserved renal function in a dose-dependent manner and prolonged mice survival [56]. Up to 47% of mice with established disease demonstrated no microscopic evidence of renal changes after high-dose fostamatinib treatment, compared with only 10% in the vehicle group [56]. In MRL/lpr mice, fostamatinib treatment for 16 weeks starting from Week 4 (prediseased state) prevented the development of renal disease at Week 20, whereas fostamatinib for 8 weeks starting from Week 16 (established disease) significantly reduced proteinuria [57]. In a human renal biopsy study, patients with diffuse proliferative LN had the highest SYK expression, whereas those with membranous LN had minimal SYK expression [32]. Several BTK inhibitors have also been shown to reduce the severity of renal disease in experimental models of LN [13]. Ibrutinib treatment for 2 months in prediseased mice (starting from 4 months) alleviated renal damage and decreased circulating antinucleosome, antihistone and anti-ssDNA autoantibodies [58]. BTK inhibitors RN486 [59] and PF-06250112 [38] both reduced the severity of established GN in NZB/NZW mice.

In murine LN, imatinib treatment starting at 5 months of age (where focal glomerular hypercellularity and immune complex deposition were evident) significantly delayed the onset of proteinuria and renal impairment, protected against abnormal histological changes and prolonged animal survival, suggesting that inhibition of PDGFR might be a potential therapeutic strategy [60]. In another in vivo study using MRL/lpr mice, higher-dose imatinib treatment starting from Week 16 (advanced stage of GN) to Week 24 significantly reduced serum IgG and antidsDNA levels, ameliorated histological changes, reduced expression of PDGFR and transforming growth-factor-β messenger RNA, reduced proteinuria, preserved renal function and prolonged survival [61]. An early IHC study showed increased EGFR expression in ~35% of LN patients [62]. Autoantibodies to the extracellular domain of EGFR have been found in Fas-defective mice and in SLE patients [63]. A recent study showed that human epidermal growth factor receptor 2 (HER-2, an RTK) was overexpressed in lupus-prone NZM2410 mice and in patients with LN, but not in other mesangioproliferative GN [64]. DDR1 was found in podocytes and crescents in renal biopsies from patients with LN and genetic inhibition of DDR1 protected mice against development of crescentic GN [48].

Janus kinases (JAKs) are NRTKs that mediate the intracellular signalling initiated by interferons (IFNs), interleukins (ILs), colony-stimulating factors and hormones. Upon activation, JAKs phosphorylate the signal transducers and activators of transcription (STAT), which in turn regulate gene transcription. A series of JAK-STAT signalling cytokines, especially type I IFNs, IL-10 and IL-6, have been implicated in the pathogenesis of SLE [65]. Treatment of lupus-prone mice with JAK2 inhibitors led to prevention or improvement of established disease [66, 67]. In MRL/lpr mice, tyrphostin AG490 (a selective JAK2 inhibitor) treatment from Week 12 to Week 20 significantly inhibited renal expression of monocytie chemotactic protein (MCP)-1 and IFN-γ, reduced renal infiltration of T cells and macrophages, reduced proteinuria and improved renal function [66]. In an elegantly designed study, Lu et al. [67] tested the efficacy of CEP-33779 (a selective JAK2 inhibitor) in age-matched MRL/lpr or BWF1 mice with established SLE or LN, respectively. In this study, reference standard treatments including dexamethasone and cyclophosphamide were included. Treatment with CEP-33779 reduced serum pro-inflammatory cytokines and renal JAK2 activity, improved renal histopathology, decreased splenomegaly and lymphomegaly and prolonged animal survival. The therapeutic effect of CEP-33779 was comparable with that of cyclophosphamide and superior to dexamethasone alone. Tofacitinib, a JAK inhibitor, has been proven efficacious in rheumatoid arthritis. It is currently being investigated in a Phase I clinical trial of SLE patients (NCT02535689). Ruxolitinib, which inhibits JAK2, has been approved for the treatment of myelofibrosis. However, it has not been used in renal disease.

**IgAN.** Despite years of effort, a representative animal model of IgAN is still lacking. We [27] and others [68] have overcome this limitation by studying the effect of IgA1 purified from IgAN patients on human mesangial cells in vitro. In particular, we showed that IgA1 from patients with IgAN (but not IgA1 from the healthy volunteers) stimulated phosphorylation of SYK, production of inflammatory cytokines and growth factors
and proliferation of mesangial cells in vitro [27]. These biological effects are similar to the pathological features of IgAN in patients. Inhibition of SYK by the active metabolite of fostamatinib or specific knockdown of SYK using siRNA reduced the synthesis of inflammatory cytokines and suppressed cell proliferation in IgA1-stimulated human mesangial cells [27]. In human IgAN, patients with endocapillary proliferation on renal biopsy had a higher level of SYK expression than those without [32].

Previous IHC study also showed that glomerular PDGFR-β expression significantly correlated with mesangial cell proliferation [69]. PDGFR inhibitor (in particular imatinib) and EGFR inhibitor reduced mesangial cell proliferation and matrix accumulation in rat acute anti-Thy-1.1 GN [40, 70]. In rat chronic anti-Thy-1.1 GN, PDGFR inhibition using B-specific oligonucleotide aptamer and neutralizing anti-PDG-F-D IgG reduced proteinuria and improved renal function [40]. In acute anti-rat Thy-1.1, early erlotinib (an EGFR inhibitor) significantly prevented progression of mesangial cell proliferation and matrix accumulation and preserved renal function [41]. It should be noted, however, that the anti-rat Thy-1.1 GN model is not a representative model of human IgAN. In IgAN patients, elevated sFlt-1 (low VEGF/sFlt-1 ratio) correlated with the severity of proteinuria and hypertension [71]. Renal biopsy of IgAN patients also showed focal loss of VEGF in podocytes [72].

### Table 2. Summary of existing evidence of tyrosine kinase involvement in immunopathogenesis of immune-mediated GN

<table>
<thead>
<tr>
<th>Tyrosine kinase</th>
<th>Disease</th>
<th>In vitro study</th>
<th>In vivo study</th>
<th>Human renal biopsy study</th>
<th>Justifiable for further clinical study</th>
</tr>
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<tbody>
<tr>
<td>Spleen tyrosine kinase</td>
<td>Anti-GBM disease</td>
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<td>IgAN</td>
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<td>No representative animal model</td>
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<td>IgAN</td>
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<td>(in anti-Thy-1.1 model)</td>
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<td>Vascular endothelial growth factor</td>
<td>IgAN</td>
<td>No data</td>
<td>No representative animal model</td>
<td>No data</td>
<td>Insufficient evidence</td>
</tr>
</tbody>
</table>

### POTENTIAL APPLICATIONS AND SAFETY CONCERNS OF TKIs IN IMMUNE-MEDIATED GN

TKIs are widely used clinically for the treatment of malignan-
cies such as chronic myeloid leukemia (CML), gastrointestinal stromal tumors (GISTs), non-small-cell lung cancer and renal cell carcinoma. There is now accumulating evidence to suggest that further clinical studies of TKIs may be justified in selected immune-mediated GN (Table 2). Multiple in vivo studies have demonstrated beneficial effects of pharmacological inhibition of tyrosine kinases in established renal disease. Some of these tyrosine kinases are also upregulated in human renal biopsies. It should be noted, however, that the pathogenesis of anti-GBM disease and AVV are complex. Although targeting tyrosine kinase signalling pathways is attractive, it is unlikely that a single selective TKI can replace traditional induction therapy. Nevertheless, it might be reasonable to consider TKIs as adjunctive induction agents such that the dosage and side effects of non-selective immunosuppressive drugs may be reduced. Using TKIs as a steroid-sparing maintenance therapy may be another possible treatment strategy. In murine LN, JAK2 inhibitor was equally effective compared with cyclophosphamide [67]. The use of TKIs as induction and maintenance therapy in human LN might be justified.
Multiple TKIs have been approved for anti-cancer therapy. Imatinib was the first Bcr-Abl TKI approved by the US Food and Drug Administration for the treatment of CML. Imatinib also has inhibitory effects on other RTKs that make it a potent immunomodulatory agent. There have been promising results with the use of imatinib in murine models of kidney disease, including experimental anti-GBM disease, anti-Thy 1.1 GN and LN [73]. Besides, a number of case reports have described its successful (off-label) use in human monoclonal proliferative GN and cryoglobulinemia [74–76].

Although the clinical outcomes of these cases are encouraging, it should be noted that imatinib may have deleterious off-target effects on the kidney. In a recent long-term study of CML patients treated with different TKIs, imatinib was associated with a higher incidence of acute kidney injury (AKI) compared with dasatinib and nilotinib [77]. Imatinib-associated AKI has been reported previously [78]. It has also been associated with tubular dysfunction causing renal potassium and phosphate wasting [79] and thrombotic microangiopathy (TMA) [80]. Imatinib may also increase serum creatinine by inhibiting tubular secretion [81]. In another study of CML patients, patients with baseline renal dysfunction had a greater incidence of transient reversible AKI after dasatinib and nilotinib treatment [82]. Dasatinib has been reported to be associated with AKI [83, 84], thrombotic thrombocytopenia purpura [85] and nephrotic range proteinuria [86].

Fostamatinib has been evaluated in >3200 rheumatoid arthritis patients enrolled in three Phase 2, one Phase 2b and three Phase 3 trials [87, 88]. It is currently the only TKI that is being studied in a Phase 2, multicentre RCT in high-risk IgAN patients (NCT02112838). This clinical trial is testing a novel SYK-targeted approach for treating IgAN and will provide important information to guide further development of novel treatment strategies. Up to 35% of subjects on fostamatinib versus 11% on placebo developed hypertension or required adjustment to their antihypertensive regimen [89]. The effect of fostamatinib on blood pressure (mean elevation of ~3 mmHg in both systolic and diastolic) appeared to be dose dependent and secondary to reduced VEGF-induced nitric oxide release from the endothelium [90]. This suggests that fostamatinib may also have off-target inhibitory effects on VEGF. Anti-VEGF therapy has been reported to be associated with hypertension, proteinuria and TMA [91]. However, previous trials of fostamatinib did not suggest an increased risk of nephrotic side effects. The current stages of development of TKIs in immune-mediated GN are summarized in Table 3.

**Table 3. Stage of development of selected TKIs in immune-mediated GN**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Target tyrosine kinase</th>
<th>Animal studies</th>
<th>Human studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fostamatinib</td>
<td>Spleen tyrosine kinase</td>
<td>Anti-GBM disease, ANCA-associated GN, lupus nephritis</td>
<td>Phase 2 clinical trial in IgAN</td>
</tr>
<tr>
<td>Ibrutinib</td>
<td>Bruton’s tyrosine kinase</td>
<td>Lupus nephritis</td>
<td>No data</td>
</tr>
<tr>
<td>Imatinib</td>
<td>Platelet-derived growth factor receptor</td>
<td>Anti-GBM, lupus nephritis, anti-Thy 1.1 GN</td>
<td>Case reports of off-label use in membranoproliferative GN and cryoglobulinemia</td>
</tr>
<tr>
<td>Tofacitinib</td>
<td>Janus kinase</td>
<td>Lupus nephritis</td>
<td>Phase 1 clinical trial in SLE</td>
</tr>
</tbody>
</table>

**CONCLUSION**

Targeting the tyrosine kinase signalling pathways represents a novel therapeutic target for the treatment of immune-mediated GN. Nonetheless, there is a persistent and even growing gap between advances in basic research and the development of clinical
trials in GN research. Collaborations between scientists and clinicians are needed to address the current unmet medical needs and provide potential solutions to speed up translation into clinical practice and implementation of biomedical science advances.

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**CONFLICT OF INTEREST STATEMENT**

F.W.K.T. is the chief investigator of the randomized controlled trial of Syk inhibitor in IgAN. He has received research project grants from AstraZeneca, Baxter Biosciences, GlaxoSmithKline, MedImmune and Roche Palo Alto and has consultancy agreements with MedImmune and Rigel Pharmaceuticals.

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