Imatinib: Novel Treatment of Immune-Mediated Kidney Injury

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ABSTRACT
The treatments for many autoimmune diseases are limited in efficacy, and long-term use is associated with severe adverse events. The tyrosine kinase inhibitors have proven to be well tolerated for long treatment periods, with minimal adverse events, in the oncology population. These agents have recently been used to treat autoimmune diseases. We review the potential mechanisms whereby tyrosine kinase inhibitors may modulate the immune response and inhibit fibrogenesis and discuss the current evidence for their use in the treatment of autoimmune diseases of the kidney.


According to 2011 data from the U.S. Renal Data System, GN caused ESRD in 10% of prevalent dialysis patients and 6% of incident patients. Almost 10,000 incident dialysis patients from 2005 to 2009 carried the histologic diagnosis of membranous nephropathy, membranoproliferative GN type I, or lupus nephritis (LN). In these inflammatory diseases, B cell–mediated antibody production, T cell proinflammatory cytokine production, macrophages, and dendritic cells all play a role in the pathogenesis of renal injury.2,3 Inflammation plays an important physiologic role in response to injury, but prolonged infiltration of lymphocytes, macrophages, and dendritic cells also leads to fibrosis through increased generation of reactive oxygen species and production of profibrotic cytokines and growth factors. Many of the drugs currently used to treat autoimmune diseases of the kidney were originally developed as antineoplastic agents; they have suboptimal efficacy, and toxic adverse effects limit their use. A major advance in oncology has been the advent of tyrosine kinase inhibitors (TKIs). This class of medication is relatively well tolerated and has immunomodulating and antifibrotic properties that may render it a valuable tool in the treatment of autoimmune diseases of the kidney.

TKIs
The TKIs are widely used clinically for the treatment of such malignancies as chronic myelogenous leukemia (CML), gastrointestinal stromal tumors (GISTs), and renal cell carcinoma. TKIs target protein tyrosine kinases (PTKs), which exist as transmembrane receptors or as intracellular nonreceptor PTKs. Fifty-eight receptor PTKs and 32 cytoplasmic PTKs exist, and they differentially modulate important cellular effects.4 Most TKIs act through competitive inhibition of the ATP binding site of the PTKs, thus blocking autophosphorylation and subsequent intracellular signal transduction, and their effects vary with type of PTK inhibition.5

Imatinib was the first TKI to be approved by the U.S. Food and Drug Administration for treatment of CML.6 Imatinib blocks nonreceptor Abelson tyrosine kinase (c-abl) that is constitutively active in CML because of the BCR-ABL fusion oncogene. Imatinib’s kinase inhibition is not specific for c-abl; it also blocks platelet-derived growth factor receptor (PDGFR), stem cell growth factor receptor (c-kit),7 discoid domain receptor (DDR) 1 and 2,8 macrophage colony-stimulating factor receptor (c-fms),9 and lymphocyte-associated kinase (lck).10 Nilotinib is a TKI that inhibits the same PTK repertoire as imatinib but has an increased potency for c-abl inhibition. Although most of this review focuses on imatinib, nilotinib should exert similar effects.

Imatinib was originally designed for the treatment of CML, but its inhibitory effects on c-abl, c-kit, DDR, c-fms, and lck render it a potent immunomodulatory agent. Imatinib may also have antifibrotic properties both by modulating inflammation and by inhibiting PDGFR, DDR, and c-abl, a downstream mediator of TGF-β–dependent matrix production.11 Imatinib’s relatively safe adverse effect profile, coupled with its immune-modulating and antifibrotic effects, serves...
as a rationale for investigating this TKI as a potential therapeutic option for immune-mediated kidney diseases. Despite promising results with the use of imatinib in murine models of kidney disease, only two case reports have described its use in human kidney disease. This review summarizes the molecular mechanisms whereby imatinib alters the immune and fibrotic responses to injury, discusses the use of imatinib in animal models of renal injury, and reports the clinical data using imatinib to treat immune-mediated diseases of the kidney.

IMMUNOMODULATION BY IMATINIB

Imatinib has various immune modulating properties on B cells, T cells, dendritic cells, and macrophages, all of which play a role in the pathogenesis of chronic immune-mediated kidney disease. Through inhibition of c-kit, lck, c-abl, and c-fms, imatinib affects development of immune cell progenitors, immune cell activation, proliferation, and function (Figure 1). We describe the cell-specific effects of imatinib on key components of the immune system.

B Cells
Dysregulated antibody production by B cell lymphocytes is important in the pathogenesis of autoimmune diseases of the kidney. c-kit is important for B cell lymphopoiesis, as shown by c-kit-deficient mice, which have a significantly reduced number of pro–B cells as adults. This effect is probably mediated through dysregulated antibody production by B cells as adults.15 Inhibition of c-kit and c-abl may compromise normal B cell development in adults. c-abl not only modulates B cell development but may also affect B cell activation. Consistent with this notion, B cells in c-abl null and mutant mice have reduced proliferation and activation in response to ligation of the B cell receptor (BCR). Also, treatment of B cells from wild-type mice with imatinib inhibits the IgM-stimulated proliferation observed in untreated B cells.20 C-abl promotes B cell activation by direct interaction with CD19, a transmembrane glycoprotein on the surface of immature and mature B cells, which acts as a co-receptor for BCR-mediated activation of B cells. Mice that underexpress or overexpress CD19 are hypoproliferative or hyperproliferative, respectively, in response to antigen.21 CD19 colocalizes with c-abl in vivo and is directly phosphorylated by c-abl in vitro. In addition, evidence suggests that c-abl plays a role in CD19-mediated B cell activation. C-abl null B cells stimulated by CD19 ligation show decreased release of intracellular calcium, a marker of B cell activation.22 C-abl also promotes B cell activation and proliferation through phosphorylation of Bruton tyrosine kinase, an important mediator of BCR’s downstream signaling. Although c-abl can phosphorylate Bruton tyrosine kinase in vitro, further studies are needed to determine its physiologic importance.

Additional clinical data support imatinib’s role as an inhibitor of B cell function, thus validating the preceding studies in murine models. Immunoprofiling of patients receiving imatinib for the treatment of CML and GIST reveals that imatinib
reduces levels of IgG, IgA, and IgM without an associated decrease in peripheral lymphocyte counts.24,25 Imatinib reduced immunoglobulin levels in 69 of 72 patients with CML and 7 of 15 patients with GISTs, and immunoglobulin levels did not decrease in 20 patients with CML not treated with imatinib.26 Furthermore, 21 of 30 patients with CML receiving treatment with imatinib demonstrate an abnormal plasma cell phenotype that correlates with decreased γ-globulin levels.27 In summary, animal models suggest that imatinib affects the B cell immune response through decreased B cell development and function, and clinical studies suggest that imatinib dampens B cell production of antibodies.

**T Cells**

T cells can mediate renal injury in autoimmune disease by direct tissue injury and through secretion of cytokines that propagate inflammation.2 Imatinib inhibits T cell development, activation, and proliferation through its inhibition of c-kit, c-abl, and lck. Signaling of c-kit is required for T cell lymphopoiesis, as illustrated by the adult c-kit null mice, which have a marked reduction in thymic T cell progenitor cells.15 In addition, the abl/m1 mouse, which contains a mutated src kinase docking site on c-abl, has a reduction in peripheral mature T cells as defined by single positivity for CD4 or CD8. The abl/m1 mouse also has diminished T cell progenitor cells, and a similar phenotype is induced by imatinib treatment of wild-type adult mice.16

Imatinib also inhibits the activation and proliferation of healthy human donor peripheral T cells in response to stimulus. One possible mechanism is through inhibition of lck, a PTK necessary for activation and proliferation of T cells.29 Engagement of the T cell receptor activates lck, which then phosphorylates the intracytoplasmic portion of the T cell receptor; this, in turn, leads to downstream signaling events required for T cell activation and proliferation. Among the downstream protein phosphorylation events inhibited by imatinib is linker for activation of T cells, whose deficiency causes profound T cell developmental defects.31 T cells secrete proinflammatory cytokines, such as IL-2, IFN-γ, and TNF-α, all of which have been implicated in autoimmune disease. IL-2 secretion has been used as a surrogate measure for downstream T cell receptor signaling, and imatinib inhibits this IL-2 secretion.10 Imatinib has also been shown to inhibit production of TNF-α and of IFN-γ in T cells from both healthy donors and patients being treated for CML.32,33 In summary, imatinib inhibits the T cell response by reducing lymphopoiesis through blockade of c-kit and c-abl and modulation of T cell function through lck, resulting in decreased activation, proliferation, and secretion of proinflammatory cytokines.

**MONOCYTES, MACROPHAGES, AND DENDRITIC CELLS**

Antigen-presenting cells, such as macrophages, monocytes, and dendritic cells, are an important component of the inflammatory response present in multiple models of kidney disease.3,34 Macrophages propagate kidney injury through secretion of inflammatory cytokines, production of profibrotic growth factors, and generation of reactive oxygen species.35–37 Macrophage depletion attenuated fibrosis in a unilateral ureteral obstruction (UUO) model of injury, suggesting that macrophage infiltration may be deleterious.38 Imatinib-treated monocyte/macrophages in vitro had reduced proliferation in response to macrophage colony stimulating factor.9 This finding was attributed to imatinib’s inhibitory effect on the phosphorylation of c-fms, the receptor for macrophage colony-stimulating factor.9 Inhibiting c-fms with a monoclonal antibody in mice reduces macrophage accumulation and proliferation in both the UUO and db/db diabetic models of injury.39,40 Furthermore, the diabetic db/db mice treated with anti-c-fms have reduced tubular apoptosis and fibrosis, suggesting that macrophages are integral to the pathophysiology of this injury model.

Imatinib also impairs the function of monocytes that differentiate into macrophages upon movement from the bloodstream to tissue. Human monocytes treated with imatinib in vitro had reduced phagocytosis, impaired formation of pseudopodia, and reduced production of proinflammatory cytokines (IL-6, TNF-α) in response to lipopolysaccharide.41 Thus, imatinib treatment potentially modulates kidney injury through inhibition of monocyte-macrophage proliferation and function.

Dendritic cells, antigen-presenting cells that modulate T cell function, are also implicated in several murine models of GN.42 Imatinib treatment inhibits the generation of dendritic cells from their CD34+ progenitors in vitro.43 Similarly, administration of imatinib to mice attenuates the expansion of dendritic cells in vivo induced by Flt3L, a dendritic cell growth factor.44 Furthermore, imatinib-treated dendritic cells are unable to induce primary T cell responses, suggesting that imatinib suppresses both dendritic cell expansion and function.43

**ANTIFIBROTIC EFFECTS OF IMATINIB**

Progressive fibrosis characterized by extracellular matrix (ECM) accumulation is the common pathway from injury of any cause to ESRD.45 Imatinib not only has the potential to reduce fibrosis indirectly through decreased inflammation but also may directly inhibit fibrogenesis by modulating TGF-β, PDGFR, and DDR signaling (Figure 2).

**TGF-β Signaling**

TGF-β is a pleiotropic growth factor with strong profibrotic effects in models of renal injury.46 TGF-β probably promotes fibrosis by stimulating fibroblast proliferation, activation, and production of ECM components as well as by inhibiting ECM degradation. TGF-β ligands bind to serine/threonine kinase receptors that signal through a canonical Smad-dependent pathway. However, many Smad-independent pathways contribute to TGF-β–mediated fibrosis, and c-abl
Fibrogenic pathways targeted by imatinib. TGF-β inhibition of c-abl inhibits a non–Smad-dependent TGF-β pathway and reduces fibrosis. This is a cell specific for mesenchymal cells. PI3K, phosphoinositide 3 kinase; PAK, p21 activated kinase. Imatinib inhibits PDGFR, thereby reducing ECM production and fibroblast proliferation DDR is the first tyrosine kinase to be shown to bind ECM. Inhibition of DDR through imatinib may result in decreased inflammation and ECM production, as demonstrated in a DDR null mouse.

**PDGFR**

PDGF, a known mitogen for mesenchymal cells, can stimulate ECM production in mesenchymal and parietal epithelial cells in vitro. The four isoforms of PDGF (A, B, C, and D) signal through the tyrosine kinase receptor PDGFR, which exists as a dimer composed of α and β chains. PDGF and its receptors are upregulated in numerous murine injury models and in human kidney disease. Inhibition of PDGF signaling through neutralizing antibodies, oligonucleotides, and chemical antagonists can reduce mesenchymal cell proliferation and ECM accumulation. Imatinib also blocks PDGF signaling and inhibits PDGF-induced mesangial proliferation in vitro. In addition, a murine model of diabetes (streptozotocin-treated apoE knockout mice) showed attenuated collagen type I and IV production when given imatinib, a finding associated with reduced expression of PDGF, PDGFR and TGF-β1. Thus, imatinib’s inhibition of PDGFR signaling is another potential mechanism whereby imatinib reduces fibrosis.

**DDR**

There are two discoidin domain receptors, DDR1 and DDR2, both of which have tyrosine kinase activity and bind collagens. In the kidney, DDRs are widely expressed in cells such as epithelia, fibroblasts, vascular smooth muscle cells, and mesangial cells. DDRs are potent mediators of inflammation and fibrosis, and DDR1 null mice have reduced collagen accumulation and infiltrating inflammatory cells in both antilgiontin II–induced renal injury and the UUO model of fibrosis. DDR1 null mice are also protected from lipopolysaccharide-induced shock, confirming its pivotal role as a mediator of inflammation. Imatinib inhibits DDRs in vitro, and this effect probably plays a role in imatinib’s attenuation of fibrosis and inflammation observed in murine models of kidney disease.

**IMATINIB IN MURINE MODELS OF KIDNEY DISEASE**

Through all of the preceding mechanisms, imatinib is a potential therapeutic agent for autoimmune mediated kidney disease. Imatinib has been investigated in various murine models of kidney disease and has been shown to improve both immunologic and fibrotic measures (Table 1).

Imatinib’s inhibitory effect on B cells was illustrated in two models of murine LN and a mouse model of cryoglobulinemia. In the two LN models, imatinib reduced anti–double-stranded DNA levels and decreased immune complex deposition. This resulted in improved survival, creatinine, and proteinuria. Cryoglobulinemia is another autoimmune disease caused by pathologic antibody secretion. A transgenic mouse model with constitutively active thymic stromal lymphopoietin resulted in cryoglobulin production and renal histologic lesions consistent with membranoproliferative GN caused by cryoglobulinemia. Imatinib treatment in this mouse model reduces the cryocrit, serum levels of all immunoglobulins, mature and immature B cells, systemic manifestations of the disease, and C3 glomerular deposition. To highlight the immunomodulatory role of imatinib, the same thymic stromal lymphopoietin g mouse model was treated with angiotensin-converting enzyme inhibition or angiotensin-receptor blocker therapy alone and showed no improvement in markers of immunologic activation. Imatinib also inhibited T cell infiltration in mouse models of anti–glomerular basement membrane (GBM) disease and chronic allograft nephropathy. Macrophage infiltration was decreased in models of chronic allograft nephropathy, streptozotocin-induced diabetic nephropathy, LN, and anti-GBM disease treated with imatinib whereas nilotinib...
Table 1. Murine models of kidney disease treated with imatinib/nilotinib

<table>
<thead>
<tr>
<th>Model of Kidney Disease</th>
<th>Intervention</th>
<th>Immune Effect</th>
<th>Effect on Fibrosis</th>
<th>Other Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti Thy 1.1 GN</td>
<td>Imatinib</td>
<td>↓T cell and macrophage infiltration</td>
<td>↓Type IV collagen αSMA</td>
<td>↓Mesangial cell activation and proliferation</td>
<td>49</td>
</tr>
<tr>
<td>Dark Agouti to Wistar Furth kidney transplant: CAN</td>
<td>Imatinib</td>
<td>Short-term and long-term administration of imatinib</td>
<td>↓CADI score TGF-β and TGF-β receptor PDGF-A and -B PDGFR-α and -β</td>
<td>↓Creatinine</td>
<td>58,60,61</td>
</tr>
<tr>
<td>ApoE knockout/ Streptozotocin: diabetes</td>
<td>Imatinib</td>
<td>↓Macrophage infiltration</td>
<td>↓Tubulointerstitial fibrosis ECM αSMA</td>
<td>↓Albuminuria</td>
<td>50</td>
</tr>
<tr>
<td>TSLP transgenic mouse: cryoglobulinemia</td>
<td>Imatinib</td>
<td>↑Macrophage infiltration</td>
<td>↓Glomerular sclerosis Collagen type I and IV, PAI1, fibronectin, PDGF-B, TGF-β αSMA</td>
<td>↓Albuminuria</td>
<td>55</td>
</tr>
<tr>
<td>5/6 nephrectomized rats: CKD</td>
<td>Nilotinib</td>
<td>↓Macrophage infiltration IL-6, IFN-γ, IL-1β, TNF-α, and MCP-1</td>
<td>↓Glomerular sclerosis Collagen type I and IV, PAI1, fibronectin, PDGF-B, TGF-β αSMA</td>
<td>↓Creatinine</td>
<td>59</td>
</tr>
<tr>
<td>Unilateral ureteral obstruction: renal fibrosis</td>
<td>Imatinib</td>
<td>No decrease in macrophage infiltration</td>
<td>↓Fibroblast proliferation αSMA ECM No decrease in PDGF-B</td>
<td>↓Proteinuria</td>
<td>11</td>
</tr>
<tr>
<td>NZ black/white mouse: LN</td>
<td>Imatinib</td>
<td>Slightly diminished anti-double-stranded DNA IC deposition ↓Monocyte/macrophage infiltration</td>
<td>↓Interstitial fibrosis αSMA TGF-β</td>
<td>↓Survival</td>
<td>53</td>
</tr>
<tr>
<td>MRL/lpr mouse: LN</td>
<td>Imatinib</td>
<td>↓Inflammatory cell infiltration IgG and anti–double-stranded DNA IFN-γ IgG deposits</td>
<td>↓PDGFR-β, TGF-β</td>
<td>↓Survival</td>
<td>54</td>
</tr>
<tr>
<td>Wistar-Kyoto rats: Anti-GBM</td>
<td>Imatinib</td>
<td>↓CD8+ but not CD3, CD4 Crescent and fibrinoid necrosis Macrophage infiltration Proinflammatory cytokines c-fms No difference in IgG deposition or levels</td>
<td>↓PDGFR-β</td>
<td>↓Proteinuria</td>
<td>57</td>
</tr>
</tbody>
</table>

CAN, chronic allograft nephropathy; CADI, chronic allograft damage index; apoE, apolipoprotein E; SMA, smooth muscle actin; TSLP, thymic stromal lymphopoietin; NZ, New Zealand; IC, immune complex; MCP, monocyte chemotactic protein; PAI1, plasminogen activator inhibitor 1.
decreased macrophage infiltration in the 5/6 nephrectomy model.50,53,54,57–59 Inflammatory cytokines were decreased by imatinib treatment in the models of anti-GBM and LN and by nilotinib treatment in the 5/6 nephrectomy model.54,57,59 These findings provide further evidence for the immunomodulatory properties of imatinib and nilotinib.

Imatinib also markedly reduced ECM deposition and fibrosis in most of these murine models of kidney disease.11,49,50,53,55,58–61 PDGF signaling was disrupted by imatinib, as shown by decreased levels of PDGF-A,58 PDGF-B,50,54,58,59, and PDGFR-β.50,54,57,58 Imatinib also decreased levels of TGF-β50,53,54,58,59 and TGF-β type I receptor58 as well. Therefore, imatinib reduces indices of fibrosis in murine injury models with associated suppression of TGF-β and PDGF signaling, suggesting that imatinib may have beneficial antifibrotic effects in addition to its immunomodulatory properties.

**HUMAN AUTOIMMUNE DISEASES TREATED WITH IMATINIB**

Few clinical trials have used imatinib to treat autoimmune diseases. Anecdotally, there has been some clinical success. Case reports document that patients receiving imatinib for CML noted significant improvement in Crohn disease for one patient62 and in severe rheumatoid arthritis for three patients.63 Imatinib improved cutaneous involvement in three cases of systemic sclerosis and has subsequently been shown to improve skin thickness scores and pulmonary function test results in a larger trial of 30 patients with scleroderma.64,65 Imatinib treatment improved skin thickening and tethering for two patients with nephrogenic sclerosing fibrosis.66 Notably, imatinib did not prove effective in the treatment of idiopathic pulmonary fibrosis.67

As for kidney disease, a patient with biopsy-proven membranoproliferative GN had improvement in proteinuria and creatinine after initiation of imatinib for concurrent CML.14 Imatinib treatment also resulted in a dramatic improvement in a patient with idiopathic type II cryoglobulinemia with kidney involvement. The patient’s creatinine level, symptoms, and cryocrit improved upon starting imatinib, worsened upon withdrawal of therapy, and dramatically improved with reinstitution of therapy.13

Table 2 lists ongoing and completed trials using imatinib or nilotinib for fibrotic diseases and rheumatoid arthritis.

**CONCLUSIONS**

Imatinib and nilotinib have improved both fibrotic and inflammatory markers of many murine models of kidney disease and, anecdotally, two clinical cases of immune-mediated kidney disease. As discussed here, the putative mechanism whereby TKI may be effective therapeutically for autoimmune renal disease involves the inhibition of their many PTK targets: c-abl, c-kit, Ick, c-fms, PDGFR, and DDR. Blockade of these targets may inhibit the immune response and suppress fibrosis, two vital effects for halting the progression of autoimmune kidney disease.

Furthermore, imatinib’s mild adverse effect profile and long-term safety record, particularly in comparison with those of current treatment options for these diseases, make this drug a potentially attractive alternative pending further studies conducted specifically in patients with autoimmune diseases. Diseases likely to benefit most from this intervention would be those necessitating chronic suppression of antibody production, such as severe membranous nephropathy, systemic lupus erythematosus, chronic humoral rejection after renal transplantation, and cryoglobulinemic vasculitis. Given the current limitations of therapies for immune-mediated kidney diseases, clinical trials are desperately needed to determine whether imatinib provides a safer and more efficacious option.

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Table 2. Current studies with imatinib/nilotinib/dasatinib in human autoimmune and fibrotic diseases

<table>
<thead>
<tr>
<th>Study</th>
<th>Investigator/Reference</th>
<th>Patient Population</th>
<th>Drug</th>
<th>Patients (n)</th>
<th>Completion Date</th>
<th>Endpoint</th>
<th>Results</th>
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<td>NCT00555581</td>
<td>Spiera et al.65</td>
<td>Systemic sclerosis</td>
<td>Imatinib</td>
<td>30</td>
<td>1/2011</td>
<td>MRSS PFT results</td>
<td>Improved MRSS and PFT results</td>
</tr>
<tr>
<td>NCT01166139</td>
<td>Spiera et al.65</td>
<td>Systemic sclerosis</td>
<td>Nilotinib</td>
<td>10</td>
<td>Recruiting</td>
<td>MRSS PFT results</td>
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<td>NCT00154336</td>
<td>Novartis</td>
<td>Rheumatoid arthritis</td>
<td>Imatinib Methotrexate</td>
<td>54</td>
<td>Completed</td>
<td>ACR20</td>
<td>Not publicly available</td>
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<tr>
<td>NCT00131274</td>
<td>Daniels et al.67</td>
<td>Idiopathic pulmonary fibrosis</td>
<td>Imatinib</td>
<td>119</td>
<td>8/2007</td>
<td>Time to 10% decline in FVC and time to death</td>
<td>No improvement</td>
</tr>
</tbody>
</table>

MRSS, Modified Rodnan Skin Score; PFT, pulmonary function test; ACR20, 20% improvement in symptoms of rheumatoid arthritis per American College of Rheumatology criteria; FVC, forced vital capacity.