Therapeutic Inhibition of VEGF Signaling and Associated Nephrotoxicities

Chelsea C. Estrada, Alejandro Maldonado, and Sandeep K. Mallipattu

Division of Nephrology, Department of Medicine, Stony Brook University, Stony Brook, New York; and Renal Section, Northport Veterans Affairs Medical Center, Northport, New York

Inhibition of vascular endothelial growth factor A (VEGFA)/vascular endothelial growth factor receptor 2 (VEGFR2) signaling is a common therapeutic strategy in oncology, with new drugs continuously in development. In this review, we consider the experimental and clinical evidence behind the diverse nephrotoxicities associated with the inhibition of this pathway. We also review the renal effects of VEGF inhibition’s mediation of key downstream signaling pathways, specifically MAPK/ERK1/2, endothelial nitric oxide synthase, and mammalian target of rapamycin (mTOR). Direct VEGFA inhibition via antibody binding or VEGF trap (a soluble decoy receptor) is associated with renal-specific thrombotic microangiopathy (TMA). Reports also indicate that tyrosine kinase inhibition of the VEGF receptors is preferentially associated with glomerulopathies such as minimal change disease and FSGS. Inhibition of the downstream pathway RAF/MAPK/ERK has largely been associated with tubulointerstitial injury. Inhibition of mTOR is most commonly associated with albuminuria and podocyte injury, but has also been linked to renal-specific TMA. In all, we review the experimentally validated mechanisms by which VEGFA-VEGFR2 inhibitors contribute to nephrotoxicity, as well as the wide range of clinical manifestations that have been reported with their use. We also highlight potential avenues for future research to elucidate mechanisms for minimizing nephrotoxicity while maintaining therapeutic efficacy.


Inhibitors of vascular development were first investigated as potential chemotherapeutic agents on the basis of study findings from the late 20th century regarding the critical role of angiogenesis in tumor development and growth. Subsequent investigations led to the discovery of vascular endothelial growth factor A (VEGFA), an essential growth factor for angiogenesis. Previous studies by Kim et al. used murine cancer cell lines to show that treatment with an mAb against VEGFA decreased tumor growth, indicating a potential therapeutic role for VEGFA inhibition in cancer treatment. Beginning with the development of bevacizumab, a recombinant IgG mAb against VEGFA, inhibiting VEGF signaling proved to be a promising approach to halting neoplastic development. Since then, many additional agents that block VEGF signaling and its downstream pathways have become available for the treatment of various cancers (Figure 1, i–vi).

Although these agents offer substantial benefit, both in the treatment of many solid tumors as well as age-related macular degeneration, their use is associated with significant nephrotoxicity. Most commonly, pharmacologic VEGF inhibition has been associated with hypertension and proteinuria. Reports describe histologic changes in the kidney primarily as glomerular endothelial injury with thrombotic microangiopathy (TMA). Nephrotic syndrome has also been observed, with the clinical manifestations varying according to mechanism and direct target of VEGF inhibition.

Current VEGF inhibitors can be classified by their target of action in the VEGFA-VEGFR2 pathway: drugs that bind to VEGFA, sequester VEGFA, inhibit receptor tyrosine kinases (RTKs), or inhibit downstream pathways. A critical review of VEGFA-VEGFR2 signaling in the kidney is necessary to fully understand the mechanisms responsible for the nephrotoxicity associated with the oncological use of VEGF inhibition.

VEGF SIGNALING IN THE KIDNEY

Filtration of plasma in nephrons occurs in the glomerular capillary beds at the glomerular filtration barrier, which consists of three layers: fenestrated endothelial cells, basement membrane, and the
foot processes of visceral epithelial cells or podocytes. In the kidney, VEGFA is expressed by both podocytes and binds to its receptor (VEGFR2) on glomerular endothelial cells. (i) Bevacizumab and ranibizumab are mAbs against VEGFA and inhibit angiogenesis through IgG antibody interaction with all of its isoforms. (ii) Aflibercept is a recombinant fusion protein comprising binding domains for VEGFR1 and VEGFR2 attached to the Fc portion of human IgG1, and acts as a soluble decoy receptor or “VEGF trap.” (iii) Ramucirumab is a fully humanized IgG1 mAb that specifically inhibits VEGFR2 by targeting its extracellular domain. (iv) TKIs such as sunitinib, pazopanib, sorafenib, and axitinib target VEGFR2, as well as interfere with the activity of additional RTKs such as PDGF receptor, fibroblast growth factor receptor, and EGF receptor, which all share a similar structure. (v) Agents such as vemurafenib and dabrafenib have been recently developed to specifically target B-Raf, a component of the intracellular MAPK/ERK intracellular pathway. (vi) mTOR inhibitors such as temsirolimus, riocorinlimus, and everolimus are used across several malignancies and act downstream of the phosphatidylinositol-3 kinase (PI3K)/AKT signal transduction pathway. Ab, antibody; BRAF, B-Raf Proto-Oncogene; EF, endothelial fenestrations; FP, foot process; GBM, glomerular basement membrane; GEnC, glomerular endothelial cell; P, Podocyte.

Figure 1. VEGFA-VEGFR2 signaling pathways and their pharmacological inhibition occur across the glomerular filtration barrier. VEGFA is released from podocytes and binds to its receptor (VEGFR2) on glomerular endothelial cells. (i) Bevacizumab and ranibizumab are mAbs against VEGFA and inhibit angiogenesis through IgG antibody interaction with all of its isoforms. (ii) Aflibercept is a recombinant fusion protein comprising binding domains for VEGFR1 and VEGFR2 attached to the Fc portion of human IgG1, and acts as a soluble decoy receptor or “VEGF trap.” (iii) Ramucirumab is a fully humanized IgG1 mAb that specifically inhibits VEGFR2 by targeting its extracellular domain. (iv) TKIs such as sunitinib, pazopanib, sorafenib, and axitinib target VEGFR2, as well as interfere with the activity of additional RTKs such as PDGF receptor, fibroblast growth factor receptor, and EGF receptor, which all share a similar structure. (v) Agents such as vemurafenib and dabrafenib have been recently developed to specifically target B-Raf, a component of the intracellular MAPK/ERK intracellular pathway. (vi) mTOR inhibitors such as temsirolimus, riocorinlimus, and everolimus are used across several malignancies and act downstream of the phosphatidylinositol-3 kinase (PI3K)/AKT signal transduction pathway. Ab, antibody; BRAF, B-Raf Proto-Oncogene; EF, endothelial fenestrations; FP, foot process; GBM, glomerular basement membrane; GEnC, glomerular endothelial cell; P, Podocyte.

The functional diversity of VEGF receptors was initially elucidated by the creation of knockout mice. Mice deficient in Vegfr2 die in utero from a defect in hematopoietic and endothelial cell development; embryonic lethality of Vegfr1 deletion is caused by endothelial cell overgrowth and disorganization. These whole-body knockout mice underscore the key role of VEGF signaling in endothelial cell proliferation, migration, and permeability.

The association of VEGFA overexpression or reduction with a wide range of glomerulopathies (Table 1) demonstrates that tight regulation of VEGFA signaling in the kidney is critical to glomerular development and the maintenance of mature glomerular function in both homeostasis and disease. For example, knockout of Vegfa during embryogenesis—including global homozygous or heterozygous knockout or podocyte-specific knockout—is uniformly lethal at or before birth. Mice with podocyte-specific partial deletion of Vegfa survive the perinatal period, but develop endotheliosis and renal failure by 9 weeks of age.

In adult mice, inducible podocyte-specific Vegfa deletion produces renal-specific TMA, which recapitulates kidney biopsy...
findings in individuals treated with VEGF inhibitors. In contrast, mice with tubule-specific deletion of Vegfa had histologically normal kidneys with some peritubular capillary density loss, emphasizing the essential role of podocyte-derived Vegfa. In studies of knockout mice, Sison et al. highlighted the importance of paracrine VEGF-VEGFR2 signaling between the podocyte and endothelial cell, showing that mice with podocyte-specific deletion of Vegfr2 did not develop glomerulosclerosis, but those with whole-body inducible deletion of Vegfr2 developed TMA, resembling mice lacking podocyte-specific Vegfa.

Although these experiments deemphasized autocrine VEGFA-VEGFR2 signaling in the podocyte, other studies have suggested the contrary. In cultured mouse podocytes, VEGF treatment reduced apoptosis as well as upregulated podocin protein expression. These results, suggesting podocyte autocrine dependence on secreted VEGF, were corroborated in cultured human podocytes treated with the BRAF inhibitor dabrafenib and the MEK1 inhibitor trametinib, which strongly inhibited VEGF release and simultaneously increased albumin permeability. Furthermore, biopsy specimens of patients treated with this combination therapy exhibited severe podocyte injury and effacement.

Interestingly, in addition to developing renal-specific TMA, mice with inducible podocyte-specific Vegfa deletion exhibited reduced glomerular complement factor H (CFH) staining and increased glomerular C3 deposition. The dependence of the expression of the complement regulatory protein CFH on VEGFA was also shown in cultured glomerular endothelial cells, where exogenous VEGF directly increased CFH expression. This relationship was not seen in other endothelial cell lines, perhaps explaining the sensitivity of glomerular endothelial cells to alterations in VEGF-VEGFR2 signaling.

Increased VEGF-VEGFR2 signaling also appears to have detrimental glomerular effects. Constitutive overexpression of Vegfa164 in podocytes leads to collapsing glomerulopathy, whereas its inducible overexpression results in glomerulomegaly with mesangial expansion. Taken together, these findings suggest that maintenance of glomerular endothelial integrity relies heavily on tight regulation of paracrine VEGF-VEGFR2 signaling between the podocyte and renal endothelium, and that administration of antiangiogenic therapeutics to disrupt this signaling and its downstream pathways directly results in renal endothelial injury, manifested primarily as proteinuria, hypertension, and renal-specific TMA. However, it should be noted that histologic diagnosis of such injury is often limited because of the underutilization of kidney biopsies. In addition,
these manifestations occur with variable onset after therapy initiation, are not dose-related, and are often reversible.

**THERAPEUTIC VEGF INHIBITION**

Classes of VEGF inhibitors act through different mechanisms to cause endothelial and glomerular injury. In the following section, we review the experimentally validated mechanisms by which VEGFA-VEGFR2 inhibitors contribute to nephrotoxicity.

**ANTI-VEGF MAB**

Pharmacologic agents that inhibit VEGFA activity through antibody binding include bevacizumab, ranibizumab, aflibercept, and ramucirumab. Bevacizumab and ranibizumab are mAbs against VEGFA and inhibit angiogenesis through IgG antibody interaction with all of its isoforms (Figure 1i). Aflibercept, a recombinant fusion protein comprising binding domains for VEGFR1 and VEGFR2 attached to the Fc portion of human IgG1, acts as a soluble decoy receptor, or VEGF trap (Figure 1ii). Ramucirumab, a fully humanized IgG1 mAb, specifically inhibits VEGFR2 by targeting its extracellular domain (Figure 1ii). Initial models of the first-in-class agent bevacizumab measured efficacy by reduced tumor growth using human breast cancer cell lines implanted into nude mice, and the adverse outcomes of proteinuria and hypertension were not observed until phase 1 trials. In murine models of direct VEGF inhibition in wild-type mice (Table 2), a single intravenous dose of anti-VEGF antibody produced significant albuminuria after 3 hours, as well as glomerular endothelial cell hypertrophy and disruption of glomerular basement membrane, seen by electron microscopy. In validation of genetic knockout studies, mice given antibody directed at recombinant VEGF during the neonatal period (on days 0, 2, 4, and 5) displayed impaired glomerulogenesis, with poor cellularity and increased extracellular matrix deposition, thereby confirming the essential role of VEGF signaling in glomerular development. Neither model showed lesions of TMA by histology, likely reflecting the short duration of treatment; however, the significant glomerular injury seen in both models underscores the importance of renal VEGF signaling during development as well as in homeostasis.

Findings from animal models demonstrate the importance of VEGF signaling during renal injury. In a rat model of crescentic GN, direct VEGF inhibition via an intramuscular injection of the soluble receptor for VEGFR1 (sFlt-1 plasmid), administered 3 days before injection of nephrotoxic serum, exacerbated crescentic formation and albuminuria. Similarly, subcutaneous injection of dRK6, which binds to VEGFA, worsened albuminuria and podocyte injury in db/db diabetic mice. Both studies demonstrated the importance of VEGF signaling in maintaining glomerular integrity in disease as well as in homeostasis.

The nephrotoxicities associated with direct VEGF inhibition in patients receiving chemotherapy is primarily derived from clinical trial data (Table 3). In a meta-analysis of 20 phase 2 and phase 3 clinical trials involving bevacizumab-based therapy in solid tumors, the incidence of all-grade hypertension was 23.6% and high-grade (grade 3 or 4) hypertension was 7.9%. Similarly, a meta-analysis of seven randomized, controlled trials (RCTs) found the incidence of proteinuria and antibody-mediated rejection in kidney transplant patients after intravitreal bevacizumab, ranibizumab, and aflibercept. In all, across multiple animal models, as well as in clinical trials, direct and systemic VEGF inhibition, via antibody binding or VEGF trap, results in hypertension, proteinuria, and a spectrum of glomerular endothelial injury.

**Tyrosine Kinase Inhibitors of VEGF Signaling**

Tyrosine kinase inhibitors (TKIs) inhibit RTKs, which consist of an extracellular binding domain, a transmembrane region, and an intracellular kinase that mediates signal transduction. TKIs interfere with the activity of one or more families of RTKs, including VEGFR, PDGF receptor (PDGFR), fibroblast growth factor receptor, and EGF receptor, which all share a similar structure. TKIs are thus commonly
### Table 2. Renal manifestations from pharmacologic VEGF inhibition in murine models

<table>
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<tr>
<th>Animal Model</th>
<th>Drug</th>
<th>Mechanism</th>
<th>Target</th>
<th>Dose, Route and Frequency</th>
<th>Effects</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>Wild-type mice, CD1, age not specified</td>
<td>Anti-VEGF antibody and mouse sFlt-1/Fc fusion protein (soluble VEGFR1)</td>
<td>Both treatments act to reduce circulating VEGF</td>
<td>VEGFA</td>
<td>3.25 and 32.5 pM, IV, single dose</td>
<td>Both treatments induced proteinuria by 3 h after administration, which resolved after 24 h. Both treatments resulted in GEnC hypertrophy and detachment from the basement membrane, starting at 3 h post-treatment and persisting to 24 h</td>
<td>17</td>
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<tr>
<td>Wild-type mice, strain not specified, neonatal</td>
<td>Antibody to recombinant human VEGF_165</td>
<td>Reduction of circulating VEGFA</td>
<td>VEGFA</td>
<td>100 μl/dose, IP, single dose on days 0, 2, 4, and 5 after birth</td>
<td>Decreased glomerular number and formation of abnormal glomeruli with poor cellularity and increased ECM. No significant changes in any nonglomerular vessels</td>
<td>21</td>
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<tr>
<td>Wistar Kyoto rats treated with antiglomerular basement membrane Ab, 12 wk</td>
<td>Mouse sFlt-1 plasmid</td>
<td>Soluble decoy receptor for VEGFR1. Reduction of circulating VEGFA</td>
<td>Soluble VEGFR1</td>
<td>500 μg, IM, 3 d before and 2 wk after injection of antiglomerular basement membrane Ab</td>
<td>Accelerated renal failure, proteinuria, interstitial fibrosis, endothelial cell loss, and downregulation of Nephrin in a model of rat crescentic GN</td>
<td>35</td>
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<tr>
<td>Wild-type mice, C57BL/6, 9–13 wk old</td>
<td>Axitinib (AG-013736)</td>
<td>Small molecule multitargeted TKI against VEGFR1–3, c-KIT, and PDGFR</td>
<td>VEGFR2, VEGFR1, VEGFR3, c-KIT, PDGFR</td>
<td>25 mg/kg, IP, twice daily for 7, 14 or 21 d</td>
<td>For dose-response studies: 1, 10, or 100 mg/kg, oral gavage, twice daily for 7 d</td>
<td>51</td>
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<tr>
<td>Wild-type mice, C57BL/6, 9–13 wk old</td>
<td>Ad-sVEGFR1</td>
<td>Adenoviral vector that expresses the extracellular domain of murine VEGFR1. Acts as soluble decoy receptor for VEGF</td>
<td>Soluble VEGFR1</td>
<td>1×10⁹ plaque-forming units, tail vein, once</td>
<td>Reduction in peritubular capillary density by 30% and glomerular capillary by 10% after 21 d of treatment. Dose dependent increase in proteinuria. Reduced glomerular capillary fenestrations. No increase in serum creatinine</td>
<td>51</td>
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Table 2. Continued

<table>
<thead>
<tr>
<th>Animal Model (Model/Transgene, Strain, Age)</th>
<th>Drug</th>
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<th>Dose, Route and Frequency</th>
<th>Effects</th>
<th>Reference</th>
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<tr>
<td>BALB/c (Bicc1/Bicc1) murine ARPKD and BALB/c wild-type controls, age not specified</td>
<td>Tesevatinib</td>
<td>TKI including EGFR, HER2/ErbB2, c-Src, and VEGFR2</td>
<td>VEGFR2, HER2, EGFR2, ERBB2</td>
<td>7.5 and 15 mg/kg, IP, daily postnatal day 4–21</td>
<td>Dose-dependent reduction in whole kidney size, total kidney weight; altered renal and liver morphology</td>
<td>48</td>
</tr>
<tr>
<td>PCK rat model (orthologous model of human ARPKD) and Sprague–Dawley wild type as control, age not specified</td>
<td>Tesevatinib</td>
<td>TKI including EGFR, HER2/ErbB2, c-Src, and VEGFR2</td>
<td>VEGFR2, HER2, EGFR2, ERBB2</td>
<td>7.5 and 15 mg/kg, oral gavage, daily for 60 d (from postnatal day 30–90)</td>
<td>Dose-dependent reduction in whole kidney size, total kidney weight; altered renal and liver morphology</td>
<td>48</td>
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<tr>
<td>UUO model and folic acid nephropathy models in male wild-type C57BL/6 mice, 6–8 wk</td>
<td>Nintedanib (BIBF11220)</td>
<td>A multitargeted TKI that blocks PDGFR, VEGFR, FGFR, and Src family kinases</td>
<td>PDGFR, VEGFR, FGFR, SRC</td>
<td>50 mg/kg, oral gavage, administered starting on day of UUO and then daily for 7 d</td>
<td>Attenuated renal fibrosis, inhibited activation of renal interstitial fibroblasts, and suppressed expression of proinflammatory cytokines after UUO</td>
<td>49</td>
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<tr>
<td>db/db and db/male C57BL/6 mice, 6 wk</td>
<td>dRK6 (a D-amino acid derivative of RK6)</td>
<td>An arginine-rich anti-VEGF hexapeptide that binds with VEGF-A, and blocks the interaction between VEGFA (mainly VEGF165 and VEGF121) and the VEGFRs</td>
<td>VEGFA</td>
<td>50 μg, SC, three times per week starting at 8 wk of age and lasting until 12 wk (short-term) and 20 wk (long-term)</td>
<td>Both short-term and long-term treatment had decreased creatinine clearance compared with control db/db mice. Long-term treatment also exacerbated albuminuria, mesangial matrix expansion, and glomerulomegaly as compared with vehicle-treated db/db and short-term dRK6-treated db/db mice</td>
<td>36</td>
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<tr>
<td>Perinatal wild-type mice (exact age not specified)</td>
<td>DC101 mAb against the extracellular portion of the VEGFR2</td>
<td></td>
<td>VEGFR2</td>
<td>0.08 mg/dose, IP, on postnatal days 2 and 4</td>
<td>Large renal cysts, impaired glomerulogenesis (hypocellular), and increased albuminuria by 3 wk of age</td>
<td>50</td>
</tr>
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</table>
called multitargeted TKIs, and have different effects depending on their specificity. TKIs targeting VEGFR in clinical use as chemotherapeutics include sunitinib, pazopanib, sorafenib, and axitinib, and differ because of their distinctive pharmacodynamic properties (Figure 1iv).

The varying effects of TKIs in various murine models that have used these agents across a spectrum of kidney diseases reflect the wide range of action and targets of TKIs (Table 2). In the bpk mouse model for autosomal recessive PKD, intraperitoneal injection of tesetakinib (a multikinase inhibitor targeting EGF receptor, HER2, and VEGFR) from postnatal days 4–21 attenuated cyst formation in the kidney and liver and reduced total kidney weight.48 Treatment with the multitargeted TKI nintedanib attenuated renal fibrosis in wild-type mice subjected to unilateral ureteral obstruction and folic acid nephropathy.49 In contrast to the renal benefit seen with multitargeted TKIs, inhibition of VEGFR2 alone with an antibody resulted in spontaneous renal cyst development when given perinatally in mice,50 and giving 10-week-old mice axitinib (a TKI targeting primarily VEGFR1, VEGFR2, and VEGFR3) resulted in loss of glomerular capillary fenestrations as well as proteinuria.51 Similarly, Wistar Kyoto rats given sunitinib, which mainly targets PDGF and VEGF receptors, exhibited proteinuria, hypertension, and endothelial injury.52

A review of 72 RCTs using TKIs targeting VEGFR1/VEGFR2 found that these drugs, like monoclonal anti-VEGF antibody therapies, were associated with an increased risk of hypertension. Among patients receiving the TKIs, the total incidence of all-grade or high-grade hypertension was 23.0% and 4.4%, respectively; sunitinib, pazopanib, cabozantinib, vandetanib, motesanib, regorafenib, cediranib, and sorafenib were associated with the highest risk.53 A separate study involving 1120 patients treated with TKIs observed a rapid and significant increase in systolic and diastolic BP after initiation of therapy, with a median onset of 29 days after first dose. Risk factors for treatment-induced hypertension in that cohort were pre-existing hypertension, body mass index >25, and age >60 years.54 Similarly, the incidence of all-grade and high-grade
proteinuria across 33 clinical trials of patients with solid tumors treated with VEGFR TKIs was 18.7% and 2.4%, respectively.56

In contrast to renal biopsy specimens from patients treated with anti-VEGF mAbs or VEGF trap, biopsy specimens for the majority of the patients receiving TKIs exhibited podocytopathies, including minimal change disease (MCD) and collapsing FSGS9 (Figure 2). In addition, a case series of four pediatric patients presented by Ruebner et al. reported that the administration of TKIs (imatinib, dasatinib, quazatinib, and sunitinib) produced severe nephrotic syndrome with marked albuminuria, edema, and decreased serum albumin. Although full evaluation was limited by a lack of biopsy in three of the patients, the biopsy sample from the fourth patient showed histology and electron microscopy findings were consistent with MCD96; another patient had laboratory evidence consistent with TMA in addition to nephrotic syndrome.56

Some evidence suggests that the increase in podocyte injury subsequent to TKIs might be mediated by tyrosine phosphorylation of
nephrin. Simons et al. showed that tyrosine phosphorylation of nephrin, an essential slit-diaphragm protein in podocytes, is critical to maintain the integrity of the filtration barrier. This was demonstrated in a murine model, in which an injection of antibody against nephrin-containing lipid rafts caused effacement of podocyte foot processes. Furthermore, New et al. demonstrated in a knock-in murine model that blocked tyrosine phosphorylation of nephrin that the mice developed proteinuria with podocyte effacement, emphasizing the importance of direct nephrin phosphorylation on the maintenance of podocyte morphology.

Interestingly, administration of the TKI sunitinib for eight consecutive days in normotensive Wistar Kyoto rats resulted in a dose-dependent decrease in expression of Nephrin mRNA; however, protein levels or phosphorylation status were not quantified. Further, in another study, induction of podocyte-specific overexpression of Vegfa in transgenic mice not only resulted in expression of VEGF2 protein in podocytes, but the VEGFR2 was also tyrosine phosphorylated and localized with nephrin, effects not seen in wild-type mice. In the same study, the researchers used whole kidney lysates to coimmunoprecipitate VEGFR2 and nephrin, demonstrating that the two physically interact in vivo. This suggests that when VEGFA availability increases, VEGFR2 is expressed in podocytes in addition to endothelial cells, and can be phosphorylated by podocyte-derived VEGFA. These changes were associated with glomerulosclerosis, basement membrane thickening, and foot process effacement.

Molecular studies of biopsied kidney samples from 29 patients with solid tumors after they were treated with either a TKI (sunitinib, axitinib, or sorafenib) or a direct anti-VEGF therapy (bevacizumab or VEGF trap) also attempted to elucidate mechanisms behind the two distinct patterns of glomerular injury, TMA and MCD/FSGS, after VEGF inhibition (Figure 2).

The authors showed that the massively upregulated RelA seen in TMA directly suppresses c-mip activity by binding to its promoter. This association of c-mip overexpression and direct podocyte injury has been characterized previously in human, murine, and in vitro studies.

In all, the diverse renal effects of the various TKIs can be ascribed to their multitargeted design. Specific mechanisms of
direct podocyte injury after treatment with TKIs can be attributed to modulation of NF-κB activity and c-mip expression, as well as nephrin phosphorylation and interaction with VEGFR2, but require validation in future studies.

**VEGF SIGNALING PATHWAYS**

To get a full picture of the nephrotoxicity associated with inhibition of VEGF signaling, it is critical to gain an understanding of the downstream mechanisms and regulatory cascades initiated by VEGFR2 activation. Key downstream signaling pathways include MAPK/ERK1/2, endothelial nitric oxide synthase (eNOS), and mammalian target of rapamycin (mTOR).

**RAF/MAPK/ERK SIGNALING**

One pathway that has been extensively studied is VEGF-induced ERK1/2 signaling, which serves to regulate endothelial differentiation and proliferation. In the kidney, MAPK/ERK signaling has diverse effects. Parietal epithelial cells in experimental FSGS show increased ERK1/2 activation, and it is also associated with increased podocyte apoptosis in diabetic conditions. In contrast to its association with pathologic consequences in renal epithelial cells, in glomerular endothelial cells, MAPK/ERK signaling is required for the protective effects of angiopoietin 1 against endoplasmic reticulum stress. In cultured glomerular endothelial cells, ERK1/2 inhibition completely suppressed VEGF-mediated proliferation, but migration was not affected.

Outside of the kidney, VEGF induces ERK1/2 phosphorylation in a time- and concentration-dependent manner and is responsible for its effects on endothelial cell hyperpermeability. This VEGF-mediated ERK1/2 signaling is dependent on the upstream mediators Raf-1 and MEK, as inhibition of either attenuated VEGF-induced endothelial cell hyperpermeability.

In addition, Takahashi et al. showed the importance of the VEGFR2/MAPK/ERK downstream pathway in cultured endothelial cells by determining that Y1175 is the major VEGFA-dependent autophosphorylation site on VEGFR2. Furthermore, mutations introduced at the Y1175 phosphorylation domain on VEGFR2 resulted in loss of the ability to tyrosine phosphorylate phospholipase C-γ, as well as a significant reduction in MAPK phosphorylation and a corresponding reduction in DNA synthesis. These effects suggest that Y1175 plays a critical role in MAPK signal transduction.

The importance of autophosphorylation of VEGFR2 was also subsequently demonstrated by Sakurai et al. who used a constitutive knock-in mouse model with phenylalanine residues substituting for tyrosine residues in the VEGFR2 gene—a change that was embryonically lethal because of loss of blood vessel differentiation. Furthermore, the critical nature of VEGF-ERK signaling was underscored in a study of a cohort of patients with hepatocellular carcinoma that found that tumors with highest expression of phospho-ERK were more responsive to VEGF inhibition via TKI.

Therapeutic inhibitors that target various portions of the MAPK/ERK are currently in development as novel chemotherapies. Some agents, such as vemurafenib and dabrafenib, specifically target B-Raf, an upstream component of the intracellular MAPK/ERK intracellular pathway (Figure 1v). Mutated B-Raf, as is found in various cancers, results in persistently elevated ERK phosphorylation. Clinically apparent renal toxicity with the use of B-Raf inhibitors most often occurs in the tubulointerstitial compartment, manifests as AKI, and is more prevalent in males, according to a recent review (Table 3). Specifically, vemurafenib treatment in eight patients with malignant melanoma resulted in severe AKI; the single biopsy performed revealed acute tubular necrosis. Interestingly, treatment with the combination of dabrafenib and the MEK inhibitor trametinib resulted in severe neoplastic syndrome with podocyte effacement and glomerular endothelial cell injury on biopsy that was reversible upon therapy discontinuation. Direct ERK1/2 inhibitors are also in development. A recently completed phase 1 trial of the first of these agents to emerge from preclinical studies, ulixertinib, noted that no adverse renal events have emerged so far.

Still, the potential nephrotoxicity associated with the inhibition of key proteins in the RAS-RAF-MEK-ERK1/2 pathway demonstrates a need to further explore downstream effectors of extracellular receptor activation that are specific to the tumor, as well as minimize nephrotoxicity.

**ENOS SIGNALING**

Some investigators have postulated a reduction in the vasodilator eNOS as a potential mechanism underlying the hypertension and endothelial injury seen subsequent to pharmacologic VEGF inhibition. Micewith eNOS deficiency (Nos3−/−) exhibit endothelial injury in the form of increased platelet aggregation, leukocyte adhesion, propensity toward thrombosis, and hypertension. Downstream of VEGF-VEGFR2 signaling in human umbilical vein cells and glomerular endothelial cells, phosphorylation of eNOS at Ser1177 induces eNOS in a time-dependent manner, leading to nitric oxide generation. Furthermore, the angiogenic effects of VEGF-VEGFR2 are mitigated in the absence of eNOS. Evidence supporting the role of VEGF in the hypertensive response comes from Tang et al. who found that a single dose of the selective VEGFR2 inhibitor SU5416 significantly downregulated lung eNOS expression in 3-day-old rats and induced pulmonary hypertension and right ventricular hypertrophy; these effects were attenuated with inhaled nitric oxide therapy. There are reports of the TKIs dasatinib and ponatinib inducing pulmonary arterial hypertension after their use in patients, and ponatinib administered to cultured human aortic endothelial cells has been similarly associated with decreased NOS3 expression.

In light of eNOS’s essential role in regulating vascular endothelial integrity through VEGF signaling, it is important to consider the contribution of other key endothelial regulatory genes in this pathway. Krüppel-like factor 2 (KLF2) and Krüppel-like factor 4 (KLF4) are well known zinc-finger transcription factors that regulate anti-inflammatory and antithrombotic pathways in the endothelium.
KLF2 induces both eNOS expression and activity in endothelial cells by regulating its promoter,88 and KLF4 has also been demonstrated to positively regulate eNOS expression.89 Interestingly, these two transcription factors appear to have discrepant effects on VEGFA-VEGFR2 signaling: KLF2 overexpression in human umbilical vein cells inhibited VEGFR2 mRNA and protein expression, as well as promoter activity, suggesting an antiangiogenic effect,90 whereas KLF4 has been demonstrated to positively regulate VEGFA expression.91 Further exploration of these signaling pathways is warranted to elucidate the mechanisms by which VEGF inhibition contributes to the nephrotoxicity observed in clinical practice.

**MTOR SIGNALING**

Another important downstream RTK target, mTOR, is part of the phosphatidylinositol 3-kinase/AKT signal transduction pathway (Figure 1vi). Inhibition of mTOR signaling blocks VEGF-mediated angiogenesis and endothelial cell proliferation at two different levels: by reducing VEGF synthesis and secretion, and by reducing VEGF2-mediated signaling.92 The mechanism responsible for the decreased VEGF production is not fully understood, but it does not appear to be due to modulation of upstream genes such as HIF-1α or TGF-β.92 Activation of mTOR occurs in conditions such as tuberous sclerosis and Peutz-Jeghers syndrome, which feature aberrant cell proliferation and a tendency to develop malignancies,93 and mTOR inhibitors such as temsirolimus, ridaforolimus, and everolimus have shown promising results in the treatment of several cancers.94

In the kidney, several studies have outlined the importance of mTOR in the maintenance of podocyte integrity, primarily through regulation of autophagy,95 as deletion of either of its two functional complexes (mTOR complex 1 or mTOR complex 2) resulted in severe glomerulosclerosis.96 Although mTOR’s role in podocytes has been more extensively studied, the mTOR autophagic pathway has a role in endothelial cells as well. The finding that endothelial-specific knockdown of Apg5, which encodes a key autophagic vesicle protein, exacerbated glomerular endothelial damage in diabetic mice97 suggests that modulation of the mTOR pathway in endothelial cells may also result in glomerular disease.

Proteinuria is the main renal effect subsequent to mTOR downregulation in murine models of podocyte injury, as well as the primary renal manifestation observed in patients after mTOR inhibition. The reported incidence of proteinuria varies widely, between 3% and 36% after everolimus therapy.98 Interestingly, the mTOR inhibitor sirolimus has been associated with *de novo* TMA in kidney transplant recipients that developed in the absence of calcineurin inhibitors, and in one case series, sirolimus correlated with a significant reduction in glomerular VEGF protein expression detected by immunostaining.99 This observation was explored further in a murine model, in which mice with podocyte-specific deletion of *mTor* developed significant proteinuria and ESRD by 5 weeks of age.100 In this model, VEGFA levels were not reduced until the mice were 3 weeks old, after the initiation of disease, suggesting multiple pathways are likely responsible for disease induction and progression.100 Accordingly, the mechanisms behind renal toxicities related to mTOR inhibition have been postulated to be related to a decrease in VEGF signaling, as well as to disruption of the autophagic pathway.100

**CLINICAL CONSIDERATIONS FOR MINIMIZING NEPHROTOXICITY**

Managing the various renal toxicities associated with VEGF inhibition will remain an important area of ongoing research because of the wide use of these agents and the steady development of novel therapeutics targeting this pathway in cancer. Experimental data from rats treated for 5 days with the TKI sunitinib demonstrated that coadministration of sunitinib with either the angiotensin-converting enzyme (ACE) inhibitor captopril or the phosphodiesterase type 5 inhibitor sildenafil reduced proteinuria and histologic evidence of endothelial injury, whereas neither had an effect on sunitinib-induced hypertension.101 The mechanism behind these renoprotective effects is undetermined, but it might involve eNOS signaling, as both ACE inhibitors and angiotensin receptor blockers have been shown to increase kidney eNOS levels after ischemia-reperfusion injury.102

Guidelines published in 2010, on the basis of recommendations to that National Cancer Institute’s Investigational Drug Steering Committee, advise conducting and documenting a formal risk assessment of cardiovascular complications before initiation of VEGF inhibition, followed by active BP monitoring during VEGF inhibition therapy, with a treatment goal of BP <140/90 mm Hg.103 The addition of antihypertensive agents when BP remains above goal is recommended, with some clinical data suggesting an added benefit of ACE inhibition over other classes.104 Temporarily discontinuing VEGF inhibition therapy or dose reduction might be necessary if BP control is not possible.103

Microalbuminuria often accompanies hypertension,38 and first-line therapy is generally renin-angiotensin-aldosterone system inhibition, as this has shown some success in mTOR antagonist–associated albuminuria after kidney transplantation.105 Albuminuria should be quantified before initiating therapy, and significant proteinuria (>2 g in 24 hours) is cause for discontinuation of therapy per 2013 guidelines,106 as is nephrotic syndrome or TMA. In the cases of nephrotic-range albuminuria, hematuria, or biochemical evidence of impaired kidney function, kidney biopsy should be pursued, as glomerular diseases associated with VEGF inhibition vary. In all, close monitoring during therapy and a thorough assessment of renal function before initiation, including microalbuminuria, hematuria, and serum creatinine, should be performed in all patients receiving VEGF inhibition therapy.

**CONCLUSIONS AND FUTURE PERSPECTIVES**

Pharmacologic inhibition of VEGF signaling and its downstream pathways is a common therapeutic strategy in oncology, but associated nephrotoxicities remain a concern. Although adverse effects such as hypertension, proteinuria, and TMA are well described in both experimental and
clinical data, strategies to mitigate them have not been established. Furthermore, long-term data are lacking regarding the effects of VEGF inhibition and risk of subsequent CKD or hypertension. Given increasing survival among patients with cancer who receive anti-VEGF therapy, this is an important area to investigate, as is identification of novel mechanisms to reduce nephrotoxicities of VEGF-inhibiting drugs without compromising the drugs’ antiangiogenic effects in cancer.

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DISCLOSURES

None.

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