Therapeutic Uses for VEGFR1 Antibodies

The present invention relates to the field of medicine. More particularly, the present invention relates to methods that may be useful for treating chronic kidney disease (CKD), and/or more particularly diabetic nephropathy (also known as diabetic kidney disease), in a patient with a VEGFR1 antibody.

Vascular endothelial growth factor receptor-1 (VEGFR-1, also known as Flt-1, SEQ ID NO: 1) is one of the three tyrosine kinase receptors (VEGFR1, VEGFR2, and VEGFR3) for VEGF family proteins. The VEGF family consists of a group of structurally related glycoproteins including VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PlGF). VEGF proteins play multiple biological and pathological roles by selectively binding to extracellular immunoglobulin-like domains of the three VEGF receptors. VEGF-A has high affinity for both VEGFR1 and VEGFR2, while VEGF-B and PlGF selectively bind to VEGFR1. VEGF-A/VEGFR2, as ligand and receptor respectively, play a major role in the signaling of vascular-angiogenic biological pathways. VEGFR1 and a soluble form of VEGFR1 (also known as sFlt-1) have been suggested by some groups to serve a decoy receptor role, sequestering VEGF-A, and preventing it from binding to its more pro-angiogenic partner, VEGFR2.

VEGF signaling is essential for vasculogenesis, angiogenesis, vascular homeostasis, inflammation, and therefore has been linked to several human diseases including cancer, diabetic complications, cardiovascular disease, and chronic inflammation. The VEGF system also plays a critical role in maintaining kidney function. While VEGF-A has been shown in some studies to have a protective effect on the kidney, the kidney has also been shown to be extremely sensitive to the effects of VEGF-A. Kidney injury has been shown to occur when VEGF-A levels are suppressed with a VEGF-A monoclonal antibody in humans (Eremina *et al.* (2008) NEJM 358:1129), and also when VEGF-A levels are elevated in mice over-expressing VEGF-A in the kidney glomerulus (Veron *et al.* (2010) Kidney Intl 77:989, and Hakroush *et al.* (2009) Am J Pathol 175:1883).

From biological studies of VEGF-A, treatment with VEGF-A has been suggested to likely be deleterious for patients with diabetes, atherosclerosis, or sepsis; these are all conditions that are either causes or complications of CKD. Increased VEGF-A levels have also been associated with diabetic nephropathy (Cha *et al.* (2000) Kidney Intl Supp 77:S104 and Hovind *et al.* (2000) Kidney Intl Supp 75:S56). Decreasing VEGF-A with VEGF-A antibodies was shown in a type 2 diabetes mouse model to improve glomerular hypertrophy and albuminuria, both markers for diabetic nephropathy (de Vriese *et al.* (2001) J AM Soc Nephol 12:993 and Flyvbjerg *et al.* (2002) Diabetes 51:3090).

sFlt-1 is a secreted form of VEGFR1 that arises from a splicing variant of the VEGFR1 gene. sFlt-1 has preserved ligand binding activity, and has been connected to the amount of VEGF-A that is available for signaling through VEGFR-2. The amount of active sFlt-1 in diabetic nephropathy has been studied, but the results have been inconsistent.

CKD is characterized by the progressive loss of kidney function. Diabetic nephropathy (also known as diabetic kidney disease) is one type of CKD, and is a chronic complication of diabetes mellitus. Increased albuminuria and gradual, progressive loss of renal function are primary manifestations in human diabetic nephropathy. Decreased renal function results in increased blood creatinine and blood urea nitrogen (BUN). Diabetes mellitus, hypertension, and glomerulonephritis are the most common causes of CKD. CKD patients experience over time an increase in albuminuria, proteinuria, serum creatinine, and renal histopathological lesions. Worsening CKD evolves into end stage renal disease (ERSD) for many patients, requiring either dialysis or kidney transplant. About 45% of ERSD patients have been estimated to have type 2 diabetes mellitus as the cause of their CKD. Glomerular filtration rate (GFR) is used to classify the severity of CKD for patients, with lower GFR corresponding to more severe CKD. Reducing the rate at which GFR declines in patients is expected to delay or prevent the development of ESRD. Angiotensin converting enzyme inhibitors or angiotensin II receptor antagonists are used as current standard of care to slow the progression of CKD to ERSD, but these have been shown inadequate to stop the ultimate progression to dialysis.

WO2006/055809 discloses the activity of VEGFR1 antibodies in multiple cancer xenograft models. Activity against certain non-neoplastic, angiogenic diseases, such as insulin-dependent diabetes mellitus and autoimmune nephritis, were also disclosed. However, to date, no antibody targeting VEGFR1 has been taught or suggested for therapeutic use for diabetic nephropathy or other forms of progressive CKD.

There remains a need to provide alternative methods to treat CKD. In particular, there remains a need to provide methods to treat diabetic nephropathy.

Accordingly, the present invention provides a method of treating chronic kidney disease in a patient, comprising administering to the patient an effective amount of a VEGFR1 antibody. In another embodiment, the present invention provides a method of treating chronic kidney disease in a patient, comprising administering to the patient an effective amount of a VEGFR1 antibody, wherein the chronic kidney disease is caused by diabetes mellitus. More particularly, the methods of the present invention provide for a patient at stage 3 or stage 4 of chronic kidney disease.

In an embodiment, the present invention provides a method of treating chronic kidney disease in a patient, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody, wherein the chronic kidney disease is diabetic nephropathy. In another embodiment, the present invention provides a method of treating chronic kidney disease in a patient, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody, wherein the chronic kidney disease is focal segmental glomerulosclerosis. In an embodiment, the present invention provides a method of treating chronic kidney disease in a patient, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody, wherein the chronic kidney disease is nephrotic syndrome.

In an embodiment, the present invention provides a method of decreasing proteinuria in a patient, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody. In a further embodiment, the present invention provides a method of decreasing proteinuria in a patient, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody, wherein the proteinuria is caused by chronic kidney disease. In a further embodiment, the present invention

provides a method of decreasing proteinuria in a patient, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody, wherein the proteinuria is caused by diabetic nephropathy.

In an embodiment, the present invention provides a method of decreasing albuminuria in a patient, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody. In a further embodiment, the present invention provides a method of decreasing albuminuria in a patient, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody, wherein the albuminuria is caused by chronic kidney disease. In a further embodiment, the present invention provides a method of decreasing albuminuria in a patient, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody, wherein the albuminuria is caused by diabetic nephropathy.

In an embodiment, the present invention provides a method of decreasing the loss of glomerular filtration rate (GFR) reflected by an increase in serum creatinine in a patient, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody. In an embodiment, the present invention provides a method of protecting against renal histopathological lesions in a patient, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody.

In an embodiment, the present invention provides a method of slowing the rate of increase in blood urea nitrogen (BUN) in a patient, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody.

In an embodiment, the present invention provides a method of slowing the rate of progression to ESRD in a patient, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody. In an embodiment, the present invention provides a method of delaying progression to ESRD in a patient, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody, wherein the progression to ESRD is delayed at least 12 months. In an embodiment, the present invention provides a method of delaying progression to ESRD in a patient, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody, wherein the progression to ESRD is delayed at least 24 months. In an

embodiment, the present invention provides a method of slowing the time to doubling of serum creatinine in a patient, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody. In an embodiment, the present invention provides a method of prolonging the time to a 30% reduction in GFR in a patient, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody.

In an embodiment, the present invention provides methods of decreasing free (unbound) sFLt-1, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody, wherein the patient has chronic kidney disease. In an embodiment, the present invention provides methods of decreasing the ability of sFLt-1 to signal and/or bind other proteins, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody, wherein the patient has chronic kidney disease.

In an embodiment, the present invention provides a VEGFR1 antibody for use in the treatment of chronic kidney disease. In a further embodiment, the present invention provides a VEGFR1 antibody for use in the treatment of chronic kidney disease, wherein the chronic kidney disease is caused by diabetes mellitus. More particularly, the present invention provides a VEGFR1 antibody for use in the treatment of chronic kidney disease, wherein the chronic kidney disease is at stage 3 or stage 4.

In an embodiment, the present invention provides a VEGFR1 antibody for use in the treatment of chronic kidney disease, wherein the chronic kidney disease is diabetic nephropathy. In another embodiment, the present invention provides a VEGFR1 antibody for use in the treatment of chronic kidney disease, wherein the chronic kidney disease is focal segmental glomerulosclerosis. In another embodiment, the present invention provides a VEGFR1 antibody for use in the treatment of chronic kidney disease, wherein the chronic kidney disease is nephrotic syndrome.

In an embodiment, the present invention provides a VEGFR1 antibody for use in decreasing proteinuria. In a further embodiment, the present invention provides a VEGFR1 antibody for use in decreasing proteinuria, wherein the proteinuria is caused by chronic kidney disease. In a further embodiment, the present invention provides a

VEGFR1 antibody for use in decreasing proteinuria, wherein the proteinuria is caused by diabetic nephropathy.

In an embodiment, the present invention provides a VEGFR1 antibody for use in decreasing albuminuria. In a further embodiment, the present invention provides a VEGFR1 antibody for use in decreasing albuminuria, wherein the albuminuria is caused by diabetic nephropathy. In a further embodiment, the present invention provides a VEGFR1 antibody for use in decreasing albuminuria, wherein the albuminuria is caused by diabetic nephropathy.

In an embodiment, the present invention provides a VEGFR1 antibody for use in decreasing the loss of glomerular filtration rate (GFR) reflected by an increase in serum creatinine. In an embodiment, the present invention provides a VEGFR1 antibody for use in protecting against renal histopathological lesions.

In an embodiment, the present invention provides a VEGFR1 antibody for use in slowing the rate of increase in blood urea nitrogen (BUN).

In an embodiment, the present invention provides a VEGFR1 antibody for use in slowing the rate of progression to ESRD. In an embodiment, the present invention provides a VEGFR1 antibody for use in delaying progression to ESRD, wherein the progression to ESRD is delayed at least 12 months. In an embodiment, the present invention provides a VEGFR1 antibody for use in delaying progression to ESRD, wherein the progression to ESRD is delayed at least 24 months. In an embodiment, the present invention provides a VEGFR1 antibody for use in slowing the time to doubling of serum creatinine. In an embodiment, the present invention provides a VEGFR1 antibody for use in prolonging the time to a 30% reduction in GFR.

In an embodiment, the present invention provides a VEGFR1 antibody for use in decreasing free (unbound) sFLt-1. In an embodiment, the present invention provides a VEGFR1 antibody for use in decreasing the ability of sFLt-1 to signal and/or bind other proteins.

In an embodiment, the present invention provides the use of a VEGFR1 antibody for the manufacture of a medicament for the treatment of chronic kidney disease. In an embodiment, the present invention provides the use of a VEGFR1 antibody for the

manufacture of a medicament for the treatment of chronic kidney disease, wherein the chronic kidney disease is caused by diabetes mellitus. More particularly, the present invention provides the use of a VEGFR1 antibody for the manufacture of a medicament for the treatment of chronic kidney disease, wherein the chronic kidney disease is at stage 3 or stage 4.

In an embodiment, the present invention provides the use of a VEGFR1 antibody for the manufacture of a medicament for the treatment of chronic kidney disease, wherein the chronic kidney disease is diabetic nephropathy. In another embodiment, the present invention provides the use of a VEGFR1 antibody for the manufacture of a medicament for the treatment of chronic kidney disease, wherein the chronic kidney disease is focal segmental glomerulosclerosis. In another embodiment, the present invention provides the use of a VEGFR1 antibody for the manufacture of a medicament for the treatment of chronic kidney disease, wherein the chronic kidney disease is nephrotic syndrome.

In an embodiment, the present invention provides the use of a VEGFR1 antibody for the manufacture of a medicament for decreasing proteinuria. In a further embodiment, the present invention provides the use of a VEGFR1 antibody for the manufacture of a medicament for decreasing proteinuria, wherein the proteinuria is caused by chronic kidney disease. In a further embodiment, the present invention provides the use of a VEGFR1 antibody for the manufacture of a medicament for decreasing proteinuria, wherein the proteinuria is caused by diabetic nephropathy.

In an embodiment, the present invention provides the use of a VEGFR1 antibody for the manufacture of a medicament for decreasing albuminuria. In a further embodiment, the present invention provides the use of a VEGFR1 antibody for the manufacture of a medicament for decreasing albuminuria, wherein the albuminuria is caused by chronic kidney disease. In a further embodiment, the present invention provides the use of a VEGFR1 antibody for the manufacture of a medicament for decreasing albuminuria, wherein the albuminuria is caused by diabetic nephropathy.

In an embodiment, the present invention provides the use of a VEGFR1 antibody for the manufacture of a medicament for decreasing the loss of glomerular filtration rate (GFR) reflected by an increase in serum creatinine. In an embodiment, the present

invention provides the use of a VEGFR1 antibody for the manufacture of a medicament for protecting against renal histopathological lesions.

In an embodiment, the present invention provides the use of a VEGFR1 antibody for the manufacture of a medicament for slowing the rate of increase in blood urea nitrogen (BUN).

In an embodiment, the present invention provides the use of a VEGFR1 antibody for the manufacture of a medicament for slowing the rate of progression to ESRD. In an embodiment, the present invention provides the use of a VEGFR1 antibody for the manufacture of a medicament for delaying progression to ESRD, wherein the progression to ESRD is delayed at least 12 months. In an embodiment, the present invention provides the use of a VEGFR1 antibody for the manufacture of a medicament for delaying progression to ESRD, wherein the progression to ESRD is delayed at least 24 months. In an embodiment, the present invention provides the use of a VEGFR1 antibody for the manufacture of a medicament for slowing the time to doubling of serum creatinine. In an embodiment, the present invention provides the use of a VEGFR1 antibody for the manufacture of a medicament for prolonging the time to a 30% reduction in GFR.

In an embodiment, the present invention provides the use of a VEGFR1 antibody for the manufacture of a medicament for decreasing free (unbound) sFLt-1. In an embodiment, the present invention provides the use of a VEGFR1 antibody for the manufacture of a medicament for decreasing the ability of sFLt-1 to signal and/or bind other proteins.

The present invention also provides a method of treating chronic kidney disease in a patient, comprising administering VEGFR1 antibodies, as described herein, in simultaneous or sequential combination with a standard of care. The present invention also provides VEGFR1 antibodies for use in the treatment of chronic kidney disease, comprising administering simultaneous or sequential combination with a standard of care.

The present invention also provides a method of treating diabetic nephropathy in a patient, comprising administering VEGFR1 antibodies, as described herein, in simultaneous or sequential combination with a standard of care. The present invention also provides VEGFR1 antibodies for use in the treatment of diabetic nephropathy,

comprising administering simultaneous or sequential combination with a standard of care. Standard of care includes, and not limited to, angiotensin converting enzyme (ACE) inhibitors or angiotensin II receptor (ARB) antagonists.

The VEGFR1 antibodies as described and used herein may be administered in simultaneous or sequential combination with a standard of care. Standard of care includes, and not limited to, angiotensin converting enzyme (ACE) inhibitors or angiotensin II receptor (ARB) antagonists.

In an embodiment, the methods and uses of the present invention comprise a VEGFR1 antibody wherein the VEGFR1 antibody has a K_D for VEGFR1 of less than 80 pM as determined by surface plasmon resonance at 25°C. The Kd values are established by a binding equilibrium at 25°C as described in Example 2. In an embodiment, the methods and uses of the present invention comprise a VEGFR1 antibody wherein the VEGFR1 antibody neutralizes VEGF-A binding *in vitro* to VEGFR1 with an IC_{50} less than 2.0 nM. In an embodiment, the methods and uses of the present invention comprise a VEGFR1 antibody wherein the VEGFR1 antibody neutralizes PlGF binding *in vitro* to VEGFR1 with an IC_{50} less than 2.0 nM. The neutralization values are established as described in Example 4 (see Table 4b). In an embodiment, the methods and uses of the present invention comprise a VEGFR1 antibody wherein the VEGFR1 antibody has a K_D for VEGFR1 of less than 80 pM as determined by surface plasmon resonance at 25°C, neutralizes VEGF-A binding *in vitro* to VEGFR1 with an IC_{50} less than 2.0 nM, and/or antibody neutralizes PlGF binding *in vitro* to VEGFR1 with an IC50 less than 2.0 nM.

In an embodiment, the present invention provides methods of treatment, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody, wherein the patient has an increase in VEGFR2 phosphorylation. In an embodiment, the present invention provides methods of treatment, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody, wherein the patient has an increase in PlGF levels. In an embodiment, the present invention provides methods of treatment, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody, wherein the patient has an increase in VEGF-A levels. In an embodiment, the present invention provides methods of

treatment, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody, wherein the patient has an increase in sFLt-1 levels. In an embodiment, the present invention provides methods of treatment, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody, wherein the patient has an increase in VEGF-B levels. In embodiments, the present invention provides methods of treatment, wherein the PlGF levels, VEGF-A levels, sFLt-1 levels, and VEGF-B levels are measured by ELISA.

In an embodiment, the present invention provides a VEGFR1 antibody for use when VEGFR2 phosphorylation is increased. In an embodiment, the present invention provides a VEGFR1 antibody for use when PLGF levels are increased. In an embodiment, the present invention provides a VEGFR1 antibody for use when VEGF-A levels are increased. In an embodiment, the present invention provides a VEGFR1 antibody for use when sFLt-1 levels are increased. In an embodiment, the present invention provides a VEGFR1 antibody for use when VEGF-B levels are increased. In embodiments, the present invention provides a VEGFR1 antibody for uses wherein the PlGF levels, VEGF-A levels, sFLt-1 levels, and VEGF-B levels are measured by ELISA.

The present invention provides a VEGFR1 antibody which is believed to cause a reduction in proteinuria with a concomitant reduction in disease progression in humans. Further, the present invention provides a VEGFR1 antibody which is believed to be effective in the treatment of chronic kidney disease in humans. Further, the present invention provides a VEGFR1 antibody which is believed to be effective in the treatment of diabetic nephropathy in humans. The present invention provides a VEGFR1 antibody which is believed to cause a reduction in albuminuria with a concomitant reduction in disease progression in humans. The present invention provides a VEGFR1 antibody which is believed to cause a reduction in serum creatinine with a concomitant reduction in disease progression in humans.

In an embodiment, the methods and uses of the present invention comprise a preferred VEGFR1 antibody wherein the VEGFR1 antibody is an antibody comprising a light chain variable region (LCVR) and a heavy chain variable region (HCVR), wherein the LCVR comprises the complementarity determining regions (CDRs) LCDR1, LCDR2,

and LCDR3, and the HCVR comprises the CDRs HCDR1, HCDR2, and HCDR3, wherein the LCDR1 is the polypeptide of RASQSVSSSYLA (SEQ ID NO: 8), the LCDR2 is the polypeptide of GASSRAT (SEQ ID NO: 9), the LCDR3 is the polypeptide of QQYGSSPLT (SEQ ID NO: 10), the HCDR1 is the polypeptide of GFAFSSYGMH (SEQ ID NO: 2), the HCDR2 is the polypeptide of VIWYDGSNKYYADSVRG (SEQ ID NO: 3), and the HCDR3 is the polypeptide of DHYGSGVHIHYFYYGLDV (SEQ ID NO: 4).

In an embodiment, the methods and uses of the present invention comprise a preferred VEGFR1 antibody wherein the VEGFR1 antibody is an antibody comprising a light chain variable region (LCVR) and a heavy chain variable region (HCVR), and wherein the LCVR is the polypeptide of SEQ ID NO: 11, and the HCVR is the polypeptide of SEQ ID NO: 5.

In an embodiment, the methods and uses of the present invention comprise a preferred VEGFR1 antibody wherein the VEGFR1 antibody comprises a light chain (LC) and a heavy chain (HC), wherein the LC is the polypeptide of SEQ ID NO: 12, and the HC is the polypeptide of SEQ ID NO: 6. In another embodiment, the methods and uses of the present invention comprise a VEGFR1 antibody wherein the VEGFR1 antibody comprises a light chain (LC) and a heavy chain (HC), wherein the LC is the polypeptide of SEQ ID NO: 12, and the HC is the polypeptide of SEQ ID NO: 7.

In an embodiment, the methods and uses of the present invention comprise a preferred VEGFR1 antibody wherein the VEGFR1 antibody comprises two light chains and two heavy chains, wherein each light chain is the polypeptide of SEQ ID NO: 12, and each heavy chain is the polypeptide of SEQ ID NO: 6. In another embodiment, the methods and uses of the present invention comprise a preferred VEGFR1 antibody wherein the VEGFR1 antibody comprises two light chains and two heavy chains, wherein each light chain is the polypeptide of SEQ ID NO: 12, and each heavy chain is the polypeptide of SEQ ID NO: 7.

In an embodiment, the present invention provides a method of treating chronic kidney disease in a patient, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody, wherein the VEGFR1 antibody comprises two

light chains and two heavy chains, wherein each light chain is the polypeptide of SEQ ID NO: 12, and each heavy chain is the polypeptide of SEQ ID NO: 6. In another embodiment, the present invention provides a method of treating chronic kidney disease in a patient, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody, wherein the VEGFR1 antibody comprises two light chains and two heavy chains, wherein each light chain is the polypeptide of SEQ ID NO: 12, and each heavy chain is the polypeptide of SEQ ID NO: 7.

In an embodiment, the present invention provides a method of treating chronic kidney disease in a patient, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody, wherein the chronic kidney disease is diabetic nephropathy, and wherein the VEGFR1 antibody comprises two light chains and two heavy chains, wherein each light chain is the polypeptide of SEQ ID NO: 12, and each heavy chain is the polypeptide of SEQ ID NO: 6. In another embodiment, the present invention provides a method of treating chronic kidney disease in a patient, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody, wherein the chronic kidney disease is diabetic nephropathy, and wherein the VEGFR1 antibody comprises two light chains and two heavy chains, wherein each light chain is the polypeptide of SEQ ID NO: 12, and each heavy chain is the polypeptide of SEQ ID NO: 7.

In an embodiment, the present invention provides a method of treating chronic kidney disease in a patient, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody, wherein the chronic kidney disease is focal segmental glomerulosclerosis, and wherein the VEGFR1 antibody comprises two light chains and two heavy chains, wherein each light chain is the polypeptide of SEQ ID NO: 12, and each heavy chain is the polypeptide of SEQ ID NO: 6. In another embodiment, the present invention provides a method of treating chronic kidney disease in a patient, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody, wherein the chronic kidney disease is focal segmental glomerulosclerosis, and wherein the VEGFR1 antibody comprises two light chains and two heavy chains, wherein

each light chain is the polypeptide of SEQ ID NO: 12, and each heavy chain is the polypeptide of SEQ ID NO: 7.

In an embodiment, the present invention provides a VEGFR1 antibody for use in the treatment of chronic kidney disease, wherein the VEGFR1 antibody comprises two light chains and two heavy chains, wherein each light chain is the polypeptide of SEQ ID NO: 12, and each heavy chain is the polypeptide of SEQ ID NO: 6. In another embodiment, the present invention provides a VEGFR1 antibody for use in the treatment of chronic kidney disease, wherein the VEGFR1 antibody comprises two light chains and two heavy chains, wherein each light chain is the polypeptide of SEQ ID NO: 12, and each heavy chain is the polypeptide of SEQ ID NO: 7.

In an embodiment, the present invention provides a VEGFR1 antibody for use in the treatment of chronic kidney disease, wherein the chronic kidney disease is diabetic nephropathy, and wherein the VEGFR1 antibody comprises two light chains and two heavy chains, wherein each light chain is the polypeptide of SEQ ID NO: 12, and each heavy chain is the polypeptide of SEQ ID NO: 6. In another embodiment, the present invention provides a VEGFR1 antibody for use in the treatment of chronic kidney disease, wherein the chronic kidney disease is diabetic nephropathy, and wherein the VEGFR1 antibody comprises two light chains and two heavy chains, wherein each light chain is the polypeptide of SEQ ID NO: 12, and each heavy chain is the polypeptide of SEQ ID NO: 7.

In an embodiment, the present invention provides a VEGFR1 antibody for use in the treatment of chronic kidney disease, wherein the chronic kidney disease is focal segmental glomerulosclerosis, and wherein the VEGFR1 antibody comprises two light chains and two heavy chains, wherein each light chain is the polypeptide of SEQ ID NO: 12, and each heavy chain is the polypeptide of SEQ ID NO: 6. In another embodiment, the present invention provides a VEGFR1 antibody for use in the treatment of chronic kidney disease, wherein the chronic kidney disease is focal segmental glomerulosclerosis, and wherein the VEGFR1 antibody comprises two light chains and two heavy chains, wherein each light chain is the polypeptide of SEQ ID NO: 12, and each heavy chain is the polypeptide of SEQ ID NO: 7.

The present invention further provides a method of treating chronic kidney disease in a patient, comprising administering to the patient an effective amount of a VEGFR1 antagonist.

Preferably, the VEGFR1 antagonist is a VEGFR1 antibody.

The general structure of an "antibody" is very well-known in the art. For an antibody of the IgG type, there are four amino acid chains (two "heavy" chains and two "light" chains) that are cross-linked via intra- and inter-chain disulfide bonds. For an antibody, one of the heavy chains forms an inter-chain disulfide bond with one of the light chains, and the other heavy chain forms an inter-chain disulfide bond with the other light chain, and one of the heavy chains forms two inter-chain disulfide bonds with the other heavy chain.

When expressed in certain biological systems, antibodies having human Fc sequences which are glycosylated in the Fc region. Antibodies may be glycosylated at other positions as well. One of skill in the art will appreciate that antibodies of the present invention may contain such glycosylation. Typically, glycosylation occurs in the Fc region of the antibody at a highly conserved N-glycosylation site. N-glycans typically attach to asparagines.

Antibody I comprises two light chains and two heavy chains, wherein each of the light chains consists of the polypeptide of SEQ ID NO: 12 and each of the heavy chains consists of the polypeptide of SEQ ID NO: 6. Antibody II comprises two light chains and two heavy chains, wherein each of the light chains consists of the polypeptide of SEQ ID NO: 12 and each of the heavy chains consists of the polypeptide of SEQ ID NO: 7. A particular DNA molecule encoding each of the heavy chains of Antibody I is SEQ ID NO: 13, and a particular DNA molecule encoding each of the light chains of Antibody I is SEQ ID NO: 15. A particular DNA molecule encoding each of the heavy chains of Antibody II is SEQ ID NO: 14, and a particular DNA molecule encoding each of the light chains of Antibody II is SEQ ID NO: 15.

Antibody I and Antibody II are antibodies against human VEGFR1; Antibody I has a IgG1 Fc, and Antibody II has a IgG4 Fc. Antibody III is a rat IgG1 antibody

against mouse VEGFR1, and Antibody IV is a chimeric antibody against mouse VEGFR1 with a rat variable region and a mouse IgG1 Fc.

An antibody for the methods and uses of the present invention can be produced using techniques well known in the art, e.g., recombinant technologies, phage display technologies, synthetic technologies, or combinations of such technologies or other technologies readily known in the art. Methods for producing and purifying antibodies are well known in the art and can be found, for example, in Harlow and Lane (1988) *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, chapters 5-8 and 15, ISBN 0-87969-314-2.

"Effective amount" means the amount of an antibody for the methods and uses of the present invention or pharmaceutical composition comprising an antibody for the methods and uses of the present invention that will elicit the biological or medical response of or desired therapeutic effect on a tissue, system, animal, mammal or human that is being sought by the researcher, medical doctor, or other clinician. An effective amount of the antibody may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody to elicit a desired response in the individual. An effective amount is also one in which any toxic or detrimental effect of the antibody is outweighed by the therapeutically beneficial effects. The effective amount can comprise an amount of about 0.001 to 20 mg/kg per single (e.g., bolus), multiple or continuous administration. In some instances, dosage levels below the lower limit of the aforesaid range may be more than adequate, while in other cases still larger doses may be employed.

The terms "treatment," "treat," "treating," and the like, are meant to include slowing or reversing the progression of a disorder. These terms also include alleviating, ameliorating, attenuating, eliminating, or reducing one or more symptoms of a disorder or condition, even if the disorder or condition is not actually eliminated and even if progression of the disorder or condition is not itself slowed or reversed. A patient refers to a mammal, preferably a human with a disease, disorder or condition that would benefit from inhibition of VEGFR1 activity.

Stage 3 or 4 of CKD has been defined to be a patient with an estimated GFR (eGFR) of between 15 and 59 ml/min/1.73m^2. Others do not use a stage definition for CKD patients, but rather define patients by their eGFR as follows: normal > 80, mild 50-80, moderate 30-50, and severe < 30.

An antibody for the methods and uses of the present invention, or pharmaceutical composition comprising the same, may be administered by parenteral routes (e.g., subcutaneous, intravenous, intraperitoneal, intramuscular, or transdermal). An antibody for the methods and uses of the present invention may be administered to a patient alone or in combination with a pharmaceutically acceptable carrier and/or diluent in single or multiple doses. Pharmaceutical compositions for the methods and uses of the present invention can be prepared by methods well known in the art (e.g., *Remington: The Science and Practice of Pharmacy*, 19th ed. (1995), A. Gennaro et al., Mack Publishing Co.) and comprise an antibody, as disclosed herein, and one or more pharmaceutically acceptable carriers, diluents, or excipients.

Example 1: Antibody Expression and Purification

The polypeptides of the variable regions of the heavy chain and light chain, the complete heavy chain and light chain amino acid sequences of Antibody I-IV, and the nucleotide sequences encoding the same, are listed below in the section entitled "Amino Acid and Nucleotide Sequences." In addition, the light chain and heavy chain CDR polypeptides are shown in Table 1.

The VEGFR1 antibodies for the methods and uses of the present invention, including, but not limited to, Antibodies I and II, can be made and purified essentially as follows. An appropriate host cell, such as HEK 293 EBNA or CHO, can be either transiently or stably transfected with an expression system for secreting antibodies using an optimal predetermined HC:LC vector ratio or a single vector system encoding both HC and LC. Clarified media, into which the antibody has been secreted, may be purified using any of many commonly-used techniques. For example, the medium may be conveniently applied to a MabSelect column (GE Healthcare), or KappaSelect column (GE Healthcare) for Fab fragment, that has been equilibrated with a compatible buffer, such as phosphate buffered saline (pH 7.4). The column may be washed to remove

nonspecific binding components. The bound antibody may be eluted, for example, by pH gradient (such as 20 mM Tris buffer pH 7 to 10 mM sodium citrate buffer pH 3.0, or phosphate buffered saline pH 7.4 to 100 mM glycine buffer pH 3.0). Antibody fractions may be detected, such as by SDS-PAGE, and then may be pooled. Further purification is optional, depending on the intended use. The antibody may be concentrated and/or sterile filtered using common techniques. Soluble aggregate and multimers may be effectively removed by common techniques, including size exclusion, hydrophobic interaction, ion exchange, multimodal, or hydroxyapatite chromatography. The product may be immediately frozen at -70°C or may be lyophilized.

Table 1: SEQ ID NOs

Antibody	Light Chain	Heavy Chain	LCVR	HCVR
I	12	6	11	5
II	12	7	11	5
III	17	16		
IV	19	18		

Antibody	LCDR1	LCDR2	LCDR3
I	RASQSVSSSYLA (SEQ ID NO: 8)	GASSRAT (SEQ ID NO: 9)	QQYGSSPLT (SEQ ID NO: 10)
II	RASQSVSSSYLA (SEQ ID NO: 8)	GASSRAT (SEQ ID NO: 9)	QQYGSSPLT (SEQ ID NO: 10)

Antibody	HCDR1	HCDR2	HCDR3
I	GFAFSSYGMH (SEQ ID NO: 2)	VIWYDGSNKYYADSVRG (SEQ ID NO: 3)	DHYGSGVHHYFYYGLDV (SEQ ID NO: 4)
II	GFAFSSYGMH (SEQ ID NO: 2)	VIWYDGSNKYYADSVRG (SEQ ID NO: 3)	DHYGSGVHHYFYYGLDV (SEQ ID NO: 4)

Example 2: Binding kinetics, affinity, and specificity of VEGFR1 antibodies

The binding kinetics, affinity, and selectivity to human VEGFR1, as well as human VEGFR2 and human VEGFR3, for antibodies for the methods and uses of the present invention, may be determined by use of a surface plasmon resonance (SPR) biosensor such as a BIAcore® 2000, BIAcore® 3000, or a BIAcore® T100 (GE HealthCare) according to methods known in the art. Binding kinetics, affinity, and specificity may be used in the analysis to demonstrate that the disclosed antibodies to mouse VEGFR1 function similarly *in vitro* to the disclosed antibodies to human VEGFR1.

Binding affinity, specificity, kinetics and stoichiometry of the antibodies to various species of VEGF receptors may be determined using a SPR assay on a Biacore instrument primed with HBS-EP running buffer (GE Healthcare #BR-1006-69) and analysis temperature may be set at either 25 °C or 37 °C. A CM4 chip (S series for the T100 at 37 °C) containing immobilized goat anti-(human or mouse) kappa (κ chain specific) or protein A may be generated using standard NHS-EDC amine coupling on two or all four flow cells (Fc) and used to employ a capture methodology. Antibody samples or VEGF-Fc receptors may be prepared at a concentration range between 1 and 10 μg/mL by dilution into running buffer. VEGFR1-His, Antibody II or Antibody IV may be prepared at different concentrations (concentration range of 200 nM to 0.078 nM in 2-fold dilution increments). Each analysis cycle may consist of (1) capturing antibody samples on immobilized protein A or anti-(human or mouse) kappa (κ chain specific) on separate flow cells (Fc2, Fc3, and Fc4), (2) injection of 250 μL (300-sec) of His-tagged receptor, Antibody II-Fab or Antibody IV over all Fc at 50 μL/min, (3) return to buffer flow for 20 min to monitor dissociation phase, (4) regeneration of chip surfaces with a 25 μL (30-sec) injection of glycine, pH 1.5, (5) equilibration of chip surfaces with a 25 μL (30-sec) injection of HBS-EP. Data may be processed using standard double-referencing and fit to a 1:1 binding model using Biacore evaluation software, version 4.1 for Biacore 2000 and 2.0.3 for Biacore T100, to determine the association rate (k_{on}, $M^{-1}s^{-1}$ units), dissociation

rate (k_{off}, s^{-1} units), and R_{max} (RU units). The equilibrium dissociation constant (K_D) may be calculated from the relationship $K_D = k_{off}/k_{on}$.

In experiments performed essentially as described in this Example 2, Antibodies I, II, III, and IV bind their corresponding species of VEGFR1 with similarly high binding affinity (K_D) (see Table 2). Antibodies II and IV both show good selectivity for VEGFR1 by not binding to VEGFR2 and VEGFR3 up to 200 nM of receptor. Antibody II has tight picomolar affinity for human and cynomolgus VEGFR1 receptors. The K_D of Antibody II for human VEGFR1 is 740 +/- 34 pM (n=3) at 37 °C and 554 +/- 29 pM (n=3) for cynomolgus VEGFR1 at 37 °C.

Table 2: *In vitro* binding parameters of Antibodies I, II, III, and IV to various species of VEGFR1, VEGFR2 or VEGFR3 receptors, using surface plasmon resonance (SPR)

Antibody	Receptor	Species	n	Temp (°C)	kon, 1/M 1/s	koff, 1/s	Kd, pM (a)
Ab I	VEGFR1	Human	3	25	(4.08 +/- 1.50) x 10^5	(1.00 +/- 0.01) x 10^{-5}	≤ 26 +/- 8.0
Ab II	VEGFR1	Human	6	25	(4.87 +/- 2.29) x 10^5	(1.42 +/- 1.0) x 10^{-5}	≤ 42 +/- 29
		Human	3	37	(3.25 +/- 0.03) x 10^5	(2.40 +/- 0.10) x 10^{-4}	740 +/- 34
		Cynomolgus	3	37	(1.49 +/- 0.04) x 10^5	(8.30 +/- 0.63) x 10^{-5}	554 +/- 29
		Mouse	1	25	*nd*	*nd*	*nd*
	VEGFR2	Human	1	25	*nd*	*nd*	*nd*
		Mouse	1	25	*nd*	*nd*	*nd*
	VEGFR3	Human	1	25	*nd*	*nd*	*nd*
		Mouse	1	25	*nd*	*nd*	*nd*
Ab III	VEGFR1	Mouse	1	25	5.77 x 10^5	1.87 x 10^{-5}	32
Ab IV	VEGFR1	Human	1	25	*nd*	*nd*	*nd*
		Mouse	4	25	(5.24 +/- 0.98) x 10^5	< 1.0 x10^{-5}[g]	≤ 18 +/- 4
	VEGFR2	Human	1	25	*nd*	*nd*	*nd*
		Mouse	1	25	*nd*	*nd*	*nd*
	VEGFR3	Human	1	25	*nd*	*nd*	*nd*
		Mouse	1	25	*nd*	*nd*	*nd*

(a) calculated as $K_D = k_{off}/k_{on}$ from each individual experiment and the final value is obtained by averaging several independent K_D values. nd = not determined because no binding was observed up to ~200 nM concentration of the receptors

Example 3: Internalization of VEGFR1 by Antibody II and Antibody IV in cultured cells expressing chimeric human VEGFR1/EpoR or mouse VEGFR1/mouseEpoR receptor

The ability of VEGFR1 antibodies to internalize, and subsequently degrade, VEGFR1 can be determined by measuring internalization of VEGFR1 in cells expressing chimeric mouse VEGFR1/mouse EpoR or human VEGFR1/ mouse EpoR receptor. VEGFR1 internalization may be used in the analysis to demonstrate that the disclosed antibodies to mouse VEGFR1 function similarly *in vitro* to the disclosed antibodies to human VEGFR1. BaF3 cells survive in the absence of IL-3 when expressing the chimeric VEGFR1/Epo receptors. Decreasing cell surface chimeric VEGFR1/EpoR by antibody mediated internalization results in cell death.

Human and mouse VEGFR1 extracellular domain and trans-membrane domain may be fused with intracellular domain of Epo receptor. The chimeric receptors may be cloned into pMSCV puro retroviral vector (Clontech, catalog number 634401). BaF3-mouseVEGFR1-mouseEpoR cells and BaF3-humanVEGFR1-mouseEpoR cells may be generated by retrovirus infection. The retrovirus may be produced by transfecting Phoenix Eco retroviral packaging cells (ATCC) with mouse VEGFR1/mouseEpoR or humanVEGFR1/mouseEpoR respectively. The retroviral particles may be used to transduce BaF3 cells. The BaF3-VEGFR1-EpoR cells may be cultured in RPMI-1640 (Thermo Scientific, #SH30255.01), 10% (v/v) FBS (Invitrogen, #10082), 1mM sodium pyruvate (Thermo Scientific, #SH30239.01), 100 U/500mL penicillin G, and 100 µg/500 mL streptomycin (Thermo Scientific, #SV30079.01), 2 µg/mL puromycin (Calbiochem #540411); 5ng/mL murine IL-3 (R & D #403-ML-CF).

To perform the internalization assay, transduced BaF3 cells may be washed three times with 20 mL of culture medium without IL-3 to wash out IL-3. 100 µl of cells in culture medium without IL-3 may be added to each well of white/clear 96-well tissue

culture plates (BD #353377) to achieve 1-2x10^4cells/well. VEGFR1 and isotype control antibodies may be serially diluted with culture medium without IL-3 to achieve 2X the final concentrations to be tested, and then 100 µl of the 2X antibody solutions may be added to each well. The plates may then be incubated at 37°C under 95% relative humidity and 5% (v/v) CO_2. After culture for six days, the number of viable cells may be determined by CellTiter-Glo Luminescent Cell Viability Assay (Promega #G7571). The plates and CellTiter-Glo substrates may be equilibrated to room temperature for 30 minutes. 100 µl of CellTiter-Glo may be added to each well. The plates may be shaken for 2 minutes on an orbital shaker to mix contents and continued to incubate at room temperature for 10 minutes to stabilize luminescent signal. The luminescence may be recorded by a Wallac Victor3TM 1420 Multilable Counter (PerkinElmer Precisely). The percentage of cell variability in VEGFR1 and control IgG antibodies treated groups may be calculated by comparing to BaF3 cells without antibody treatment. The average of triplicate treatments of each dose may be used as mean and for standard error calculation. T-test may be used to compare data between VEGFR1 and control antibodies at same doses. A P value of less than 0.05 may be considered as statistical significant.

In experiments performed essentially as described in this Example 3, with incubation of different doses of Antibody II, the antibody inhibits BaF3-mouseVEGFR1-mouseEpoR cell proliferation as exhibited by the significant decrease in viability compared to human IgG4 control antibody (Table 3). Similarly, after six days of incubation with different dose of Antibody IV, the BaF3-mouse VEGFR1-mouse EpoR cells showed more cell death as compared to mouse IgG1 control antibody (Table 3). These results demonstrate internalization of the cell surface VEGFR1 receptor by both Antibody II and IV. Furthermore, the results indicate that the binding of Antibody II and IV to the chimeric VEGFR1 on BaF3 cells does not stimulate receptor activation and cell proliferation.

Table 3: Antibody II and IV decrease cell viability in BaF3 cells expressing chimeric mouse and human VEGFR1/EpoR

Antibody II (nM)	0.0003	0.001	0.006	0.03	0.16	0.8	4	20	100
Cell viability (%)	88.02 +/-7.06	95.30 +/- 6.09	74.62 +/- 4.17	59.24 +/- 8.78	35.75 +/- 1.22	33.22 +/- 5.28	34.28 +/- 1.77	23.35 +/- 2.23	26.53 +/- 2.85
Antibody IV (nM)	0.0003	0.001	0.006	0.03	0.16	0.8	4	20	100
Cell viability (%)	97.50 +/-6.88	108.28 +/- 19.14	93.9 +/- 8.21	84.37 +/- 3.61	54.77 +/- 7.21	13.29 +/- 1.66	7.17 +/- 1.05	4.28 +/- 0.35	2.60 +/- 0.37

Data displays the average and standard error of triplicate treatments and is representative of two experiments.

Example 4: Neutralization of human VEGF-A and PlGF binding to human VEGFR1 by Antibody II measured by solid phase ELISA and cell based VEGFR1 phosphorylation assays

The ability of VEGFR1 antibodies to bind to VEGFR1 and neutralize binding of VEGF-A and PlGF to VEGFR1 can be measured by solid phase ELISA and by cell-based VEGFR1 phosphorylation assays. VEGF-A and PlGF neutralization may be used in the analysis to demonstrate that the disclosed antibodies to mouse VEGFR1 function similarly *in vitro* to the disclosed antibodies to human VEGFR1.

For the solid phase ELISA, a 96-well plate may be coated with 1 µg/mL of hVEGFR1-Fc (R&D 321-fl 050/cf) in PBS at 50 µl/well, and then incubated overnight at 4°C. The solution may then be removed from the wells, and then the wells may be blocked for 1 hour with 100 µl of 1% casein at room temperature. Antibodies (including control Ron antibody (R&D MAB691)) may be titrated in a microtiter plate against PBSt (PBS containing Tween 20) starting at 50 µg/mL, and diluting with 1:3 dilutions to 0.023 µg/mL (see Table 4a). The ELISA plate may be washed 3 times with PBSt, and then 50 µl/well of pre-titrated antibody may be added. After a one hour incubation at 37 °C, 50 µl of biotinylated ligand (2 nM hPLGF1 (Pepro Tech #AF-100 06) or 2 nM hVEGFA-

165 (R&D #293-VE) may be added to each well, and incubation continued at 37°C for 30 min. The plate may be washed 3 times with PBSt, and then 50 µl/well of NA-AP (1:1000 dilution) may be added, with an incubation at room temperature of 15 min. After washing three more times, 100 µl/well of PMP (1:35 diluted in water) may be added. The plate may then be developed for 30 min and read at OD_{560}.

For the cell-based VEGFR1 phosphorylation assay, PAE-R1 cells expressing human VEGFR1 may be seeded into 24-well tissue culture plates at 100,000 cells/well, and cultured overnight at 37°C with 5% CO_2. The culture medium may then be changed to 500 µl of starvation medium (DMEM/F12 containing 0.1%BSA), and cultured overnight. Antibodies may then be added into the medium, and the cells cultured for 30 minutes. Human VEGF-A165 (R&D cat#293-VE) or human PlGF (R&D cat#264-PG) may then be added and the cells incubated for another 10 minutes. Phosphorylated human VEGFR1 may be determined using an ELISA (R&D Duo Set IC ELISA development kit, #DYC4170-2).

Data may be expressed as mean +/- standard error. JMP8 may be used for ANOVA analysis followed by Dunnett's comparison. IC_{50} may be calculated using GraphPad Prism version 4. A P value less than 0.05 may be considered as statistically significant.

In experiments performed essentially as described above in this Example 4, Antibody II neutralizes human VEGF-A and PlGF binding to VEGFR1, and also suppresses human VEGF-A and PlGF stimulated VEGFR1 phosphorylation. Antibody II neutralizes PlGF and VEGF-A binding to VEGFR1 with an IC_{50} of 0.43 nM and 0.14 nM, respectively (Table 4a).

Data points at low antibody concentrations are required to accurately calculate the IC_{50}. Accordingly, the experiment is repeated at antibody concentrations starting at 5 µg/mL and diluting with 1:3 dilutions to 0.0023 µg/mL. The experimental procedure may be as described below.

For the solid phase ELISA, a 96-well plate may be coated with 1 µg/mL of hVEGFR1-Fc (R&D 321-fl 050/cf) in PBS at 50 µl/well, and then incubated overnight at 4°C. The solution may then be removed from the wells, and then the wells may be

blocked for 1 hour with 100 µl of 1% casein at room temperature. Antibodies (including control Ron antibody (R&D MAB691)) may be titrated in a microtiter plate against PBSt (PBS containing Tween 20) starting at 5 µg/mL, and diluting with 1:3 dilutions to 0.0023 µg/mL (see Table 4b). The ELISA plate may be washed 3 times with PBSt, and then 50 µl/well of pre-titrated antibody may be added. After a one hour incubation at 37 °C, 50 µl of biotinylated ligand (4 nM hPLGF1 (R&D Cat# 264-PG-010) or 2 nM hVEGFA-165 (R&D #293-VE) may be added to each well, and incubation continued at 37°C for 30 min. The plate may be washed 3 times with PBSt, and then 50 µl/well of NA-AP (1:1000 dilution) may be added, with an incubation at room temperature of 15 min. After washing three more times, 100 µl/well of PMP (1:35 diluted in water) may be added. The plate may then be developed for 30 min and read at OD_{560}.

Data may be expressed as mean +/- standard error. JMP8 may be used for ANOVA analysis followed by Dunnett's comparison. IC_{50} may be calculated using GraphPad Prism version 6. A P value less than 0.05 may be considered as statistically significant.

In the repeated experiments performed essentially as described above, Antibody II neutralizes human VEGF-A and PlGF binding to VEGFR1, and also suppresses human VEGF-A and PlGF stimulated VEGFR1 phosphorylation. Antibody II neutralizes PlGF and VEGF-A binding to VEGFR1 with an IC_{50} of 0.31 nM and 1.02 nM, respectively (Table 4b).

Antibody II blocks both VEGF and PlGF stimulated VEGFR1 phosphorylation in vitro as seen in Table 5.

Table 4a: Neutralization of human VEGF-A and PlGF binding

Antibody II blocks human PlGF binding to VEGFR1								
Ab II (µg/ml)	50.000	16.667	5.556	1.852	0.617	0.206	0.069	0.023
OD-Ab II	0.130	0.126	0.129	0.127	0.120	0.123	0.150	0.213
Control Ab (µg/ml)	50.000	16.667	5.556	1.852	0.617	0.206	0.069	0.023
OD-Control mAb	0.339	0.304	0.298	0.300	0.295	0.315	0.283	0.296

Antibody II blocks human VEGF-A binding to VEGFR1								
Ab II (µg/ml)	50.000	16.667	5.556	1.852	0.617	0.206	0.069	0.023
OD-Ab II	0.135	0.134	0.144	0.181	0.166	0.194	0.296	0.414
Control Ab (µg/ml)	50.000	16.667	5.556	1.852	0.617	0.206	0.069	0.023
OD-Control Ab	0.515	0.566	0.535	0.530	0.535	0.554	0.519	0.506

Table 4b: Neutralization of human VEGF-A and PlGF binding

Antibody II blocks human PlGF binding to VEGFR1								
Ab II (µg/ml)	5.0000	1.6667	0.5556	0.1852	0.0617	0.0206	0.0069	0.0023
OD-Ab II	0.1366	0.1325	0.1350	0.1645	0.2346	0.3747	0.3955	0.4291
Control Ab (µg/ml)	5.0000	1.6667	0.5556	0.1852	0.0617	0.0206	0.0069	0.0023
OD-Control mAb	0.4068	0.4318	0.4363	0.4052	0.4076	0.3886	0.370	0.3593
Antibody II blocks human VEGF-A binding to VEGFR1								
Ab II (µg/ml)	5.0000	1.6667	0.5556	0.1852	0.0617	0.0206	0.0069	0.0023
OD-Ab II	0.3614	0.4569	0.5745	0.8829	1.2888	1.5889	1.4418	1.598
Control Ab (µg/ml)	5.0000	1.6667	0.5556	0.1852	0.0617	0.0206	0.0069	0.0023
OD-Control Ab	1.5116	1.4165	1.4701	1.4571	1.5133	1.6151	1.5255	1.5426

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Table 5: Phosphorylation in cultured PAE-R1 cells expressing human VEGFR1

	pVEGFR1 (OD)
SFM	0.2590 +/- 0.0090
Human IgG4	0.2390 +/- 0.0090
Antibody II	0.2445 +/- 0.0075
VEGF-A	0.5430 +/- 0.0270
Antibody II + VEGF-A	0.3150 +/- 0.0040
PlGF	0.5330 +/- 0.0240
Antibody II + PlGF	0.2735 +/- 0.0015

$P < 0.001$. Comparisons with a control (SFM) using Dunnett's Method.

Example 5: Antibody II and IV attenuate the suppressing effect of sFlt1 on VEGF-A mediated ERK phosphorylation

The ability of VEGFR1 antibodies to attenuate the suppression of VEGF-A mediated ERK phosphorylation by sFlt1 can be measured in cultured hUVEC and bEnd3 cells. Suppression of VEGF-A mediated ERK phosphorylation may be used in the analysis to demonstrate that the disclosed antibodies to mouse VEGFR1 function similarly *in vitro* to the disclosed antibodies to human VEGFR1.

For measurement in bEnd3 cells, bEnd3 cells may be purchased from ATCC (#CRL-2299). The bEnd3 cells may be resuspended to $3x10^5$ cells/mL in DMEM/high glucose medium (HyClone #SH30243.01) containing Anti/Anti (Thermo #SV30079.01) and 10% fetal bovine serum (Invitrogen #10082-147). 0.5 mL of resuspended bEnd.3 cells (containing approximately $1.5x10^5$ cells) may be added to each well of a 24-well microtiter plates (Costar #3524) and incubated at 37°C, 5% CO_2, for 24 hrs. Medium may then be aspirated, and then cells may be starved in 500 µL/well of DMEM/high glucose medium containing Anti/Anti and 0.1% bovine serum albumin (BSA) (Sigma #A7979) at 37°C, 5% CO_2, for 15 hours. Before adding VEGF-A, sFlt1, and/or VEGFR1 antibody, the medium may be changed to fresh starvation medium with 0.1% BSA.

For VEGF-A treatment, 50 µL of 10X concentrations of mouse VEGF164 (8 ng/mL final) (R&D #493-MV/CF) may be added to each well and cells incubated for 10 min at 37°C, 5% CO_2. For VEGF-A plus sFlt1 treatment, 30 µL of 20X concentrations of mouse VEGF164 (8 ng/mL final) may be pre-mixed with 30 µL of mouse sFlt1 (40 ng/mL final, R&D #471-F1) for 30 min at 37°C, then the 50 µL of mixture may be added to each well and the cells incubated for 10 min at 37°C, 5% CO_2. For VEGF-A plus sFlt1 and Antibody IV, 20 µL of 30X concentrations of Antibody IV (500 ng/mL final) may be pre-mixed with mouse sFlt1 (40 ng/mL final, R&D 471-F1) for 30 min at 37°C. Next, 40 µL of 30X concentrations of mouse VEGF164 (8ng/mL final) may be added to the mixture of Antibody IV and sFlt1. 50 µL of the final mixture may be added to each well and cells incubated for 10 min at 37°C, 5% CO_2. For the serum free medium control group, 50 µL of starvation medium with 0.1% BSA may be added to each well and cells incubated for 10 min at 37°C, 5% CO_2.

For measurement in hUVE cells, hUVE cells may be resuspended to $3x10^5$ cells/mL in EGM-2 medium (Lonza # CC-3156) with supplements (Lonza # CC-4176). 0.5 mL of resuspended hUVE cells may be added to each well of 24-well microtiter plates (Costar #3524) ($1.5x10^5$ cells/well) and incubated at 37°C, 5% CO_2, for 24 hours. The medium may then be changed to 500 µL/well EGM-2 medium containing 0.1% bovine serum albumin (BSA) (Sigma #A7979) for starvation for 15 hours at 37°C, 5% CO_2. Before adding treatment compound, the medium may be changed to fresh starvation medium with 0.1% BSA. For VEGF-A treatment, 50 µL of 10X concentrations of human VEGF165 (1.5ng/mL final) (R&D 293-VE) may be added and cells incubated at 37°C, 5% CO_2, for 10 min. For VEGF-A plus sFlt1, 30 µL of 20X concentrations of human VEGF165 (1.5ng/mL final) may be mixed with human sFlt1 (40ng/mL final, R&D 321-FL/CF) at 37°C for 30 min. 50ul of the mixture may then be added to the well and cells incubated at 37°C, 5% CO_2, for10 min. For VEGF-A plus sFlt1 and Antibody II, 20 µL of 30X concentrations of Antibody II (73 µg/mL final) may be pre-mixed with human sFlt1 (40ng/mL final) for 30 min at 37°C, and 40 µL of 30X concentrations of human VEGF165 (1.5ng/mL final) may then be added to the mixture of VEGF-A and sFlt1. 50uL of the final mixture may be added to the well and cells incubated at 37°C, 5% CO_2, for 10 min. For the serum free medium control group, 50 µL of starvation medium with 0.1% BSA may be added to each well and cells incubated at 37°C, 5% CO_2, for 10 min.

For cell lysate preparation, 10 mL of Tris Lysis buffer (MSD) may be mixed with 200 µL protease inhibitor solution (50x stock), 100 µL phosphatase inhibitor I (100x stock), 100 µL phosphatase inhibitor II (100x stock) , 40 µL phenylmethanesulfonylfuoride (PMSF) in DMSO (250x stock), and 100 µL SDS (10% stock). PMSF and SDS may be added immediately before using. After removing treatment medium, 50 µL of cell lysis buffer may be added to each well. The cells may be incubated with lysis buffer on ice for 10 min and then shaken at 4°C for 30 min. The protein concentration of the lysate may be determined using Pierce BCA protein assay kit, cat#23227.

For the ERK1/2 phosphorylation assay, phosphorylated ERK1/2 may be determined following the protocol of a sandwich immunoassay developed by MSD

(Phospho-ERK1/2 (Thr202/Tyr204; Thr185/Tyr187) Assay Whole Cell Lysate Kit; MSD #K151DWD-1). The assay may use a plate pre-coated with capture antibody for phosphorylated ERK1/2 (Thr202/Tyr204; Thr185/Tyr187). After adding samples, a solution containing the detection antibody, anti-total ERK1/2 conjugated with an electrochemiluminescent compound (MSD SULFO-TAG label), may be added. The MSD SECTOR Imager may be used for measuring intensity of emitted light correlated with phosphorylated ERK1/2 level in the sample. Both capture and detection antibodies cross react with human and mouse whole cell lysates.

Data may be expressed as mean +/- standard error. JMP8 may be used for ANOVA analysis followed by Dunnett's comparison.

In experiments performed essentially as described in this Example 5, Antibody II and Antibody IV attenuate sFlt1 activity in cultured hUVEC and bEnd3 cells, respectively (Table 6). Pre-incubation of sFlt1 with Antibody IV before mixing with mouse VEGF-A164 significantly prevents sFlt1's suppressive effect on VEGF-A stimulated ERK1/2 phosphorylation in bEnd3 cells. Final concentration of Antibody IV is 500 ng/mL in the study. Similar to Antibody IV, pre-incubation of sFlt with Antibody II before mixing with human VEGF-A165 significantly decreases VEGF-A165 stimulated ERK1/2 phosphorylation in hUVE cells. Final concentration of Antibody II is 73 μg/mL in the study. These results demonstrate that both Antibody II and IV block the Flt1 trapping of VEGF-A, which may result in increased accessibility of VEGF-A to VEGFR2.

Table 6: Antibody II and IV attenuate sFlt1 activity in bEnd3 and hUVE cells

hUVEC	pERK1/2 (OD)	**bEnd3**	pERK1/2(OD)
Control	2940.5 +/- 358.5	Control	2907.0 +/- 426.5
hVEGF-A	24418.0 +/- 2300.0*	mVEGF-A	17106.5 +/- 1185.5**
hVEGF-A+sFlt1	10532.5 +/- 3080.5	mVEGF-A+sFlt1	3064.0 +/- 42.0
hVEGFA+sFlt1 +Antibody II	20877.5 +/-1354.5*	mVEGFA+sFlt1 +Antibody IV	16438.5 +/- 346.5**

* $p<0.01$, ** $p<0.001$. Comparisons with a control (SFM) using Dunnett's Method.

Example 6: Antibody III and IV decreases albuminuria and improves renal histopathological lesions in remnant kidney mouse model

The potential for VEGFR1 antibodies to improve albuminuria and renal histopathological lesions, both indicators of CKD, can be measured in vivo in a remnant kidney mouse model (Leelahavanichkul, et al. (2010) Kidney Int. 78(11): 1136–1153).

The remnant kidney model may be generated by surgically remove three-quarters of the mouse kidney mass at approximately 8 weeks of age. The model resembles human chronic kidney disease (CKD) developing albuminuria, hypertension, and renal lesions including mesangial expansion, glomerulosclerosis, and interstitial fibrosis. Remnant kidney surgery may be conducted in 129S6/SvEvTac male mice at 8-9 weeks of age. A modified one stage procedure for three-quarters reduction of total renal mass may be used where two poles of one kidney may be excised using cauterization, followed by a nephrectomy of the second kidney. Surgical staples may be removed one week post surgery. Mice may be housed individually on High Density racks in micro-isolator caging, sawdust bedding nestles for enrichment, in a room maintained at 75 ^{o}F on a 12 hour light/dark cycle and humidity 50%. Mice may have free access to bottle water and Purina 5008 diet. House water may be tap water filtered through reverse osmosis and chlorinated, with pH 6.5-7. The remnant kidney mice may be randomized by baseline urinary ACR and body weight 2 weeks post surgery.

For urine collection and urinary albumin and creatinine measurement, spot urine may be collected by placing a single animal on a 96 well Corning #3359 polypropylene microtiter plate. A plexiglas housing chamber may be secured over the mouse and plate. The urine may be transferred into a 1.5mL Eppendorf tube on ice using a micropipette, and centrifuged at 10,000 rpm for 5 minutes. Urinary albumin may be measured using an internally validated assay and urinary creatinine may be measured using an enzymatic method.

For renal pathology, kidneys may be collected at the end of studies, fixed in formalin, and processed for paraffin sectioning according to standard methodology. Sections of kidney may be evaluated for renal lesions by a pathologist. Mesangial matrix, glomerular fibrosis, and interstitial fibrosis, may be semi-quantitatively scored using the

following scale: none (0), minimal (1), slight (2), moderate (3), marked (4) and severe (5). Glomerular mesangial matrix expansion and basement membrane thickening may be scored using H&E and PAS stained sections. Masson's trichrome stained sections of kidney may be evaluated to determine the degree of fibrosis (interstitial and glomerular).

For measurement of systolic blood pressure, blood pressure may be measured using tail cuff method (Coda System, Kent Scientific) where mice may be acclimated to the restraint by placing them in the mouse holder with tail cuff attached for 5 minutes daily, 3-5 days prior to the actual measurement. The equipment room temperature may be increased to 75 °F to provide additional warmth during the blood pressure collection process. Mice may be selected and randomized. The mice may be placed in a holder/restrainer and set on top of the Coda warming pad unit (31-33 °C). The tail may be placed through the tail cuffs and each mouse may be restrained for approximately 30 minutes. The tail cuff may be inflated, compressing the tail tightly enough to momentarily interrupt arterial blood flow, and then gradually loosened by deflation to observe the return of the arterial pulse. On return of arterial pulse, the cuff may be fully deflated. Average of repeat measurements from one mouse may be used as level of systolic blood pressure for that mouse.

Data may be presented as mean +/- SE. GraphPad Prism or JMP may be used for ANOVA or unpaired t-test analysis. For analysis, urinary ACR data may be transformed to logarithm values. Graphpad Prism or JMP may be used for ANOVA analysis followed by Dunnett's multiple comparison test. A P value less than 0.05 may be considered statistically significant.

In experiments performed essentially as described in this Example 6, three studies are performed. For Study 1, six groups are included: (1) PBS, three time a week (tiw), n = 12; (2) mouse IgG1 at 10 mg/kg, tiw, n = 12; (3) Antibody IV at 10 mg/kg, tiw, n = 12; (4) Antibody IV at 3 mg/kg, tiw, n = 12; (5) Antibody IV at 3 mg/kg, qw, n = 12; and (6) Antibody IV at 1 mg/kg, tiw, n = 12. 0.2mL of testing or control compounds is injected subcutaneously (sc) at the doses and intervals indicated above for sixteen weeks. Spot urine and body weight are collected at baseline (before treatment) and again at weeks 3, 6, 10, 12 and 16 of dosing. Systolic blood pressures are collected at week 16 of dosing.

Samples collected at the end of the eight weeks of study include EDTA-anticoagulated whole blood and kidneys. EDTA plasma is used to measure BUN, creatinine, and other parameters. One half of the coronally sectioned remnant kidney is fixed in 10% nBF (neutral buffered formalin) for histopathological examination, the other half of remnant kidney is flash frozen in -80°C for future analysis.

For Study 2, five groups are included in this study: (1) sham control (n = 4, without treatment); (2) PBS control (n = 10); (3) rat IgG1 at 10 mg/kg (n = 10); (4) Antibody III at 10 mg/kg, (n = 10); and (5) Antibody III at 3mg/kg (n = 10). All groups are dosed three times (tiw) a week intraperitoneally (i.p.) at a volume of 0.2mL/mouse, for eight weeks. Spot urine is collected and body weight measured at randomization (baseline) and again at weeks 4, 6 and 8 of dosing. Systolic blood pressures are collected at weeks 4, and 8 of dosing. Blood pressure is determined using tail cuff. Endpoint samples collection is the same as described in Study 1.

For Study 3, nine groups are included: (1) sham operation group, no treatment, n = 4; (2) PBS control, tiw, n = 6; (3) rat IgG1 at 30 mg/kg, biw, n = 10; (4) Antibody III at 30 mg/kg, biw, n = 10; (5) Antibody III at 10 mg/kg, tiw, n = 10; (6) Antibody III at 10 mg/kg, qw, n = 10; (7) Antibody III at 3 mg/kg, tiw, n = 10; (8) Antibody III at 3 mg/kg, qw, n = 10; and (9) Antibody III at 1 mg/kg, qw, n = 10. Compounds are injected subcutaneously (sc) at a volume of 0.2 mL/mouse for six weeks. Spot urine and body weight are collected at baseline (before treatment) and at weeks 2, 4 and 6 of dosing. Systolic blood pressures are collected at week 6 of dosing using tail cuff method. Endpoint sample collection is the same as described in Study 1.

Studies 1, 2, and 3 demonstrate that Antibody III (3 and 10 mg/kg) and Antibody IV (1, 3, and 10 mg/kg) significantly decrease albuminuria in comparison to the control at the corresponding time point, as measured by urinary albumin/creatinine (ACR), in remnant kidney mice (Tables 7-9). Antibody III and Antibody IV at the 10 mg/kg, 30 mg/kg, and 3 mg/kg (tiw) dosing groups also improve renal histopathological scores, as measured by renal glomerular fibrosis, interstitial fibrosis, and Masson's scores, in remnant kidney mice (Tables 10-11).

Table 7: Antibody IV decreases ACR in remnant kidney mice (Study 1)

Group	Baseline (µg/mg)	Week 3 (µg/mg)	Week 6 (µg/mg)	Week 10 (µg/mg)	Week 16 (µg/mg)
PBS Control	1255.5 +/- 584.2	2008.6 +/- 655.5	3363.0 +/- 1396.7	3090.1 +/- 1060.3	3926.3 +/- 1780.4
mIgG1 10mg/kg tiw	1051.5 +/- 501.0	1780.0 +/- 571.6	2628.3 +/- 795.3	3559.3 +/- 1414.3	2269.3 +/- 646.1
Antibody IV 10mg/kg tiw	1069.9 +/- 369.0	674.9 +/- 355.4	125.5 +/- 37.6	253.7 +/- 71.5	231.2 +/- 111.2
Antibody IV 3mg/kg tiw	992.5 +/- 308.8	410.1 +/- 259.3	509.5 +/- 170.2	780.7 +/- 224.1	538.3 +/- 165.7
Antibody IV 3mg/kg qw	923.9 +/- 241.1	1654.1 +/- 654.5	1804.3 +/- 612.1	2107.4 +/- 619.7	1591.3 +/- 488.3
Antibody IV 1mg/kg tiw	1015.7 +/- 331.9	577.0 +/- 151.8	947.0 +/- 154.1	1188.0 +/- 284.2	926.9 +/- 218.8

Data presented as mean +/- SE

Table 8: Antibody III decreases ACR in remnant kidney mice (Study 2)

Group	n	baseline (µg/mg)	week 4 (µg/mg)	week 6 (µg/mg)	week 8 (µg/mg)
Sham Control	4	19.2 +/- 4.4	12.5 +/- 1.2	12.1 +/-2.0	9.0 +/- 1.0
PBS Control	10	1423.0 +/- 511.3	3406.6 +/- 861.5	2966.1 +/- 1136.5	2426.7 +/- 872.6
rIgG1 10mg/kg, tiw	10	1293.0 +/- 408.5	5780.9 +/- 2170.2	5390.9 +/- 2387.3	6771.5 +/- 3277.7
Antibody III 10mg/kg, tiw	10	1529.4 +/- 808.7	138.3 +/- 61.4	53.9 +/- 21.4	80.5 +/- 35.5
Antibody III 3mg/kg, tiw	10	1792.7 +/- 772.5	937.3 +/- 507.5	2249.8 +/- 1501.5	2054.8 +/- 1024.8

Data presented as mean +/- SE

Table 9: Antibody III decreases ACR in remnant kidney mice (Study 3)

Group	n	Baseline (μg/mg)	week2 (μg/mg)	week 4 (μg/mg)	week 6 (μg/mg)
Sham	4	19.5 +/- 2.7	14.2 +/- 0.5	11.1 +/- 1.1	11.8 +/- 2.6
Saline	6	1650.7 +/- 1047.1	1007.5 +/- 242.0	1502.5 +/- 335.0	1724.1 +/- 66.7
rIgG1 30mg/kg biw	10	1444.3 +/- 358.6	1940.8 +/- 374.0	2594.3 +/- 617.3	2855.6 +/- 565.0
Ab III 30mg/kg biw	10	1644.7 +/- 318.0	642.1 +/- 141.1	217.7 +/- 81.8	164.4 +/- 72.0
Ab III 10mg/kg tiw	10	2246.3 +/- 657.1	1066.0 +/- 301.5	337.8 +/- 99.0	649.0 +/- 299.6
Ab III 10mg/kg qw	10	1663.0 +/- 336.2	1002.1 +/- 194.4	593.7 +/- 223.1	1380.5 +/- 536.5
Ab III 3mg/kg tiw	10	1833.9 +/- 496.9	717.1 +/- 180.9	250.9 +/- 71.6	555.2 +/- 106.9
Ab III 3mg/kg qw	10	1741.6 +/- 605.7	1223.4 +/- 270.5	1617.1 +/- 205.7	3023.9 +/- 426.5
Ab III 1mg/kg qw	10	1938.7 +/- 464.3	1880.2 +/- 651.0	2561.8 +/- 827.9	4085.9 +/- 1249.1

Data presented as mean +/- SE

Table 10: Renal histopathological scores in remnant kidney mice (Study 2)

Group	Glomerular fibrosis score	Interstitial fibrosis score	Systolic BP (mmHg)
mIgG1 10mg/kg tiw	0.8 +/- 0.3	1.3 +/- 0.3	170 +/- 1.4
Ab III 10mg/kg tiw	0.0 +/- 0.0	0.3 +/- 0.1	145 +/- 2.2

Table 11: Renal histopathological scores in remnant kidney mice (Study 3)

Group	n	Interstitial fibrosis score	Masson's score	Glomerular fibrosis score
Sham	4	0	0	0
Saline	6	1.17 +/- 0.17	1.17 +/- 0.17	0.50 +/- 0.22
rIgG1 30mg/kg biw	10	1.50 +/- 0.27	1.30 +/- 0.15	0.30 +/- 0.15
Ab III 30mg/kg biw	10	0.30 +/- 0.15	0.30 +/- 0.15	0
Ab III 10mg/kg tiw	10	0.50 +/- 0.17	0.50 +/- 0.17	0
Ab III 10mg/kg qw	10	0.40 +/- 0.22	0.40 +/- 0.22	0.30 +/- 0.15
Ab III 3mg/kg tiw	10	0.30 +/- 0.15	0.30 +/- 0.15	0.10 +/- 0.10
Ab III 3mg/kg qw	10	1.20 +/- 0.33	1.20 +/- 0.33	0.50 +/- 0.17
Ab III 1mg/kg qw	10	1.20 +/- 0.25	1.20 +/- 0.25	0.20 +/- 0.13

Example 7: Antibody III decreases albuminuria and improves renal histological lesions in diabetic db/db and uninephrectomized db/db mice

The potential for VEGFR1 antibodies to improve albuminuria and renal histological lesions, both indicators of CKD, can be measured in vivo in diabetic db/db and uninephrectomized db/db mice. db/db mice represent a type 2 diabetic mouse model developing albuminuria and renal histopathological lesions resembling human diabetic nephropathy (Sharma *et al.* (2003) *Am J Physiol Renal Physiol* 284: F1138). Uninephrectomized db/db mice develop more albuminuria and severe renal structural lesions than db/db mice without uninephrectomy (Ninichuk *et al.* (2007) Eur J Med Res 12:351).

For urine collection and urinary albumin and creatinine measurement, spot urine may be collected by placing a single animal on a 96 well Corning #3359 polypropylene microtiter plate. A plexiglas housing chamber may be secured over the mouse and plate. The urine may be transferred into a 1.5mL Eppendorf tube on ice using a micropipette, and centrifuged at 10,000 rpm for 5 minutes. Urinary albumin may be measured using an internally validated assay and urinary creatinine may be measured using an enzymatic method.

For renal pathology, kidneys may be collected at the end of studies, fixed in formalin, and processed for paraffin sectioning according to standard methodology. Sections of kidney may be evaluated for renal lesions by a pathologist. Mesangial matrix, glomerular fibrosis, and interstitial fibrosis, may be semi-quantitatively scored using the following scale: none (0), minimal (1), slight (2), moderate (3), marked (4) and severe (5). Glomerular mesangial matrix expansion and basement membrane thickening may be scored using H&E and PAS stained sections. Masson's trichrome stained sections of kidney may be evaluated to determine the degree of fibrosis (interstitial and glomerular).

Data may be presented as mean +/- SE. GraphPad Prism or JMP may be used for ANOVA analysis followed by Dunnett's multiple comparison test. A P value less than 0.05 may be considered statistically significant.

In experiments performed essentially as described in this Example 7, two studies are performed. In Study 4, male db/db male mice (BKS.Cg-+ *Lepr* ^{db}/+ *Lepr* ^{db}/OlaHsd)

are purchased from Harlan Laboratories (Indianapolis, IN), and randomized at seven weeks of age to receive PBS, control rat IgG1 at 10mg/kg, Antibody III at 10mg/kg, and Antibody III at 3mg/kg, respectively, three time per week (tiw) for six weeks. Mouse number in each group is ten except for PBS group with eight mice. Albumin/creatinine (ACR) is determined in spot urine collected at week 4 and 6 of treatment. Blood parameters and kidney histology are examined at the end of the six week study.

In Study 5, uninephrectomy surgery is conducted in db/db mice (BKS.Cg-+ Lepr db/+ Lepr db/OlaHsd) at 4 weeks of age, according to IACUC and their institutional guidelines. The mice are randomized at approximately 8-9 weeks of age. Seven groups are included in the study: (1) PBS control, n = 6; (2) rat IgG1 at 30 mg/kg, biw, n = 10; (3) Antibody III at 30 mg/kg, biw, n = 10; (4) Antibody III at 10 mg/kg, tiw, n = 10; (5) Antibody III at 10 mg/kg, qw, n = 10; (6) Antibody III at 3 mg/kg, qw, n = 10; and (7) Antibody III at 1 mg/kg, qw, n = 10. Animals are dosed at 0.2 mL/injection, subcutaneously (sc), for six weeks. Blood glucose, body weight, spot urine ACR are examined periodically as indicated in Tables 13 and 15. Blood creatinine, BUN, and renal histology are examined at the end of the six week study.

In Studies 4 and 5, Antibody III significantly decreased urinary albumin/creatinine (ACR) in comparison to the control antibody for both 10 mg/kg, tiw, and 3 mg/kg, tiw, doses in db/db mice, and for all tested doses (30mg/kg, tiw; 10mg/kg, tiw; 10mg/kg, qw; 3mg/kg, tiw; 3mg/kg, qw, and 1mg/kg, qw) in uninephrectomized db/db mice (Tables 12-13). Antibody III also improves renal histopathological scores, as measured by Mesangial Matrix Scores, and decreases blood urea nitrogen (BUN) in uninephrectomized db/db mice (Table 14 and 15).

Table 12: Antibody III decreases ACR in db/db mice (Study 4)

Group	n	Baseline (µg/mg)	Week 4 (µg/mg)	Week 6 (µg/mg)
PBS	8	273.3 +/- 61.5	438.8 +/- 64.6	520.3 +/- 64.5
rat IgG1 10mg/kg, tiw	10	281.3 +/- 49.9	540.8 +/- 61.4	519.1 +/- 66.6
Antibody III 10mg/kg, tiw	10	279.5 +/- 53.1	172.2 +/- 39.2	110.9 +/- 12.2
Antibody III 3mg/kg, tiw	10	287.4 +/- 52.6	265.4 +/- 57.3	150.1 +/- 24.0

Table 13: Antibody III decreases ACR in uninephrectomized db/db mice (Study 5)

Group	n	Baseline (µg/mg)	Week 2 (µg/mg)	Week 4 (µg/mg)	Week 6 (µg/mg)
Saline	6	477.9 +/- 166.4	622.5 +/- 108.8	1577.3 +/- 430.9	2199.5 +/- 327.4
Rat IgG 30mg/kg biw	10	459.7 +/- 103.4	1430.3 +/- 261.4	2501.9 +/- 284.2	2461.4 +/- 235.4
Antibody III 30mg/kg biw	10	471.5 +/- 94.7	576.1 +/- 217.9	467.0 +/- 88.5	468.3 +/- 155.8
Antibody III 10mg/kg tiw	10	445.0 +/- 78.9	609.2 +/- 110.6	631.2 +/- 132.9	646.0 +/- 123.9
Antibody III 10mg/kg qw	10	467.0 +/- 128.9	669.9 +/- 150.9	1377.6 +/- 386.1	726.1 +/- 91.7
Antibody III 3mg/kg tiw	10	433.9 +/- 148.1	1028.6 +/- 377.2	1308.7 +/- 246.9	1759.1 +/- 340.8
Antibody III 1 mg/kg qw	10	387.2 +/- 73.8	511.8 +/- 101.5	1367.9 +/- 247.5	1700.6 +/- 239.7

Table 14: Antibody III improves renal histological lesions in uninephrectomized db/db mice

	Animal number (n)	Mesangial Matrix Scores
Saline	6	1.8+/-0.2
Rat IgG1-30mg/kg biw	10	1.7+/-0.2
Antibody III - 30mg/kg biw	10	1.1+/-0.1
Antibody III -10mg/kg tiw	10	1.4+/-0.2
Antibody III -10mg/kg qw	10	1.1+/-0.1
Antibody III -3mg/kg qw	10	1.1+/-0.1
Antibody III -1mg/kg qw	10	1.0

Table 15: Effect of Antibody III on body weight, glucose, cholesterol, BUN, and FFA on uninephrectomized db/db mice

	Saline	Rat IgG1 30mg/kg biw	Ab III 30mg/kg biw	Ab III 10mg/kg tiw	Ab III 10mg/kg qw	Ab III 3mg/kg qw	Ab III 1mg/kg qw
Body weight (g)	56.1 +/- 1.6	54.4 +/- 2.9	58.4 +/- 1.0	52.8 +/- 0.8	57.4 +/- 0.6	56.4 +/- 0.6	56.4 +/- 0.8
Blood glucose (mg/dL)	551.2 +/- 23.5	581.6 +/- 9.9	585.1 +/- 9.5	588.5 +/- 10.9	601 +/- 0.0	548.2 +/- 19.2	535.4 +/- 24.4
Plasma BUN (mg/dL)	37.9 +/- 1.1	40.2 +/- 1.6	33.2 +/- 1.1	30.2 +/- 1.5	31.3 +/- 1.3	35.2 +/- 1.0	33.1 +/- 1.0
Cholesterol (mg/dL)	221.8 +/- 5.4	227.9 +/- 9.3	194.5 +/- 5.3	199.9 +/- 9.4	195.1 +/- 7.4	216.6 +/- 11.8	231.5 +/- 7.7
FFA (mmol/dL)	2.5 +/- 0.4	2.8 +/- 0.3	2.0 +/- 0.1	1.9 +/- 0.1	2.0 +/- 0.1	2.1 +/- 0.1	2.6 +/- 0.2

Example 8: Antibody IV decreases albuminuria, prevents serum creatinine increase, and decreases mortality in diabetic db/db-eNOS deficient mice

The potential for VEGFR1 antibodies to improve albuminuria and renal function, both indicators of CKD, can be measured in vivo in diabetic db/db-eNOS deficient mice. db/db-eNOS knockout mice represent a diabetic kidney injury model resembling a more

advanced stage human diabetic nephropathy. The mice develop hyperglycemia, albuminuria, arteriolar hyalinosis, GBM thickness, mesangial expansion, mesangiolysis, focal segmental and early nodular glomerulosclerosis, as well as decline of glomerular filtration rate (GFR) (Zhao et al. (2006) J Am Soc Nephrol 17:2664). Without treatment, the mice exhibit high mortality after 16-20 weeks of age.

For urine collection and urinary albumin and creatinine measurement, spot urine may be collected by placing a single animal on a 96 well Corning #3359 polypropylene microtiter plate. A plexiglas housing chamber may be secured over the mouse and plate. The urine may be transferred into a 1.5mL Eppendorf tube on ice using a micropipette, and centrifuged at 10,000 rpm for 5 minutes. Urinary albumin may be measured using an internally validated assay and urinary creatinine may be measured using an enzymatic method.

In experiments performed essentially as described in this Example 8, two studies examining Antibody IV efficacy are conducted in the diabetic db/db-eNOS knocking-out mice (Study 6 and Study 7).

For Study 6, the db/db- eNOS knock-out mice (BKS.Cg-Lepr<db>-Nos3<tm1Unc/Rhrs>) at 8 to 22 weeks of age are randomized into two groups: control group consisting of 6 male and five female mice receiving 10mg/kg of mouse IgG1 and treatment group consisting of 6 male and 4 female mice receiving 10mg/kg of Antibody IV. The antibodies are administrated three times a week (tiw), subcutaneously (sc), at a volume of 0.2 mL/injection, for 12 weeks. Serum creatinine is measured at the end of the study.

For Study 7, the db/db- eNOS knock-out mice are randomized into three groups to receive PBS, control mouse IgG1 at 10 mg/kg, and Antibody IV at 10 mg/kg, respectively. The PBS group consists of 11 mice including 5 male and 6 female mice. The Control IgG1 group consists of 11 mice including 6 male and 5 female mice. The Antibody IV group consists of 7 male and 5 female mice at the beginning of treatment. The Antibody IV and control reagents are administered three times a week (tiw), sc, for 12 weeks. Spot urine ACR levels are measured at baseline and week 2, 4, 6, 6, 10, and 12

of treatment. Serum creatinine is examined at baseline, and week 6 and week 12 of treatment.

Antibody IV significantly decreases albuminuria, as measured by urinary albumin/creatinine (ACR) in comparison to the control at the corresponding time point, in db/db-eNOS knockout mice (Table 16). Antibody IV also improved renal function, as measured by prevention of serum creatinine increase in diabetic db/db-eNOS knockout mice (Table 16). Among mice that survived to endpoint of the study, 69% in control group (PBS+IgG1, n=13) increased serum creatinine greater than 50% versus 10% in Antibody IV group (n=10; P=0.016 in chi-square test). Furthermore, 30% of mice in control group doubled serum creatinine while no mouse did in Antibody IV group. Antibody IV treatment decreases mortality in db/db-eNOS knock-out mice (Table 17).

Table 16: Antibody IV decreases ACR in diabetic db/db-eNOS deficient mice

Group	**Week 0 baseline** (μg/mg)	**Week 2** (μg/mg)	**Week 4** (μg/mg)	**Week 6** (μg/mg)	**Week 8** (μg/mg)	**Week 10** (μg/mg)	**Week 12** (μg/mg)
PBS	11502.6 +/- 1451.1	33350.3 +/- 2880.5	51168.3 +/- 7966.4	58561.6 +/- 9937.8	90540.6 +/- 19688.1	95784.9 +/- 17895.9	49514.8 +/- 8284.1
Control IgG1 10 mg/kg, tiw	13186.0 +/- 2112.4	31400.7 +/- 4660.2	45277.1 +/- 6009.7	58445.8 +/- 5991.3	63692.3 +/- 10104.8	92968.7 +/- 16816.8	137806.2 +/- 29612.0
Ab IV 10 mg/kg, tiw	16972.3 +/- 2693.0	21815.3 +/- 4686.3	28199.8 +/- 7232.3	25653.3 +/- 7353.8	19753.9 +/- 4596.1	28873.2 +/- 9990.1	36793.8 +/- 14604.3

Table 17: Survival rate (%) in Antibody IV and control antibody treated diabetic db/db-eNOS knockout mice (Study 6 and 7)

	week 0	week 4	week 8	week 10	week 12
control IgG1 10 mg/kg, tiw	100	93.94	72.73	57.58	45.45
Antibody IV 10 mg/kg, tiw	100	90.91	86.36	81.82	81.82

Example 9: Antibody II and III effect on blood PlGF levels in monkey and mouse

The ability of VEGFR1 antibodies to affect *in vivo* blood PlGF levels can be measured in monkey and mouse. For measurement of PlGF in monkey plasma, a PlGF ELISA assay (R&D Systems #DPG00) may be used. For measurement of PlGF in mouse blood, a PlGF ELISA assay (R&D Systems, Quantikine Mouse PlGF-2 immunoassay; catalog # MP200) may be used. Serum and plasma samples may be diluted 2-20 fold into calibrator diluent (R&D Systems #RD5-17) prior to assay. Standard curve may range from 23.4 to 1500 pg/ml for mouse PlGF, and 15.6 to 1000pg/ml for monkey PlGF. Data may be presented as mean +/- SE; and GraphPad Prism 4 may be used for data analysis.

In a Cynomolgus monkey study, the *in vivo* response of blood PlGF to Antibody II may be measured using plasma collected from four groups of monkeys (each group consisting of 4 male and 4 female monkeys) at 2.5-4.5 years of age. The monkeys may receive Antibody II at doses of 0, 3, 20, or 65 mg/kg, once a week (qw) for 13 weeks. The blood samples may be collected at the end of the study.

In a mouse study, the *in vivo* response of blood PlGF to Antibody III may be measured using blood collected from remnant mice or uninephrectomized db/db mice. In a remnant kidney mouse study, Antibody III may be dosed at 10 mg/kg and 3 mg/kg, three times a week (tiw), for eight weeks. In a uninephrectomized db/db study, Antibody III may be administrated at 30mg/kg biw, 10mg/kg tiw, 10mg/kg qw, 3mg/kg qw, and 1mg/kg qw, for 6 weeks. Plasma samples may be collected at the end of the studies.

In experiments performed essentially as described in this Example 9, Antibody II significantly elevates plasma PlGF levels in cynomolgus monkeys at doses of 3, 20, and

65 mg/kg, once a week, for 13 weeks (Table 18). Antibody III dose dependently increases blood PlGF in both remnant kidney and uninephrectomized mice (Table 19).

Table 18: Blood PlGF in Cynomolgus Monkey treated with Antibody II for 13 weeks

	Plasma PlGF (pg/mL)
Vehicle	4.3 +/- 1.7
Antibody II 3 mg/kg, qw	527.6 +/- 94.9
Antibody II 20 mg/kg, qw	805.4 +/- 56.9
Antibody II 65 mg/kg, qw	807.5 +/- 129.4

$P < 0.01$ vs. Vehicle group

Table 19: Blood PlGF in mice treated with Antibody III

Uninephrectomized db/db mouse study		**Remnant kidney mouse study**	
	Plasma PlGF (pg/ml)		**Plasma PlGF (pg/ml)**
Saline	12.6+/- 4.8	PBS	88.4+/-20.5
rIgG1-30mg/kg, biw	20.2+/-2.6	rIgG1-10mg/kg, tiw	173.6+/-30.1
Ab III-30mg/kg, biw	5849.3+/-530.4	Ab III -10mg/kg, tiw	5523.4+/-230.2
Ab III -10mg/kg, tiw	1605.1+/-201.7	Ab III -3mg/kg, tiw	1888.6+/-538.1
Ab III -10mg/kg, qw	520.8+/-117.3		
Ab III -3mg/kg, qw	20.1+/-2.4		
Ab III -1mg/kg, qw	21.6+/-1.8		

$P < 0.01$

Example 10: Renal VEGFR2 phosphorylation in mouse and monkey kidneys

The ability of VEGFR1 antibodies to affect renal VEGFR2 phosphorylation can be measured in the kidneys of monkey and mouse.

Monkey kidneys may be collected from a Cynomolgus monkey study in which four groups of monkeys (each group consisting of 4 male and 4 female monkeys) at 2.5-4.5 years of age may be treated with Antibody II at doses of 0, 3, 20, and 65 mg/kg, respectively, once a week (qw), for 13 weeks. Monkeys in dose 0 group may receive a vehicle (PBS, pH7.4) injection. The kidney samples may be collected and frozen at the end of the study.

Monkey kidney homogenate may be prepared using QIAGEN TissueLyser where approximately 150 µg of monkey kidney tissue may be placed in a 2ml QIAGEN tube on ice and then add 500 µL Sample Buffer (MSD phosphor-VEGFR2 cat# K151DJD-1). The tube may be shaken on a QIAGEN TissueLyser with stainless steel beads (5mm, QIAGEN #69989) at 6.5 speeds for 60 seconds. The tube may be incubated on ice for 5 minutes followed by rotating tubes at 4°C for 30 minutes. The tube may then be centrifuged at 4°C and 8,000 rpm for 10 minutes. The supernatant may be removed to a fresh Eppendorf tube. Protein concentration in 1:100 diluted homogenate may be determined using BCA assay (Pierce BCA protein assay kit cat# 23227). The sample may be stored at -80°C.

Phosphorylated VEGFR2 levels may be determined using the Phospho-VEGFR-2 (Tyr1054) assay whole cell lysate kit (MSD #K151DJD-1). Monkey kidney homogenate containing 400µg total proteins may be loaded to each well.

Mouse kidneys may be collected from a remnant kidney study as described in Example 6, a uninephrectomized db/db mouse study as described in Example 7, or in normal 129 mice. In the 129 mouse study, male 129S6/SvEvTac mice from Taconic Farm may be randomized into two groups at approximately 10 weeks of age to receive either Antibody III or control rat IgG1 antibody. Both Antibody III and control IgG1 antibodies may be administrated at 10 mg/kg, three times a week (tiw), sc, for 16 weeks. Kidneys may be collected at the end of the 16 week study.

Mouse kidney homogenate may be prepared by adding approximately 50 µg of mouse kidney to 300 µL of Sample Buffer (MSD mouse phospho-KDR(Tyr1175). Tube may be placed on ice and shaken on a FastPrep at 6.5 speed for 40 seconds. After 5 minutes of incubation on ice, tissues may be disrupted using the FastPrep. The tubes may be rotated at 4°C for 30 minutes, followed by centrifuging at 4°C and 8,000 rpm for 10 minutes. The supernatant may be removed to a fresh Eppendorf tube. The protein concentration in 1:100 diluted homogenate may be determined using the BCA method (Pierce BCA protein assay kit cat# 23227). The sample may be stored at -80°C.

Phosphorylated mouse VEGFR2 may be determined using the Mouse phospho-KDR (Tyr1175) assay whole cell lysate kit (MSD custom#N45CA-1). Mouse kidney homogenate containing 400µg total protein may be loaded to each well. OD may be read using the SECTOR Imager.

In experiments performed essentially as described in this Example 10, Antibody II increases renal VEGFR2 phosphorylation in Cynomolgus monkeys receiving 20mg/kg, qw dose for 13 weeks (Table 20). Similarly, Antibody III increases renal VEGFR2 phosphorylation in remnant kidney mice receiving six weeks of Antibody III treatment (Table 21), in uninephrectomized db/db mice receiving six weeks of Antibody III treatment (Table 22), as well as in 129 mice receiving 16 weeks of Antibody III treatment (Table 23).

Table 20: VEGFR2 phosphorylation in monkey kidney after treatment with Antibody II

	pVEGFR2 (OD)
Vehicle	93.38 +/- 8.702
Antibody II	177.88 +/- 25.423

$P < 0.05$

Table 21: Antibody III increases renal VEGFR2 phosphorylation in remnant mouse kidneys.

	pVEGFR2 (OD) +/- Standard Error	pVEGFR2 (OD) +/- Standard Median Error
rIgG1-30mg/kg biw		513.00+/- 26.985
Antibody III -30mg/kg biw		848.25+/- 78.996
Antibody III -10mg/kg qw	657.25 +/- 535.24	657.25+/- 65.984
Antibody III -3mg/kg qw	464 +/- 341.99	464+/- 34.945

$P < 0.003$ vs. rIgG1 control group, by ANOVA and Dunnett's comparison

Table 22: Antibody III increases VEGFR2 phosphorylation in uninephrectomized db/db mouse kidneys.

	pVEGFR2 (OD) +/- Standard Error	pVEGFR2 (OD) +/- Standard Median Error
rIgG1 30mg/kg, biw	466.75+/- 73.87	466.75+/- 36.44
Antibody III 30mg/kg, biw	1056.00+/- 73.87	1056.00+/- 110.94
Antibody III 10mg/kg, tiw	590.50+/- 60.32	590.50+/- 49.84
Antibody III 10mg/kg, qw	550.00+/- 85.30	550.00+/- 108.67
Antibody III 3mg/kg, qw	373.00+/- 73.87	373.00+/- 57.91

$P < 0.0005$ vs. control IgG1 group, by ANOVA and Dunnett's comparison

Table 23: Antibody III increases renal VEGFR2 phosphorylation in 129S6/SvEvTac mouse kidneys

	pVEGFR2 (OD)
rIgG, 10mg/kg tiw	484.25 +/- 95.33
Antibody III, 10mg/kg tiw	1039.00 +/- 60.29

$P < 0.001$ vs. control IgG1 group.

Amino Acid and Nucleotide Sequences

SEQ ID NO: 1 (hVEGFR1)

MVSYWDTGVLLCALLSCLLLTGSSSGSKLKDPELSLKGTQHIMQAGQTLHLQCR
GEAAHKWSLPEMVSKESERLSITKSACGRNGKQFCSTLTLNTAQANHTGFYSCK
YLAVPTSKKKETESAIYIFISDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITV
TLKKFPLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGHLYKTNYLTHR
QTNTIIDVQISTPRPVKLLRGHTLVLNCTATTPLNTRVQMTWSYPDEKNKRASVR
RRIDQSNSHANIFYSVLTIDKMQNKDKGLYTCRVRSGPSFKSVNTSVHIYDKAFIT
VKHRKQQVLETVAGKRSYRLSMKVKAFPSPEVVWLKDGLPATEKSARYLTRGY
SLIIKDVTEEDAGNYTILLSIKQSNVFKNLTATLIVNVKPQIYEKAVSSFPDPALYP
LGSRQILTCTAYGIPQPTIKWFWHPCNHNHSEARCDFCSNNEESFILDADSNMGN
RIESITQRMAIIEGKNKMASTLVVADSRISGIYICIASNKVGTVGRNISFYITDVPNG
FHVNLEKMPTEGEDLKLSCTVNKFLYRDVTWILLRTVNNRTMHYSISKQKMAIT
KEHSITLNLTIMNVSLQDSGTYACRARNVYTGEEILQKKEITIRDQEAPYLLRNLS
DHTVAISSSTTLDCHANGVPEPQITWFKNNHKIQQEPGIILGPGSSTLFIERVTEED
EGVYHCKATNQKGSVESSAYLTVQGTSDKSNLELITLTCTCVAAT LFWLLLTLFI
RKMKRSSSEIKTDYLSIIMDPDEVPLDEQCERLPYDASKWEFARERLKLGKSLGR
GAFGKVVQASAFGIKKSPTCRTVAVKMLKEGATASEYKALMTELKILTHIGHHL
NVVNLLGACTKQGGPLMVIVEYCKYGNLSNYLKSKRDLFFLNKDAALHMEPKK
EKMEPGLEQGKKPRLDSVTSSESFASSGFQEDKSLSDVEEEEDSDGFYKEPITMED
LISYSFQVARGMEFLSSRKCIHRDLAARNILLSENNVVKICDFGLARDIYKNPDYV
RKGDTRLPLKWMAPESIFDKIYSTKSDVWSYGVLLWEIFSLGGSPYPGVQMDED
FCSRLREGMRMRAPEYSTPEIYQIMLDCWHRDPKERPRFAELVEKLGDLLQANV
QQDGKDYIPINAILTGNSGFTYSTPAFSEDFFKESISAPKFNSGSSDDVRYVNAFKF
MSLERIKTFEELLPNATSMFDDYQGDSSTLLASPMLKRFTWTDSKPKASLKIDLR
VTSKSKESGLSDVSRPSFCHSSCGHVSEGKRRFTYDHAELERKIACCSPPPDYNSV
VLYSTPPI

SEQ ID NO: 2 (HCDR1 – Antibody 1 and 2)

GFAFSSYGMH

SEQ ID NO: 3 (HCDR2 - Antibody 1 and 2)
VIWYDGSNKYYADSVRG

SEQ ID NO: 4 (HCDR3 - Antibody 1 and 2)
DHYGSGVHHYFYYGLDV

SEQ ID NO: 5 (HCVR - Antibody 1 and 2)
QAQVVESGGGVVQSGRSLRLSCAASGFAFSSYGMHWVRQAPGKGLEWVAVIW
YDGSNKYYADSVRGRFTISRDNSENTLYLQMNSLRAEDTAVYYCARDHYGSGV
HHYFYYGLDVWGQGTTVTVSS

SEQ ID NO: 6 (HC - Antibody 1)
QAQVVESGGGVVQSGRSLRLSCAASGFAFSSYGMHWVRQAPGKGLEWVAVIW
YDGSNKYYADSVRGRFTISRDNSENTLYLQMNSLRAEDTAVYYCARDHYGSGV
HHYFYYGLDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF
PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP
SNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL
NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF
SCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 7 (HC - Antibody 2)
QAQVVESGGGVVQSGRSLRLSCAASGFAFSSYGMHWVRQAPGKGLEWVAVIW
YDGSNKYYADSVRGRFTISRDNSENTLYLQMNSLRAEDTAVYYCARDHYGSGV
HHYFYYGLDVWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFP
EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKP
SNTKVDKRVESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVD

VSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV
MHEALHNHYTQKSLSLSLG

SEQ ID NO: 8 (LCDR1 - Antibody 1 and 2)
RASQSVSSSYLA

SEQ ID NO: 9 (LCDR2 - Antibody 1 and 2)
GASSRAT

SEQ ID NO: 10 (LCDR3 - Antibody 1 and 2)
QQYGSSPLT

SEQ ID NO: 11 (LCVR - Antibody 1 and 2)
EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRA
TGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPLTFGGGTKVEIK

SEQ ID NO: 12 (LC - Antibody 1 and 2)
EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRA
TGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPLTFGGGTKVEIKRTVA
APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 13 (HC DNA- Antibody 1)
CAGGCGCAGGTGGTGGAGTCTGGGGGAGGCGTGGTCCAGTCTGGGAGGTCCC
TGAGACTCTCCTGTGCAGCGTCTGGATTCGCCTTCAGTAGCTACGGCATGCAC
TGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATGGT
ATGATGGAAGTAATAAATACTATGCAGACTCCGTGAGGGGCCGATTCACCAT
CTCCAGAGACAATTCCGAGAACACGCTGTATCTGCAAATGAACAGCCTGAGA

GCCGAGGACACGGCTGTATATTACTGTGCGAGAGATCACTATGGTTCGGGGG
TGCACCACTATTTCTACTACGGTCTGGACGTCTGGGGCCAAGGGACCACGGTC
ACCGTCTCCTCAGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTC
CTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGAC
TACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCG
GCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGC
AGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCA
ACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCA
AATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTG
GGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGAT
CTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGAC
CCTGAGGTCAAGTTCAACTGGTATGTGGACGGCGTGGAGGTGCATAATGCCA
AGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCG
TCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAA
GGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCC
AAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGG
AGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCC
CAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTA
CAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTATAGCA
AGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTC
CGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTG
TCCCCGGGTAAA

SEQ ID NO: 14 (HC DNA- Antibody 2)

CAGGCGCAGGTGGTGGAGTCTGGGGGAGGCGTGGTCCAGTCTGGGAGGTCCC
TGAGACTCTCCTGTGCAGCGTCTGGATTCGCCTTCAGTAGCTACGGCATGCAC
TGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATGGT
ATGATGGAAGTAATAAATACTATGCAGACTCCGTGAGGGGCCGATTCACCAT
CTCCAGAGACAATTCCGAGAACACGCTGTATCTGCAAATGAACAGCCTGAGA
GCCGAGGACACGGCTGTATATTACTGTGCGAGAGATCACTATGGTTCGGGGG

TGCACCACTATTTCTACTACGGTCTGGACGTCTGGGGCCAAGGGACCACGGTC
ACCGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCGCTAGCGCCCTG
CTCCAGGAGCACCTCCGAGAGCACAGCCGCCCTGGGCTGCCTGGTCAAGGAC
TACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCG
GCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGC
AGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACGAAGACCTACACCTGCA
ACGTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGTCCA
AATATGGTCCCCCATGCCCACCCTGCCCAGCACCTGAGGCCGCCGGGGGACC
ATCAGTCTTCCTGTTCCCCCCAAAACCCAAGGACACTCTCATGATCTCCCGGA
CCCCTGAGGTCACGTGCGTGGTGGTGGACGTGAGCCAGGAAGACCCCGAGGT
CCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCATAATGCCAAGACAAAG
CCGCGGGAGGAGCAGTTCAACAGCACGTACCGTGTGGTCAGCGTCCTCACCG
TCCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAA
CAAAGGCCTCCCGTCCTCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAG
CCCCGAGAGCCACAGGTGTACACCCTGCCCCCATCCCAGGAGGAGATGACCA
AGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACAT
CGCCGTGGAGTGGGAAAGCAATGGGCAGCCGGAGAACAACTACAAGACCAC
GCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAGGCTAACCG
TGGACAAGAGCAGGTGGCAGGAGGGGAATGTCTTCTCATGCTCCGTGATGCA
TGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCTGGGT

SEQ ID NO: 15 (LC DNA- Antibody 1 and 2)

GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAG
AGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCAGCTACTTAGCCT
GGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCC
AGCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAGTGGGTCTGGGACAG
ACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTTGCAGTGTATTAC
TGTCAGCAGTATGGTAGCTCACCGCTCACTTTCGGCGGAGGGACCAAGGTGG
AGATCAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGAT
GAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTA

TCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGT
AACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGC
CTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCT
ACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTT
CAACAGGGGAGAGTGTTAG

SEQ ID NO: 16 (HC - Antibody 3)

QVQLKESGPGLVRPSETLSLTCTVSGFSLSDYSLSWVRRPSGKGPEWLGRLWFDG
DTTYNSAFKSRLTISRDTSKDQVFLKMNSLQTDDTGTYYCTRDDRDFDYWGQG
VMVTVSSAETTAPSVYPLAPGTALKSNSMVTLGCLVKGYFPEPVTVTWNSGALS
SGVHTFPAVLQSGLYTLTSSVTVPSSTWPSQTVTCNVAHPASSTKVDKKIVPRNC
GGDCKPCICTGSEVSSVFIFPPKPKDVLTITLTPKVTCVVVDISQDDPEVHFSWFVD
DVEVHTAQTRPPEEQFNSTFRSVSELPILHQDWLNGRTFRCKVTSAAFPSPIEKTIS
KPEGRTQVPHVYTMSPTKEEMTQNEVSITCMVKGFYPPDIYVEWQMNGQPQEN
YKNTPPTMDTDGSYFLYSKLNVKKEKWQQGNTFTCSVLHEGLHNHHTEKSLSHS
PGK

SEQ ID NO: 17 (LC - Antibody 3)

DIVMTQTPVSMSVSLGGQVSISCRSSQSLVNNNGNTYLSWYIQKPSQSPQLLIYKV
SNRVSGISDRFSGSGSGTDFTLKINKIEPDDLGVYYCGQNTQYPLTFGSGTKLEIK
RADAAPTVSIFPPSTEQLATGGASVVCLMNNFYPRDISVKWKIDGTERRDGVLDS
VTDQDSKDSTYSMSSTLSLTKADYESHNLYTCEVVHKTSSSPVVKSFNRNEC

SEQ ID NO: 18 (HC - Antibody 4)

QVQLKESGPGLVRPSETLSLTCTVSGFSLSDYSLSWVRRPSGKGPEWLGRLWFDG
DTTYNSAFKSRLTISRDTSKDQVFLKMNSLQTDDTGTYYCTRDDRDFDYWGQG
VMVTVSSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLS
SGVHTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIVPRDC
GCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVDISKDDPEVQFSWFVDDV
EVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISK

TKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYK
NTQPIMDTDGSYFVYSKLNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSHSPG

SEQ ID NO: 19 (LC - Antibody 4)

DIVMTQTPVSMSVSLGGQVSISCRSSQSLVNNNGNTYLSWYIQKPSQSPQLLIYKV
SNRVSGISDRFSGSGSGTDFTLKINKIEPDDLGVYYCGQNTQYPLTFGSGTKLEIK
RADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNS
WTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC

WE CLAIM:

1. A method of treating chronic kidney disease in a patient, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody, wherein the VEGFR1 antibody is an antibody comprising a light chain variable region (LCVR) and a heavy chain variable region (HCVR), wherein the LCVR comprises the complementarity determining regions (CDRs) LCDR1, LCDR2, and LCDR3, and the HCVR comprises the CDRs HCDR1, HCDR2, and HCDR3, wherein the LCDR1 is the polypeptide of RASQSVSSSYLA (SEQ ID NO: 8), the LCDR2 is the polypeptide of GASSRAT (SEQ ID NO: 9), the LCDR3 is the polypeptide of QQYGSSPLT (SEQ ID NO: 10), the HCDR1 is the polypeptide of GFAFSSYGMH (SEQ ID NO: 2), the HCDR2 is the polypeptide of VIWYDGSNKYYADSVRG (SEQ ID NO: 3), and the HCDR3 is the polypeptide of DHYGSGVHHYFYYGLDV (SEQ ID NO: 4).

2. The method according to Claim 1, wherein the chronic kidney disease is caused by diabetes mellitus.

3. The method according to Claim 1 or 2, wherein the patient is at stage 3 or stage 4 of chronic kidney disease.

4. The method according to any one of Claims 1-3, wherein the chronic kidney disease is diabetic nephropathy or focal segmental glomerulosclerosis.

5. A method of decreasing proteinuria in a patient, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody,

wherein the VEGFR1 antibody is an antibody comprising a light chain variable region (LCVR) and a heavy chain variable region (HCVR), wherein the LCVR comprises the complementarity determining regions (CDRs) LCDR1, LCDR2, and LCDR3, and the HCVR comprises the CDRs HCDR1, HCDR2, and HCDR3, wherein the LCDR1 is the polypeptide of RASQSVSSSYLA (SEQ ID NO: 8), the LCDR2 is the polypeptide of GASSRAT (SEQ ID NO: 9), the LCDR3 is the polypeptide of QQYGSSPLT (SEQ ID NO: 10), the HCDR1 is the polypeptide of GFAFSSYGMH (SEQ ID NO: 2), the HCDR2 is the polypeptide of VIWYDGSNKYYADSVRG (SEQ ID NO: 3), and the HCDR3 is the polypeptide of DHYGSGVHHYFYYGLDV (SEQ ID NO: 4).

6. The method according to Claim 5, wherein the proteinuria is caused by diabetic nephropathy.

7. A method of decreasing albuminuria in a patient, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody, wherein the VEGFR1 antibody is an antibody comprising a light chain variable region (LCVR) and a heavy chain variable region (HCVR), wherein the LCVR comprises the complementarity determining regions (CDRs) LCDR1, LCDR2, and LCDR3, and the HCVR comprises the CDRs HCDR1, HCDR2, and HCDR3, wherein the LCDR1 is the polypeptide of RASQSVSSSYLA (SEQ ID NO: 8), the LCDR2 is the polypeptide of GASSRAT (SEQ ID NO: 9), the LCDR3 is the polypeptide of QQYGSSPLT (SEQ ID NO: 10), the HCDR1 is the polypeptide of GFAFSSYGMH (SEQ ID NO: 2), the HCDR2 is the polypeptide of VIWYDGSNKYYADSVRG (SEQ ID NO: 3), and the HCDR3 is the polypeptide of DHYGSGVHHYFYYGLDV (SEQ ID NO: 4).

8. The method according to Claim 7, wherein the albuminuria is caused by diabetic nephropathy.

9. A method of decreasing the loss of glomerular filtration rate reflected by an increase in serum creatinine in a patient, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody, wherein the VEGFR1 antibody is an antibody comprising a light chain variable region (LCVR) and a heavy chain variable region (HCVR), wherein the LCVR comprises the complementarity determining regions (CDRs) LCDR1, LCDR2, and LCDR3, and the HCVR comprises the CDRs HCDR1, HCDR2, and HCDR3, wherein the LCDR1 is the polypeptide of RASQSVSSSYLA (SEQ ID NO: 8), the LCDR2 is the polypeptide of GASSRAT (SEQ ID NO: 9), the LCDR3 is the polypeptide of QQYGSSPLT (SEQ ID NO: 10), the HCDR1 is the polypeptide of GFAFSSYGMH (SEQ ID NO: 2), the HCDR2 is the polypeptide of VIWYDGSNKYYADSVRG (SEQ ID NO: 3), and the HCDR3 is the polypeptide of DHYGSGVHHYFYYGLDV (SEQ ID NO: 4).

10. A method of protecting against renal histopathological lesions in a patient, comprising administering to a patient in need thereof, an effective amount of a VEGFR1 antibody, wherein the VEGFR1 antibody is an antibody comprising a light chain variable region (LCVR) and a heavy chain variable region (HCVR), wherein the LCVR comprises the complementarity determining regions (CDRs) LCDR1, LCDR2, and LCDR3, and the HCVR comprises the CDRs HCDR1, HCDR2, and HCDR3, wherein the LCDR1 is the polypeptide of RASQSVSSSYLA (SEQ ID NO: 8), the LCDR2 is the polypeptide of GASSRAT (SEQ ID NO: 9), the LCDR3 is the polypeptide of QQYGSSPLT (SEQ ID NO: 10), the HCDR1 is the polypeptide of GFAFSSYGMH (SEQ ID NO: 2), the HCDR2 is the polypeptide of VIWYDGSNKYYADSVRG (SEQ ID NO:

3), and the HCDR3 is the polypeptide of DHYGSGVHHYFYYGLDV (SEQ ID NO: 4).

11. The method according to any one of Claims 1-10, wherein the VEGFR1 antibody has a K_D for VEGFR1 of less than 80 pM as determined by surface plasmon resonance.

12. The method according to any one of Claims 1-11, wherein the VEGFR1 antibody neutralizes VEGF-A binding *in vitro* to VEGFR1 with an IC_{50} less than 2.0 nM.

13. The method according to any one of Claims 1-12, wherein the VEGFR1 antibody neutralizes PlGF binding *in vitro* to VEGFR1 with an IC_{50} less than 2.0 nM.

14. The method according to any one of Claims 1-13, wherein the VEGFR1 antibody comprises a LCVR that is the polypeptide of SEQ ID NO: 11, and a HCVR that is the polypeptide of SEQ ID NO: 5.

15. The method according to any one of Claims 1-14, wherein the VEGFR1 antibody comprises a light chain (LC) and a heavy chain (HC), wherein the LC is the polypeptide of SEQ ID NO: 12, and the HC is the polypeptide of SEQ ID NO: 6.

16. The method according to any one of Claims 1-14, wherein the VEGFR1 antibody comprises a light chain (LC) and a heavy chain (HC), wherein the LC is the polypeptide of SEQ ID NO: 12, and the HC is the polypeptide of SEQ ID NO: 7.

17. The method according to any one of Claims 1-15, wherein the VEGFR1 antibody comprises two light chains and two heavy chains, wherein each light chain is the polypeptide of SEQ ID NO: 12, and each heavy chain is the polypeptide of SEQ ID NO: 6.

18. The method according to any one of Claims 1-14 or 16, wherein the VEGFR1 antibody comprises two light chains and two heavy chains, wherein each light chain is the polypeptide of SEQ ID NO: 12, and each heavy chain is the polypeptide of SEQ ID NO: 7.

19. A VEGFR1 antibody for use in the treatment of chronic kidney disease, wherein the VEGFR1 antibody is an antibody comprising a light chain variable region (LCVR) and a heavy chain variable region (HCVR), wherein the LCVR comprises the complementarity determining regions (CDRs) LCDR1, LCDR2, and LCDR3, and the HCVR comprises the CDRs HCDR1, HCDR2, and HCDR3, wherein the LCDR1 is the polypeptide of RASQSVSSSYLA (SEQ ID NO: 8), the LCDR2 is the polypeptide of GASSRAT (SEQ ID NO: 9), the LCDR3 is the polypeptide of QQYGSSPLT (SEQ ID NO: 10), the HCDR1 is the polypeptide of GFAFSSYGMH (SEQ ID NO: 2), the HCDR2 is the polypeptide of VIWYDGSNKYYADSVRG (SEQ ID NO: 3), and the HCDR3 is the polypeptide of DHYGSGVHHYFYYGLDV (SEQ ID NO: 4).

20. A VEGFR1 antibody for use according to Claim 19, wherein the chronic kidney disease is caused by diabetes mellitus.

21. A VEGFR1 antibody for use according to Claim 19 or 20, wherein the chronic kidney disease is at stage 3 or stage 4.

22. A VEGFR1 antibody for use according to any one of Claims 19 or 21, wherein the chronic kidney disease is diabetic nephropathy or focal segmental glomerulosclerosis.

23. A VEGFR1 antibody for use in decreasing proteinuria, wherein the VEGFR1 antibody is an antibody comprising a light chain variable region (LCVR) and a heavy chain variable region (HCVR), wherein the LCVR comprises the complementarity determining regions (CDRs) LCDR1, LCDR2, and LCDR3, and the HCVR comprises the CDRs HCDR1, HCDR2, and HCDR3, wherein the LCDR1 is the polypeptide of RASQSVSSSYLA (SEQ ID NO: 8), the LCDR2 is the polypeptide of GASSRAT (SEQ ID NO: 9), the LCDR3 is the polypeptide of QQYGSSPLT (SEQ ID NO: 10), the HCDR1 is the polypeptide of GFAFSSYGMH (SEQ ID NO: 2), the HCDR2 is the polypeptide of VIWYDGSNKYYADSVRG (SEQ ID NO: 3), and the HCDR3 is the polypeptide of DHYGSGVHHYFYYGLDV (SEQ ID NO: 4).

24. A VEGFR1 antibody for use according to Claim 23, wherein the proteinuria is caused by diabetic nephropathy.

25. A VEGFR1 antibody for use in decreasing albuminuria, wherein the VEGFR1 antibody is an antibody comprising a light chain variable region (LCVR) and a heavy chain variable region (HCVR), wherein the LCVR comprises the complementarity determining regions (CDRs) LCDR1, LCDR2, and LCDR3, and the HCVR comprises the CDRs HCDR1, HCDR2, and HCDR3, wherein the LCDR1 is the polypeptide of RASQSVSSSYLA (SEQ ID NO: 8), the LCDR2 is the polypeptide of GASSRAT (SEQ ID NO: 9), the LCDR3 is the polypeptide of QQYGSSPLT (SEQ ID NO: 10), the HCDR1 is the polypeptide of GFAFSSYGMH (SEQ ID NO: 2), the HCDR2 is the polypeptide of

VIWYDGSNKYYADSVRG (SEQ ID NO: 3), and the HCDR3 is the polypeptide of DHYGSGVHHYFYYGLDV (SEQ ID NO: 4).

26. A VEGFR1 antibody for use according to Claim 25, wherein the albuminuria is caused by diabetic nephropathy.

27. A VEGFR1 antibody for use in decreasing the loss of glomerular filtration rate reflected by an increase in serum creatinine, wherein the VEGFR1 antibody is an antibody comprising a light chain variable region (LCVR) and a heavy chain variable region (HCVR), wherein the LCVR comprises the complementarity determining regions (CDRs) LCDR1, LCDR2, and LCDR3, and the HCVR comprises the CDRs HCDR1, HCDR2, and HCDR3, wherein the LCDR1 is the polypeptide of RASQSVSSSYLA (SEQ ID NO: 8), the LCDR2 is the polypeptide of GASSRAT (SEQ ID NO: 9), the LCDR3 is the polypeptide of QQYGSSPLT (SEQ ID NO: 10), the HCDR1 is the polypeptide of GFAFSSYGMH (SEQ ID NO: 2), the HCDR2 is the polypeptide of VIWYDGSNKYYADSVRG (SEQ ID NO: 3), and the HCDR3 is the polypeptide of DHYGSGVHHYFYYGLDV (SEQ ID NO: 4).

28. A VEGFR1 antibody for use in protecting against renal histopathological lesions, wherein the VEGFR1 antibody is an antibody comprising a light chain variable region (LCVR) and a heavy chain variable region (HCVR), wherein the LCVR comprises the complementarity determining regions (CDRs) LCDR1, LCDR2, and LCDR3, and the HCVR comprises the CDRs HCDR1, HCDR2, and HCDR3, wherein the LCDR1 is the polypeptide of RASQSVSSSYLA (SEQ ID NO: 8), the LCDR2 is the polypeptide of GASSRAT (SEQ ID NO: 9), the LCDR3 is the polypeptide of QQYGSSPLT (SEQ ID NO: 10), the HCDR1 is the polypeptide of GFAFSSYGMH (SEQ ID NO: 2), the HCDR2 is the polypeptide of

VIWYDGSNKYYADSVRG (SEQ ID NO: 3), and the HCDR3 is the polypeptide of DHYGSGVHHYFYYGLDV (SEQ ID NO: 4).

29. A VEGFR1 antibody for use according to any one of Claims 19-28, wherein the VEGFR1 antibody has a K_D for VEGFR1 of less than 80 pM as determined by surface plasmon resonance.

30. A VEGFR1 antibody for use according to any one of Claims 19-29, wherein the VEGFR1 antibody neutralizes VEGF-A binding *in vitro* to VEGFR1 with an IC_{50} less than 2.0 nM.

31. A VEGFR1 antibody for use according to any one of Claims 19-30, wherein the VEGFR1 antibody neutralizes PlGF binding *in vitro* to VEGFR1 with an IC_{50} less than 2.0 nM.

32. A VEGFR1 antibody for use according to any one of Claims 19-31, wherein the VEGFR1 antibody is an antibody comprising a LCVR that is the polypeptide of SEQ ID NO: 11, and a HCVR that is the polypeptide of SEQ ID NO: 5.

33. A VEGFR1 antibody for use according to any one of Claims 19-32, wherein the VEGFR1 antibody is an antibody comprising a light chain (LC) and a heavy chain (HC), wherein the LC is the polypeptide of SEQ ID NO: 12, and the HC is the polypeptide of SEQ ID NO: 6.

34. A VEGFR1 antibody for use according to any one of Claims 19-32, wherein the VEGFR1 antibody is an antibody comprising a light chain (LC) and a heavy chain (HC), wherein the LC is the polypeptide of SEQ ID NO: 12, and the HC is the polypeptide of SEQ ID NO: 7.

35. A VEGFR1 antibody for use according to any one of Claims 19-33, wherein the VEGFR1 antibody comprises two light chains and two heavy chains, wherein each light chain is the polypeptide of SEQ ID NO: 12, and each heavy chain is the polypeptide of SEQ ID NO: 6.

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36. A VEGFR1 antibody for use according to any one of Claims 19-32 or 34, wherein the VEGFR1 antibody comprises two light chains and two heavy chains, wherein each light chain is the polypeptide of SEQ ID NO: 12, and each heavy chain is the polypeptide of SEQ ID NO: 7.

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