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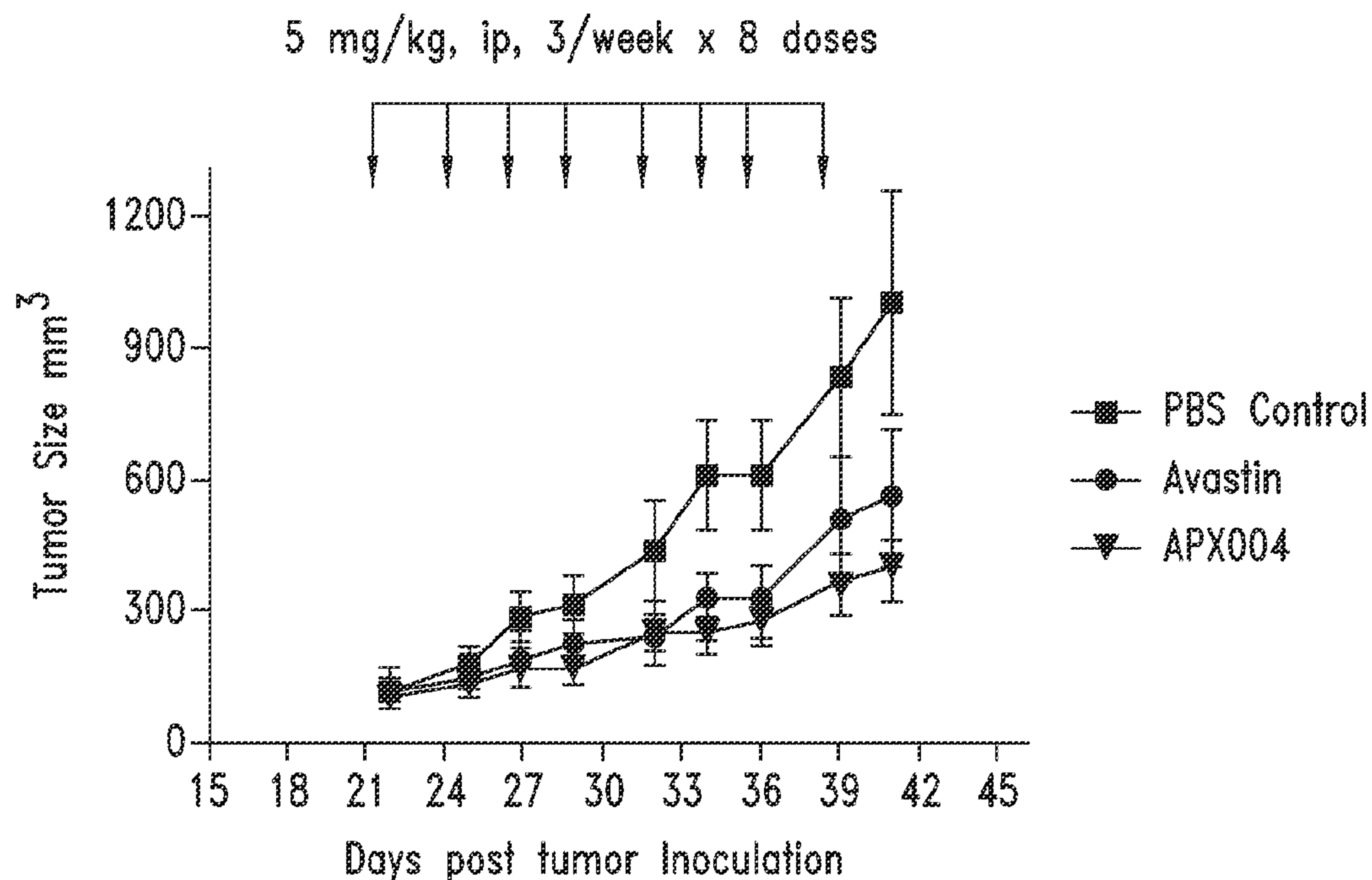


FIG. 7A

(57) Abrégé/Abstract:

The present invention provides anti-KDR monoclonal antibodies and related compositions, which may be used in any of a variety of therapeutic methods for the treatment of a variety of cancers, rheumatoid arthritis, diabetic retinopathy and other diseases associated with aberrant VEGF or KDR expression and/or activity.

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(54) Title: ANTI-KDR ANTIBODIES AND METHODS OF USE

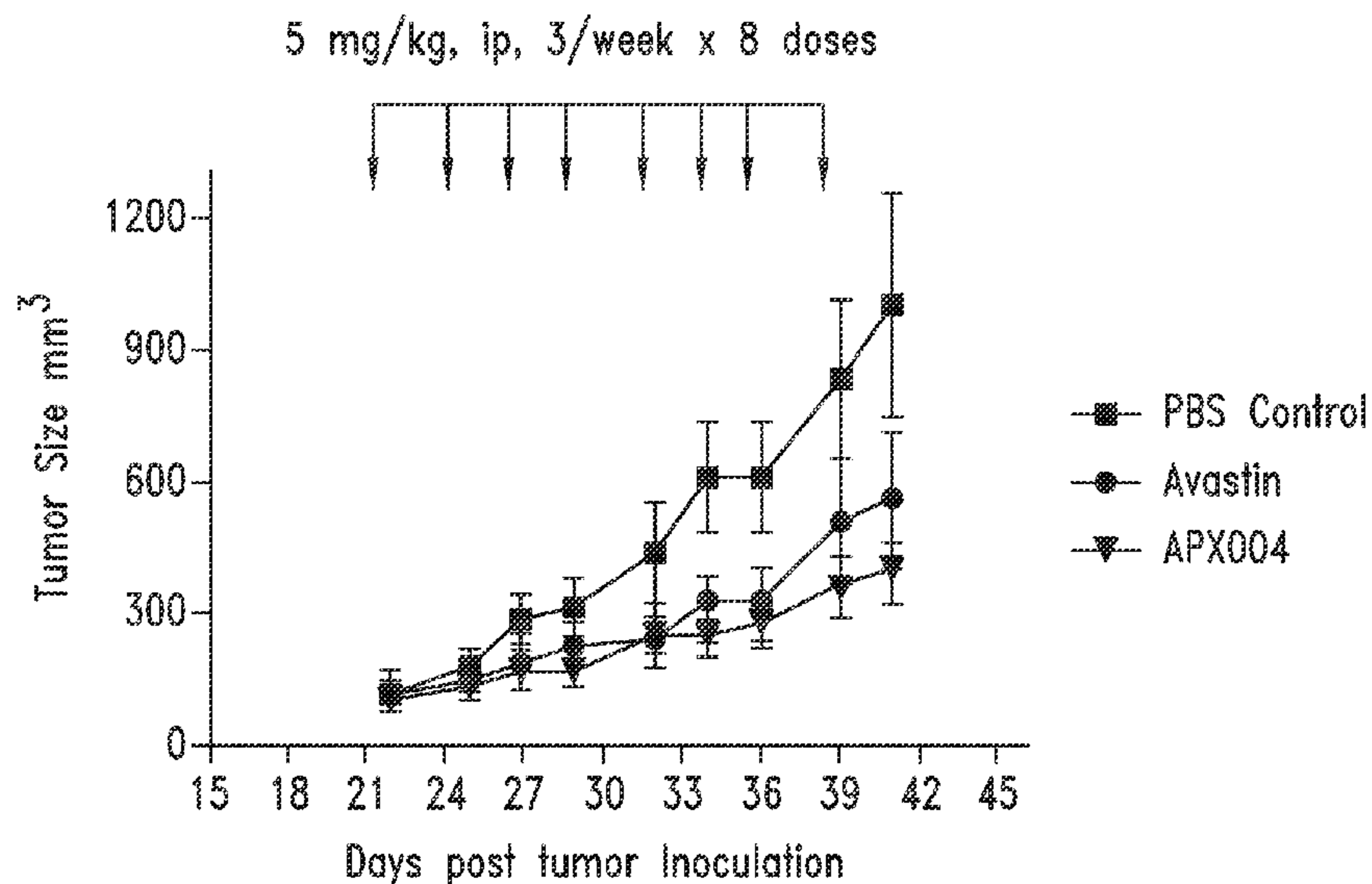


FIG. 7A

(57) Abstract: The present invention provides anti-KDR monoclonal antibodies and related compositions, which may be used in any of a variety of therapeutic methods for the treatment of a variety of cancers, rheumatoid arthritis, diabetic retinopathy and other diseases associated with aberrant VEGF or KDR expression and/or activity.

WO 2013/067098 A1**Declarations under Rule 4.17:**

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ANTI-KDR ANTIBODIES AND METHODS OF USE

CROSS REFERENCES TO RELATED APPLICATIONS

This application claims priority to US Provisional Application No. 61/554,758, filed November 2, 2011, and US Provisional Application No. 61/609,581, filed March 12, 2012, both of which are incorporated by reference herein in their entirety.

STATEMENT REGARDING SEQUENCE LISTING

The Sequence Listing associated with this application is provided in text format in lieu of a paper copy, and is hereby incorporated by reference into the specification. The name of the text file containing the Sequence Listing is APEX_015_02WO_ST25.txt. The text file is 103 KB, was created on November 1, 2012, and is being submitted electronically via EFS-Web.

BACKGROUND

15 Technical Field

The present invention relates generally to anti-VEGF Receptor 2 (VEGFR2; aka kinase insert domain-containing receptor, or KDR) antibodies, compositions and methods of using same. Such antibodies are useful, for example, in methods for treating and inhibiting a variety of disorders including age-related macular degeneration (AMD), diabetes and ischemic retinopathies, rheumatoid arthritis, psoriasis and a variety of oncological diseases including renal cell carcinoma, metastatic gastric or gastro-esophageal junction adenocarcinoma, breast cancer, hepatocellular carcinoma, colorectal cancer, prostate cancer, non-small cell lung cancer, ovarian cancers, melanoma, and recurrent glioblastoma multiforme, leukemias and solid tumors.

Description of the Related Art

VEGFR2/KDR is the primary angiogenic receptor and binds VEGF isoforms A, C, D and E, and is important for endothelial cell differentiation, as well as the mitogenic, angiogenic and permeability-enhancing effects of VEGF. An
5 anti-KDR antibody may prevent all known VEGF isoforms from binding to VEGFR2/KDR and initiating signaling. In addition, because tumors secrete many more molecules of VEGF while the number of receptors remains relatively constant, targeting the receptor increases the probability of completely suppressing signaling even in the presence of very high levels of VEGF isoforms.

10

Angiogenesis

Angiogenesis, the formation of new blood vessels from existing vasculature, is a tightly regulated event and plays an important role in normal physiology such as embryonic development, follicular growth, wound healing, as
15 well as in pathological conditions such as tumor growth and progression (1, 2).

Growth and metastasis of primary tumors is dependent on formation of new blood vessels. In the absence of neovascularization, tumors become necrotic or apoptotic and/or fail to grow beyond 2–3 mm³ in size (3). Tumor angiogenesis involves several processes, including endothelial cell activation,
20 proliferation, migration, and tissue infiltration from preexisting blood vessels that are triggered by specific angiogenic growth factors produced by tumor cells and the surrounding stroma (1– 4).

VEGF and VEGF Receptors

25 Several growth factors have been identified as possible regulators of angiogenesis (5). Among these factors, vascular endothelial growth factor (VEGF) and its receptors have been shown to play a key role in tumor angiogenesis (6 –9).

VEGF is a homodimeric 34–42 kDa heparin-binding glycoprotein with potent angiogenic, mitogenic, and vascular permeability-enhancing activities (10,

11). VEGF regulates vasculogenesis during embryonic development and angiogenic processes during adult life (12, 13). VEGF family members include VEGF-A, VEGF-B, VEGF-C, VEGF-D, and VEGF-E. VEGFs bind to and mediate activity through the VEGF receptors (VEGFRs). There are 3 VEGFRs including
5 VEGFR1 (Flt-1), VEGFR2 (Flk-1/KDR) and VEGFR3 (14-16).

The physiological importance of VEGF and the VEGF receptors in blood vessel formation has been clearly demonstrated in gene knockout experiments (17-18). The VEGFR1 tyrosine kinase exhibits all the conserved motifs that are required for kinase activity. However, the level of phosphorylation of
10 VEGFR1 in response to VEGF-A is low (37, 38). The function of VEGFR-1 is less well defined, it may act as a dummy/decoy receptor to sequester VEGF from VEGFR-2 binding and modulate VEGFR-2 signaling. It has been shown that VEGFR-3 may mediate lymphangiogenesis in response to VEGF-C and VEGF-D. VEGFR2/KDR is the primary angiogenic receptor and binds VEGF isoforms A, C,
15 D and E, and is important for endothelial cell differentiation and mitogenesis.

Structure and Biology of KDR

The VEGFRs are receptor tyrosine kinases and belong to the same family of receptors as the PDGFs and fibroblast growth factors (FGFs).
20 VEGFR2/KDR is a 200 kDa glycoprotein that consists of 7 Ig-like loops in the extracellular domain, a transmembrane domain, and two intracellular tyrosine kinase domains split by a kinase insert. The second and third Ig-like loops are high-affinity ligand-binding domains for VEGF while the first and fourth Ig-like loops regulate ligand binding and receptor dimerization, respectively. VEGF binds KDR
25 with a Kd of 75-250 pM as compared to a Kd of 25 pM for VEGFR1.

KDR is primarily expressed on the cell surface of vascular endothelial cells. KDR is also found on the cell surface of hematopoietic cells, vascular smooth muscle cells (VSMCs), and some malignant cells.

KDR is the primary receptor in developmental angiogenesis and hematopoiesis and is the major mediator of the mitogenic, angiogenic and permeability-enhancing effects of VEGF. VEGFR2^{-/-} knockout mice showed embryonic lethality at E8.5–9.5 with defective blood-island formation and vasculogenesis (41). Physiologically, the binding of VEGF to KDR results in endothelial cell activation, proliferation, migration, invasion and survival. Upon binding to VEGF, KDR receptors dimerize, leading to activation of kinase domains and transduction of KDR receptor signaling. Like many other receptor tyrosine kinases, the major intracellular signaling pathways that lead to angiogenesis include MAPK and PI3 kinase activation.

KDR as Molecular Target for Antibody Therapy

VEGFR2/VEGF axis is a predominant pathway in tumor angiogenesis. Numerous studies have shown that overexpression of VEGF and KDR are strongly associated with invasion and metastasis in human malignancies (6). VEGF receptors have been implicated in angiogenesis that occurs in many human solid tumors, including bladder (21), breast (22, 23), colon (24, 25), gastrointestinal (26), glioma (12, 27), renal (28), melanoma (29), and neuroblastoma (30).

The important role for KDR in tumor angiogenesis was directly demonstrated in studies in which expression of a dominant-negative KDR receptor resulted in decreased endothelial cell mitogenesis and growth inhibition of subcutaneous glioma tumors in athymic mice (31). Other studies confirmed this role using neutralizing soluble VEGF receptor (34, 35) as well as using KDR inhibitors including small molecule tyrosine kinase inhibitor (TKI) and KDR-specific Abs.

In addition to its effect on tumor angiogenesis, KDR is also found on some tumor cells such as leukemia cells and may directly mediate tumorigenesis through an autocrine loop that stimulates leukemia growth (42, 43).

Inhibition of KDR signaling can reduce angiogenesis and retards tumor growth (35, 36). The vast majority of current treatments targeting KDR are small-molecule tyrosine kinase inhibitors (TKIs). TKIs interfere with the binding of ATP or other substrates to the tyrosine kinases and disrupt the kinase catalytic activity. All of the TKIs developed to date (like Sunitinib) bind reversibly to the ATP binding site of the KDR kinase domain.

VEGF is expressed at high levels in various types of tumors (12, 19), and newly sprouting capillaries are clustered around VEGF-producing tumor cells (12). VEGF expression is strongly up-regulated under hypoxic conditions, such as those associated with rapidly growing tumors (20). Neutralizing VEGF by an antibody such as Avastin (33, 34) is a clinically approved therapy to inhibit cancer or other angiogenesis diseases such AMD. However, resistance to VEGF blockade has been found even when given in combination with chemotherapy. This resistance may be associated with remodeled vasculature and with increased expression of other angiogenic factors. As such, there remains a need in the art for improved compositions and methods for inhibiting cancer and other diseases associated with angiogenesis.

Although both TKI and anti-KDR antibodies can inhibit KDR-mediated angiogenesis, the antibody approach has advantages over TKIs. In contrast to TKIs, an anti-KDR antibody is a more specific KDR targeting agent (*i.e.*, it does not inhibit other VEGF receptors). Because of its high specificity, an anti-KDR antibody may be able to limit and/or avoid the off-target effects and toxicities caused by the less specific TKIs (44).

VEGF is expressed at high levels in various types of tumors (12, 19), and newly sprouting capillaries are clustered around VEGF-producing tumor cells (12). VEGF expression is strongly up-regulated under hypoxic conditions, such as those associated with rapidly growing tumors (20). Neutralizing VEGF by an antibody such as Avastin (33, 34) is a clinically approved therapy to inhibit cancer or other angiogenesis diseases such AMD. However, resistance to VEGF

blockade has been found even when given in combination with chemotherapy. This resistance may be associated with remodeled vasculature and with increased expression of other angiogenic factors.

In contrast to Avastin which binds one of the ligands (VEGF-A) only,
 5 an anti-KDR antibody is expected to prevent all known VEGFs from binding to VEGFR2/KDR. This may have a more profound inhibitory effect on tumor angiogenesis than just blocking VEGF-A. It is possible that therapy with an anti-KDR antibody may be effective in cases of Avastin resistance. The potential advantage of targeting KDR over VEGF is summarized in Table 1.

10

Table 1. Advantages of antibody therapy targeting KDR over VEGF

Advantages over anti-VEGF antibodies	Mechanism/Reasons
Fewer antibodies are needed to achieve effective inhibition of VEGF-KDR pathway	1) Overexpression/abundant production of VEGF in tumors 2) KDR expression on endothelial cells is more constant than VEGF from tumor and cancer-related stroma
Specifically blocks KDR pathway from activation by several VEGF ligands	An anti-KDR antibody selectively blocks KDR signalling from activation by related ligands (VEGF-A, E, C, D)
Provides an option for combination therapy with anti-VEGF for more efficient inhibition	Combination therapy may overcome the acquired resistance by anti-VEGF therapy

Therefore, an antibody that targets KDR and blocks KDR signaling may have higher specificity and more complete target inhibition and therefore may
 15 have broad applications in both solid and liquid tumors as well as have the potential to overcome Avastin resistance.

Anti-KDR Therapeutic Antibodies in Development

Ramucirumab (IMC-1121B)

Ramucirumab, which is being developed by ImClone Systems/ Eli Lilly, is a fully human IgG1 mAb that binds human KDR (KD $\approx$ 50 pM) and blocks VEGF binding, thus inhibiting angiogenesis. Because Ramucirumab does not cross react with mouse KDR, a surrogate anti-mouse KDR antibody (DC-101) was
5 generated and used for POC preclinical studies.

In phase I clinical trials in patients with advanced cancers, ramucirumab was well tolerated on weekly dosing schedules. Mechanism-related dose limiting toxicities were hypertension and deep vein thrombosis. Data from a phase II trial as a monotherapy in patients with metastatic renal cell carcinoma
10 following KDR tyrosine kinase inhibitor therapy was reported recently (39). Patients with progressive disease or intolerance to either sorafenib, sunitinib or both were administered 8 mg/kg ramucirumab IV biweekly. Tumor assessments were performed every six weeks. A total of 40 patients were enrolled and 39 were treated. Nineteen patients (49%) had stable disease that lasted for more than 5
15 months; preliminary median progression free survival was 6 months. More phase II trials are ongoing in combination with dacarbazine in melanoma, with mitoxantrone/prednisone in patients with prostate cancer, with carboplatin/paclitaxel in patients with NSCLC and with oxaliplatin/folinic acid/5-fluorouracil in patients with colorectal cancer.

20 Ramucirumab is currently being evaluated in patients with breast cancer, gastric cancer or gastroesophageal junction adenocarcinoma and hepatocellular carcinoma in 3 Phase III studies. Three additional Phase 3 studies of ramucirumab with or without paclitaxel in metastatic gastric adenocarcinoma, in second line metastatic colorectal cancer and in second line non-small cell lung
25 cancer are ongoing.

33C3

33C3 developed by AstraZeneca is a fully human anti-KDR antibody generated using XenoMouseTM technology. 33C3 binds the Ig domains 4-7 of

KDR, and so has no impact on VEGF-A binding to KDR. It does not compete with antibody that interacts at the ligand binding site. 33C3 has high affinity for KDR (KD <1nM) and inhibits VEGF-A induced phosphorylation of KDR. *In vitro*, 33C3 potently inhibits both tube length and number of branch points in a 2D angiogenesis assay and endothelial tube formation in a 3D assay. *In vivo*, 33C3 is a very effective inhibitor of angiogenesis in both a human endothelial angiogenesis assay and in a human skin chimera model (40). 33C3 is now at the early preclinical stage of development. 33C3 has not been tested in *in vivo* tumor models, due to lack of cross reactivity to mouse KDR.

TTAC-0001

TTAC-0001, a fully human anti-KDR antibody generated by phage display, is now in the preclinical stage of developed by PharmAbcine. The antibody showed potent anti-angiogenic efficacy against various cancer mouse models (45).

15 BRIEF SUMMARY

One aspect of the present disclosure provides an isolated antibody, or an antigen-binding fragment thereof, that binds to KDR, comprising (i) a heavy chain variable region comprising the VHCDR1 region set forth in SEQ ID NO:3 or 11, the VHCDR2 region set forth in SEQ ID NO:4 or 12, and the VHCDR3 region set forth SEQ ID NO:5; and (ii) a light chain variable region comprising the VLCDR1 region set forth in SEQ ID NO:6, the VLCDR2 region set forth in SEQ ID NO:7, and the VLCDR3 region set forth in SEQ ID NO: 8; or a variant of said antibody, or an antigen-binding fragment thereof, comprising heavy and light chain variable regions identical to the heavy and light chain variable regions of (i) and (ii) except for up to 8 amino acid substitutions in said CDR regions. In one embodiment, an isolated antibody, or antigen-binding fragment thereof as disclosed herein comprises the heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:1. In another embodiment, an isolated

antibody, or antigen-binding fragment thereof as disclosed herein comprises the light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:2. In one embodiment, the anti-KDR antibodies disclosed herein bind to human KDR and crossreact with mouse or monkey KDR.

5 Another aspect of the present disclosure provides an isolated antibody, or an antigen-binding fragment thereof, that binds to KDR, comprising (i) a heavy chain variable region comprising a VHCDR1, a VHCDR2, and a VHCDR3 of an antibody as set forth in Figure 11; and (ii) a corresponding light chain variable region comprising a VLCDR1, a VLCDR2 and a VLCDR3 of an antibody as set
10 forth in Figure 11; or a variant of said antibody, or an antigen-binding fragment thereof, comprising heavy and light chain variable regions identical to the heavy and light chain variable regions of (i) and (ii) except for up to 8 amino acid substitutions in said CDR regions. In one embodiment, the isolated antibody, or antigen-binding fragment thereof, comprises a heavy chain variable region
15 comprising any one of the amino acid sequences set forth in SEQ ID NOs:17-42. In another embodiment, the isolated antibody, or antigen-binding fragment thereof, comprises a light chain variable region comprising any one of the amino acid sequences set forth in SEQ ID NO:43-68.

Another aspect of the present disclosure provides an isolated
20 antibody, or an antigen-binding fragment thereof, that binds to KDR, comprising a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:1. In one embodiment, the isolated antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:1 and comprises a light chain variable region which comprises an amino acid sequence
25 having at least 90% identity to the amino acid sequence set forth in SEQ ID NO:2. In one embodiment, such an antibody comprises a light chain variable region which comprises the amino acid sequence set forth in SEQ ID NO:2.

Yet another aspect of the present disclosure provides an isolated antibody, or an antigen-binding fragment thereof, that binds to KDR, comprising a

light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:2. In one embodiment, the isolated antibody, or an antigen-binding fragment thereof, that binds to KDR, comprises a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:2 and comprises a heavy chain
5 variable region which comprises an amino acid sequence having at least 90% identity to the amino acid sequence set forth in SEQ ID NO:1.

In certain embodiments of the present disclosure, the anti-KDR antibodies described herein are humanized. Illustrative VH and VL regions of the humanized antibodies that bind KDR as described herein are set forth in SEQ ID
10 NOs:9 and 10.

In another embodiment of the anti-KDR antibodies described herein, the antibody may comprise a single chain antibody, a ScFv, a univalent antibody lacking a hinge region, a minibody, a Fab, a Fab' fragment, a F(ab')₂ fragment or a whole antibody.

15 In certain embodiments, the isolated antibodies described herein comprise a human IgG constant domain. In this regard, the IgG constant domain may comprise an IgG1 CH1 domain, such as the IgG1 CH1 domain amino acid sequence as set forth in SEQ ID NO:16. In certain embodiments, the antibodies described herein comprise an IgG constant domain which comprises an IgG1 Fc
20 region.

Another aspect of the present disclosure provides an isolated antibody, or an antigen-binding fragment thereof, that competes with an anti-KDR antibody as described herein for binding to human KDR.

Yet another aspect of the present disclosure provides an isolated
25 antibody, or antigen-binding fragment thereof, that binds KDR with a high affinity, such as an affinity of a KD of 5.3×10^{-11} M or lower. In this regard, the affinity of an anti-KDR antibody as disclosed herein may be about 3.5, 4, 4.5, 5, or 5.5×10^{-11} M. In certain embodiments, the isolated antibodies of the present disclosure crossreact with murine KDR or non-human primate KDR.

Another aspect of the present disclosure provides an isolated antibody, or antigen-binding fragment thereof wherein the isolated antibody or antigen-binding fragment thereof blocks VEGF binding to KDR; inhibits KDR signaling; inhibits endothelial cell proliferation; inhibits tumor angiogenesis; inhibits tumor cell growth; or a combination of any one or more of the aforementioned functions. In one embodiment, the isolated antibody or antigen-binding fragment thereof, blocks VEGF binding to KDR, inhibits KDR signaling, inhibits endothelial cell proliferation, inhibits tumor angiogenesis, and inhibits tumor cell growth.

In one aspect of the invention, an isolated antibody or antigen-binding fragment thereof directly inhibits tumor growth.

The present disclosure also provides isolated polynucleotides encoding the anti-KDR antibodies as described herein and expression vectors comprising such isolated polynucleotides and isolated host cells comprising such vectors.

Another aspect of the present disclosure provides a composition comprising a physiologically acceptable carrier and a therapeutically effective amount of the isolated antibody or antigen-binding fragment thereof as described herein.

Yet another aspect of the present disclosure provides a method for treating a patient having a cancer associated with aberrant VEGF or KDR expression or activity, comprising administering to the patient a composition comprising a physiologically acceptable carrier and a therapeutically effective amount of an isolated antibody or antigen-binding fragment thereof as described herein, thereby treating the cancer associated with aberrant VEGF or KDR expression or activity. In this regard, the antibodies described herein may be useful for the treatment of cancers including, but not limited to, angiosarcoma, renal cell carcinoma, gastrointestinal cancer, metastatic gastric or gastro-esophageal junction adenocarcinoma, breast cancer, bladder cancer, hepatocellular carcinoma, colorectal cancer, prostate cancer, non-small cell lung cancer,

neuroblastoma, ovarian cancer, melanoma, recurrent glioblastoma multiforme, and leukemia.

Another aspect of the present invention provides a method for treating a patient afflicted by an inflammatory disease, comprising administering to
5 the patient a therapeutically effective amount of a composition comprising any one or more of the antibodies disclosed herein, thereby treating the patient afflicted with the inflammatory disease.

Another aspect of the present disclosure provides a method for treating a patient having rheumatoid arthritis comprising administering to the
10 patient a composition comprising a physiologically acceptable carrier and a therapeutically effective amount of an isolated antibody or antigen-binding fragment thereof as described herein, thereby treating the patient having rheumatoid arthritis.

Another aspect of the present disclosure provides a method for
15 treating a patient having psoriasis comprising administering to the patient a composition comprising a physiologically acceptable carrier and a therapeutically effective amount of an isolated antibody or antigen-binding fragment thereof as described herein, thereby treating the patient having psoriasis.

A further aspect of the present disclosure provides a method for
20 treating a patient afflicted by an angiogenesis mediated disease, comprising administering to the patient a therapeutically effective amount of a composition comprising any one or more of the antibodies disclosed herein, thereby treating the patient afflicted with the angiogenesis mediated disease. In this regard in certain embodiments the patient is afflicted by age related macular degeneration.

25 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: shows a graph of the screening results for most potent antibodies that block KDR binding to VEGF.

Figure 2: Screening for antibodies that inhibit the phosphorylation of KDR. (A) Inhibition of KDR phosphorylation in ELISA assay; (B) Inhibition of KDR phosphorylation in Western blot. Ab9530 is a control KDR neutralizing antibody from Abcam (Cambridge, MA, USA).

5 Figure 3: APX004 binds selectively to human and mouse KDR but not to other human VEGFR family proteins or VEGF.

Figure 4: APX004 potently blocks the binding of KDR to VEGF with IC50 of 2.6 nM.

10 Figure 5: APX004 inhibited the KDR phosphorylation induced by VEGF.

Figure 6: APX004 inhibits HUVEC proliferation in a dose-dependent manner.

15 Figure 7A and 7B: APX004 exhibited more potent anti-tumor activity (77% inhibition) than Avastin (69% inhibition) by the termination of an *in vivo* study (Day 41).

Figure 8: APX004 demonstrated a significant anti-tumor activity at a dose of 2.5 mg/kg ($p < 0.01$) in the H460 tumor model.

Figure 9: APX004 significantly inhibits A375 tumor growth at 3 mg/kg. Volume = (width)² x length/2. Symbols and bars mean + standard deviation.

20 Figure 10: APX004 significantly inhibits HT29 tumor growth at 5 mg/kg and 10 mg/kg. Tumor volumes were calculated according to the following equation: Volume = (width)² x length/2. Symbols and bars, mean + standard deviation.

25 Figure 11 is an alignment of the VH and VL regions of the anti-KDR antibodies identified in Example 1. Figure 11A1 shows amino acids 1-100 of the VH region. Figure 11A2 shows the remaining amino acids of the VH. Figure 11B1 shows the alignment of amino acids 1-70 of the VL region. Figure 11B2 shows the alignment of the remaining amino acids of the VL region. SEQ ID Nos for the VH regions shown in the alignment are provided in SEQ ID Nos:1 and 17-42; the SEQ

ID Nos for the VL regions shown in the alignment are provided in SEQ ID Nos:2 and 43-68, as summarized in the “Brief Description of the Sequences” section below and in Table 3. The CDRs are underlined.

BRIEF DESCRIPTION OF THE SEQUENCES

5 SEQ ID NO:1 is the amino acid sequence of the VH region of the clone 36 rabbit anti-KDR antibody.

 SEQ ID NO:2 is the amino acid sequence of the VL region of the clone 36 rabbit anti-KDR antibody.

 SEQ ID NO:3 is the amino acid sequence of the VHCDR1 region of
10 the clone 36 rabbit anti-KDR antibody.

 SEQ ID NO:4 is the amino acid sequence of the VHCDR2 region of the clone 36 rabbit anti-KDR antibody.

 SEQ ID NO:5 is the amino acid sequence of the VHCDR3 region of the clone 36 rabbit anti-KDR antibody.

15 SEQ ID NO:6 is the amino acid sequence of the VLCDR1 region of the clone 36 rabbit anti-KDR antibody.

 SEQ ID NO:7 is the amino acid sequence of the VLCDR2 region of the clone 36 rabbit anti-KDR antibody.

 SEQ ID NO:8 is the amino acid sequence of the VLCDR3 region of
20 the clone 36 rabbit anti-KDR antibody.

 SEQ ID NO:9 is the amino acid sequence of the humanized sequence of the VH region of the clone 36 rabbit anti-KDR antibody.

 SEQ ID NO:10 is the amino acid sequence of the humanized sequence of the VL region of the clone 36 rabbit anti-KDR antibody.

25 SEQ ID NO:11 is the amino acid sequence of the VHCDR1 region of the humanized clone 36 anti-KDR antibody (APX004).

 SEQ ID NO:12 is the amino acid sequence of the VHCDR2 region of the humanized clone 36 anti-KDR antibody (APX004).

SEQ ID NO:13 is the polynucleotide sequence encoding the human CK region.

SEQ ID NO:14 is the amino acid sequence of human CK.

5 SEQ ID NO:15 is the polynucleotide sequence encoding the human IgG1 CH1 region.

SEQ ID NO:16 is the amino acid sequence of the human IgG1 CH1 region.

SEQ ID NOs:17-42 are the amino acid sequences of the VH of rabbit anti-KDR antibody clones as summarized in Table 3.

10 SEQ ID NOs:43-68 are the amino acid sequences of the VL of rabbit anti-KDR antibody clones as summarized in Table 3.

SEQ ID Nos:69-95 are the amino acid sequences of the VHCDR1 for the rabbit anti-KDR antibody clones as shown in Figure 11.

15 SEQ ID Nos:96-122 are the amino acid sequences of the VHCDR2 for the rabbit anti-KDR antibody clones as shown in Figure 11.

SEQ ID Nos:123-149 are the amino acid sequences of the VHCDR3 for the rabbit anti-KDR antibody clones as shown in Figure 11.

SEQ ID Nos:150-176 are the amino acid sequences of the VLCDR1 for the rabbit anti-KDR antibody clones as shown in Figure 11.

20 SEQ ID Nos:177-203 are the amino acid sequences of the VLCDR2 for the rabbit anti-KDR antibody clones as shown in Figure 11.

SEQ ID Nos:204-230 are the amino acid sequences of the VLCDR3 for the rabbit anti-KDR antibody clones as shown in Figure 11.

DETAILED DESCRIPTION

25 The anti-KDR antibodies described herein have high binding affinity (53 pM) and selectivity to KDR. The antibodies described herein block the interaction of KDR with VEGF and inhibit VEGF-induced KDR phosphorylation and endothelial cell proliferation. In certain embodiments, the antibodies described

herein cross-react with mouse KDR, thus enabling the antibodies to be tested in *in vivo* animal models without a need for a surrogate antibody. The antibodies described herein demonstrate potent activity at inhibiting tumor growth in human tumor xenograft models. Because of cross-reactivity, the anti-KDR antibodies
5 described herein can be fully evaluated and characterized *in vivo* in terms of PK, PD, biomarker development even potential toxicities in preclinical mouse models before testing it in non-human primates and humans.

The present disclosure relates to antibodies and antigen-binding fragments thereof that specifically bind to KDR, in particular antibodies having
10 specific epitopic specificity and functional properties. One embodiment of the invention encompasses specific humanized antibodies and fragments thereof capable of binding to KDR, blocking KDR binding with VEGF and inhibiting VEGF induced downstream cell signaling and biological effects. In more specific embodiments of the invention, the antibodies described herein specifically bind to
15 KDR with affinity of about 5.3×10^{-11} M and block KDR binding to VEGF.

In further embodiments, the antibodies described herein directly inhibit tumor growth. In this regard, certain tumors express KDR and may use the VEGF-KDR pathway as an autocrine loop to grow. Thus, in certain embodiments, the anti-tumor effect mediated by the antibodies described herein may include (i)
20 anti-angiogenesis and/or (ii) direct inhibition of tumor growth.

Embodiments of the invention pertain to the use of anti-KDR antibodies or antigen-binding fragments thereof for the diagnosis, assessment and treatment of diseases and disorders associated with VEGF or aberrant expression thereof. The subject antibodies are used in the treatment or prevention of disorders
25 associated with VEGF or KDR expression and/or activity, including but not limited to, rheumatoid arthritis, diabetes and ischemic retinopathies, age-related macular degeneration, psoriasis and glomerular hypertrophy associated with proteinuria and a variety of oncological diseases including angiosarcoma, renal cell carcinoma, gastrointestinal cancer, metastatic gastric or gastro-esophageal junction

adenocarcinoma, breast cancer, bladder cancer, hepatocellular carcinoma, colorectal cancer, prostate cancer, non-small cell lung cancer, neuroblastoma, ovarian cancers, melanoma, and recurrent glioblastoma multiforme, leukemias and solid tumors, among other diseases.

5 The practice of the present invention will employ, unless indicated specifically to the contrary, conventional methods of virology, immunology, microbiology, molecular biology and recombinant DNA techniques within the skill of the art, many of which are described below for the purpose of illustration. Such techniques are explained fully in the literature. See, *e.g.*, *Current Protocols in*
10 *Molecular Biology* or *Current Protocols in Immunology*, John Wiley & Sons, New York, N.Y.(2009); Ausubel *et al.*, *Short Protocols in Molecular Biology*, 3rd ed., Wiley & Sons, 1995; Sambrook and Russell, *Molecular Cloning: A Laboratory Manual* (3rd Edition, 2001); Maniatis *et al.* *Molecular Cloning: A Laboratory Manual* (1982); *DNA Cloning: A Practical Approach*, vol. I & II (D. Glover, ed.);
15 *Oligonucleotide Synthesis* (N. Gait, ed., 1984); *Nucleic Acid Hybridization* (B. Hames & S. Higgins, eds., 1985); *Transcription and Translation* (B. Hames & S. Higgins, eds., 1984); *Animal Cell Culture* (R. Freshney, ed., 1986); Perbal, *A Practical Guide to Molecular Cloning* (1984) and other like references.

 As used in this specification and the appended claims, the singular
20 forms "a," "an" and "the" include plural references unless the content clearly dictates otherwise.

 Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of
25 elements or integers but not the exclusion of any other element or integer or group of elements or integers.

 Each embodiment in this specification is to be applied *mutatis mutandis* to every other embodiment unless expressly stated otherwise.

Standard techniques may be used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques may be performed according to manufacturer's specifications or as commonly
5 accomplished in the art or as described herein. These and related techniques and procedures may be generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. Unless specific definitions are provided, the nomenclature utilized in connection with, and the
10 laboratory procedures and techniques of, molecular biology, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques may be used for recombinant technology, molecular biological, microbiological, chemical syntheses, chemical analyses, pharmaceutical
15 preparation, formulation, and delivery, and treatment of patients.

Embodiments of the present invention relate to antibodies that bind to the KDR. In particular, the antibodies described herein specifically bind to KDR with unexpectedly high affinity, block VEGF binding to the KDR, block VEGF activity and have therapeutic utility for the treatment of diseases associated with
20 aberrant expression or activity of VEGF. The antibodies described herein also have advantageous properties such as the ability to inhibit a variety of VEGF/KDR-mediated biological effects (e.g., phosphorylation of KDR, angiogenesis, endothelial cell proliferation, and other VEGF/KDR-mediated effects known to the skilled person). The antibodies described herein may also have effects on KDR
25 receptor internalisation.

Sequences of illustrative antibodies, or antigen-binding fragments, or complementarity determining regions (CDRs) thereof, are set forth in SEQ ID NOs:1-12 and 17-230.

As is well known in the art, an antibody is an immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one epitope recognition site, located in the variable region of the immunoglobulin molecule. As used
5 herein, the term encompasses not only intact polyclonal or monoclonal antibodies, but also fragments thereof (such as dAb, Fab, Fab', F(ab')₂, Fv), single chain (ScFv), synthetic variants thereof, naturally occurring variants, fusion proteins comprising an antibody portion with an antigen-binding fragment of the required specificity, humanized antibodies, chimeric antibodies, and any other modified
10 configuration of the immunoglobulin molecule that comprises an antigen-binding site or fragment (epitope recognition site) of the required specificity. "Diabodies", multivalent or multispecific fragments constructed by gene fusion (WO94/13804; P. Holliger et al., Proc. Natl. Acad. Sci. USA 90 6444-6448, 1993) are also a particular form of antibody contemplated herein. Minibodies comprising a scFv
15 joined to a CH3 domain are also included herein (S. Hu et al., Cancer Res., 56, 3055-3061, 1996). See e.g., Ward, E. S. *et al.*, Nature 341, 544-546 (1989); Bird et al., Science, 242, 423-426, 1988; Huston et al., PNAS USA, 85, 5879-5883, 1988); PCT/US92/09965; WO94/13804; P. Holliger et al., Proc. Natl. Acad. Sci. USA 90 6444-6448, 1993; Y. Reiter et al., Nature Biotech, 14, 1239-1245, 1996;
20 S. Hu et al., Cancer Res., 56, 3055-3061, 1996.

The term "antigen-binding fragment" as used herein refers to a polypeptide fragment that contains at least one CDR of an immunoglobulin heavy and/or light chains that binds to the antigen of interest, in particular to the KDR. In this regard, an antigen-binding fragment of the herein described antibodies may
25 comprise 1, 2, 3, 4, 5, or all 6 CDRs of a VH and VL sequence set forth herein from antibodies that bind KDR. An antigen-binding fragment of the KDR-specific antibodies described herein is capable of binding to KDR. In certain embodiments, an antigen-binding fragment or an antibody comprising an antigen-binding fragment, prevents or inhibits VEGF binding to the KDR and subsequent signalling

events. In certain embodiments, the antigen-binding fragment binds specifically to and/or inhibits or modulates the biological activity of human KDR.

The term "antigen" refers to a molecule or a portion of a molecule capable of being bound by a selective binding agent, such as an antibody, and
5 additionally capable of being used in an animal to produce antibodies capable of binding to an epitope of that antigen. An antigen may have one or more epitopes.

The term "epitope" includes any determinant, preferably a polypeptide determinant, capable of specific binding to an immunoglobulin or T-cell receptor. An epitope is a region of an antigen that is bound by an antibody. In
10 certain embodiments, epitope determinants include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl or sulfonyl, and may in certain embodiments have specific three-dimensional structural characteristics, and/or specific charge characteristics. In certain
15 embodiments, an antibody is said to specifically bind an antigen when it preferentially recognizes its target antigen in a complex mixture of proteins and/or macromolecules. An antibody is said to specifically bind an antigen when the equilibrium dissociation constant is $\leq 10^{-7}$ or 10^{-8} M. In some embodiments, the equilibrium dissociation constant may be $\leq 10^{-9}$ M or $\leq 10^{-10}$ M.

In certain embodiments, antibodies and antigen-binding fragments
20 thereof as described herein include a heavy chain and a light chain CDR set, respectively interposed between a heavy chain and a light chain framework region (FR) set which provide support to the CDRs and define the spatial relationship of the CDRs relative to each other. As used herein, the term "CDR set" refers to the three hypervariable regions of a heavy or light chain V region. Proceeding from
25 the N-terminus of a heavy or light chain, these regions are denoted as "CDR1," "CDR2," and "CDR3" respectively. An antigen-binding site, therefore, includes six CDRs, comprising the CDR set from each of a heavy and a light chain V region. A polypeptide comprising a single CDR, (*e.g.*, a CDR1, CDR2 or CDR3) is referred to herein as a "molecular recognition unit." Crystallographic analysis of a number

of antigen-antibody complexes has demonstrated that the amino acid residues of CDRs form extensive contact with bound antigen, wherein the most extensive antigen contact is with the heavy chain CDR3. Thus, the molecular recognition units are primarily responsible for the specificity of an antigen-binding site.

5 As used herein, the term "FR set" refers to the four flanking amino acid sequences which frame the CDRs of a CDR set of a heavy or light chain V region. Some FR residues may contact bound antigen; however, FRs are primarily responsible for folding the V region into the antigen-binding site, particularly the FR residues directly adjacent to the CDRs. Within FRs, certain amino residues and
10 certain structural features are very highly conserved. In this regard, all V region sequences contain an internal disulfide loop of around 90 amino acid residues. When the V regions fold into a binding-site, the CDRs are displayed as projecting loop motifs which form an antigen-binding surface. It is generally recognized that there are conserved structural regions of FRs which influence the folded shape of
15 the CDR loops into certain "canonical" structures—regardless of the precise CDR amino acid sequence. Further, certain FR residues are known to participate in non-covalent interdomain contacts which stabilize the interaction of the antibody heavy and light chains.

 The structures and locations of immunoglobulin variable domains
20 may be determined by reference to Kabat, E. A. et al., Sequences of Proteins of Immunological Interest. 4th Edition. US Department of Health and Human Services. 1987, and updates thereof, now available on the Internet (immuno.bme.nwu.edu).

 A "monoclonal antibody" refers to a homogeneous antibody
25 population wherein the monoclonal antibody is comprised of amino acids (naturally occurring and non-naturally occurring) that are involved in the selective binding of an epitope. Monoclonal antibodies are highly specific, being directed against a single epitope. The term "monoclonal antibody" encompasses not only intact monoclonal antibodies and full-length monoclonal antibodies, but also fragments

thereof (such as Fab, Fab', F(ab')₂, Fv), single chain (ScFv), variants thereof, fusion proteins comprising an antigen-binding portion, humanized monoclonal antibodies, chimeric monoclonal antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen-binding fragment
5 (epitope recognition site) of the required specificity and the ability to bind to an epitope. It is not intended to be limited as regards the source of the antibody or the manner in which it is made (e.g., by hybridoma, phage selection, recombinant expression, transgenic animals, etc.). The term includes whole immunoglobulins as well as the fragments etc. described above under the definition of "antibody".

10 The proteolytic enzyme papain preferentially cleaves IgG molecules to yield several fragments, two of which (the F(ab) fragments) each comprise a covalent heterodimer that includes an intact antigen-binding site. The enzyme pepsin is able to cleave IgG molecules to provide several fragments, including the F(ab')₂ fragment which comprises both antigen-binding sites. An Fv fragment for
15 use according to certain embodiments of the present invention can be produced by preferential proteolytic cleavage of an IgM, and on rare occasions of an IgG or IgA immunoglobulin molecule. Fv fragments are, however, more commonly derived using recombinant techniques known in the art. The Fv fragment includes a non-covalent V_H::V_L heterodimer including an antigen-binding site which retains much
20 of the antigen recognition and binding capabilities of the native antibody molecule. Inbar *et al.* (1972) *Proc. Nat. Acad. Sci. USA* 69:2659-2662; Hochman *et al.* (1976) *Biochem* 15:2706-2710; and Ehrlich *et al.* (1980) *Biochem* 19:4091-4096.

In certain embodiments, single chain Fv or scFV antibodies are contemplated. For example, Kappa bodies (Ill *et al.*, *Prot. Eng.* 10: 949-57 (1997);
25 minibodies (Martin *et al.*, *EMBO J* 13: 5305-9 (1994); diabodies (Holliger *et al.*, *PNAS* 90: 6444-8 (1993); or Janusins (Traunecker *et al.*, *EMBO J* 10: 3655-59 (1991) and Traunecker *et al.*, *Int. J. Cancer Suppl.* 7: 51-52 (1992), may be prepared using standard molecular biology techniques following the teachings of the present application with regard to selecting antibodies having the desired

specificity. In still other embodiments, bispecific or chimeric antibodies may be made that encompass the ligands of the present disclosure. For example, a chimeric antibody may comprise CDRs and framework regions from different antibodies, while bispecific antibodies may be generated that bind specifically to
5 KDR through one binding domain and to a second molecule through a second binding domain. These antibodies may be produced through recombinant molecular biological techniques or may be physically conjugated together.

A single chain Fv (sFv) polypeptide is a covalently linked $V_H:V_L$ heterodimer which is expressed from a gene fusion including V_H - and V_L -encoding
10 genes linked by a peptide-encoding linker. Huston *et al.* (1988) *Proc. Nat. Acad. Sci. USA* 85(16):5879-5883. A number of methods have been described to discern chemical structures for converting the naturally aggregated—but chemically separated—light and heavy polypeptide chains from an antibody V region into an sFv molecule which will fold into a three dimensional structure
15 substantially similar to the structure of an antigen-binding site. See, e.g., U.S. Pat. Nos. 5,091,513 and 5,132,405, to Huston *et al.*; and U.S. Pat. No. 4,946,778, to Ladner *et al.*

In certain embodiments, an KDR binding antibody as described herein is in the form of a diabody. Diabodies are multimers of polypeptides, each
20 polypeptide comprising a first domain comprising a binding region of an immunoglobulin light chain and a second domain comprising a binding region of an immunoglobulin heavy chain, the two domains being linked (e.g. by a peptide linker) but unable to associate with each other to form an antigen binding site: antigen binding sites are formed by the association of the first domain of one
25 polypeptide within the multimer with the second domain of another polypeptide within the multimer (WO94/13804).

A dAb fragment of an antibody consists of a V_H domain (Ward, E. S. *et al.*, Nature 341, 544-546 (1989)).

Where bispecific antibodies are to be used, these may be conventional bispecific antibodies, which can be manufactured in a variety of ways (Holliger, P. and Winter G. *Current Opinion Biotechnol.* 4, 446-449 (1993)), e.g. prepared chemically or from hybrid hybridomas, or may be any of the bispecific
5 antibody fragments mentioned above. Diabodies and scFv can be constructed without an Fc region, using only variable domains, potentially reducing the effects of anti-idiotypic reaction.

Bispecific diabodies, as opposed to bispecific whole antibodies, may also be particularly useful because they can be readily constructed and expressed
10 in *E. coli*. Diabodies (and many other polypeptides such as antibody fragments) of appropriate binding specificities can be readily selected using phage display (WO94/13804) from libraries. If one arm of the diabody is to be kept constant, for instance, with a specificity directed against antigen X, then a library can be made where the other arm is varied and an antibody of appropriate specificity selected.
15 Bispecific whole antibodies may be made by knobs-into-holes engineering (J. B. B. Ridgeway et al., *Protein Eng.*, 9, 616-621, 1996).

In certain embodiments, the antibodies described herein may be provided in the form of a UniBody®. A UniBody® is an IgG4 antibody with the hinge region removed (see GenMab Utrecht, The Netherlands; see also, e.g.,
20 US20090226421). This proprietary antibody technology creates a stable, smaller antibody format with an anticipated longer therapeutic window than current small antibody formats. IgG4 antibodies are considered inert and thus do not interact with the immune system. Fully human IgG4 antibodies may be modified by eliminating the hinge region of the antibody to obtain half-molecule fragments
25 having distinct stability properties relative to the corresponding intact IgG4 (GenMab, Utrecht). Halving the IgG4 molecule leaves only one area on the UniBody® that can bind to cognate antigens (e.g., disease targets) and the UniBody® therefore binds univalently to only one site on target cells. For certain cancer cell surface antigens, this univalent binding may not stimulate the cancer

cells to grow as may be seen using bivalent antibodies having the same antigen specificity, and hence UniBody® technology may afford treatment options for some types of cancer that may be refractory to treatment with conventional antibodies. The small size of the UniBody® can be a great benefit when treating some forms
5 of cancer, allowing for better distribution of the molecule over larger solid tumors and potentially increasing efficacy.

In certain embodiments, the antibodies of the present disclosure may take the form of a nanobody. Nanobodies are encoded by single genes and are efficiently produced in almost all prokaryotic and eukaryotic hosts *e.g. E. coli* (see
10 *e.g.* U.S. Pat. No. 6,765,087), moulds (for example *Aspergillus* or *Trichoderma*) and yeast (for example *Saccharomyces*, *Kluyvermyces*, *Hansenula* or *Pichia* (see *e.g.* U.S. Pat. No. 6,838,254). The production process is scalable and multi-kilogram quantities of nanobodies have been produced. Nanobodies may be formulated as a ready-to-use solution having a long shelf life. The Nanoclone
15 method (see, *e.g.*, WO 06/079372) is a proprietary method for generating Nanobodies against a desired target, based on automated high-throughput selection of B-cells.

In certain embodiments, the anti-KDR antibodies or antigen-binding fragments thereof as disclosed herein are humanized. This refers to a chimeric
20 molecule, generally prepared using recombinant techniques, having an antigen-binding site derived from an immunoglobulin from a non-human species and the remaining immunoglobulin structure of the molecule based upon the structure and/or sequence of a human immunoglobulin. The antigen-binding site may comprise either complete variable domains fused onto constant domains or only
25 the CDRs grafted onto appropriate framework regions in the variable domains. Epitope binding sites may be wild type or modified by one or more amino acid substitutions. This eliminates the constant region as an immunogen in human individuals, but the possibility of an immune response to the foreign variable region remains (LoBuglio, A. F. *et al.*, (1989) *Proc Natl Acad Sci USA* 86:4220-4224;

Queen *et al.*, *PNAS* (1988) 86:10029-10033; Riechmann *et al.*, *Nature* (1988) 332:323-327). Illustrative methods for humanization of the anti-KDR antibodies disclosed herein include the methods described in U.S. patent no. 7,462,697.

Illustrative humanized antibodies according to certain embodiments of the present invention comprise the humanized sequences provided in SEQ ID NOs:9, 10, 19 and 20.

Another approach focuses not only on providing human-derived constant regions, but modifying the variable regions as well so as to reshape them as closely as possible to human form. It is known that the variable regions of both heavy and light chains contain three complementarity-determining regions (CDRs) which vary in response to the epitopes in question and determine binding capability, flanked by four framework regions (FRs) which are relatively conserved in a given species and which putatively provide a scaffolding for the CDRs. When nonhuman antibodies are prepared with respect to a particular epitope, the variable regions can be "reshaped" or "humanized" by grafting CDRs derived from nonhuman antibody on the FRs present in the human antibody to be modified. Application of this approach to various antibodies has been reported by Sato, K., *et al.*, (1993) *Cancer Res* 53:851-856. Riechmann, L., *et al.*, (1988) *Nature* 332:323-327; Verhoeyen, M., *et al.*, (1988) *Science* 239:1534-1536; Kettleborough, C. A., *et al.*, (1991) *Protein Engineering* 4:773-3783; Maeda, H., *et al.*, (1991) *Human Antibodies Hybridoma* 2:124-134; Gorman, S. D., *et al.*, (1991) *Proc Natl Acad Sci USA* 88:4181-4185; Tempest, P. R., *et al.*, (1991) *Bio/Technology* 9:266-271; Co, M. S., *et al.*, (1991) *Proc Natl Acad Sci USA* 88:2869-2873; Carter, P., *et al.*, (1992) *Proc Natl Acad Sci USA* 89:4285-4289; and Co, M. S. *et al.*, (1992) *J Immunol* 148:1149-1154. In some embodiments, humanized antibodies preserve all CDR sequences (for example, a humanized mouse antibody which contains all six CDRs from the mouse antibodies). In other embodiments, humanized antibodies have one or more CDRs (one, two, three, four, five, six) which are

altered with respect to the original antibody, which are also termed one or more CDRs "derived from" one or more CDRs from the original antibody.

In certain embodiments, the antibodies of the present disclosure may be chimeric antibodies. In this regard, a chimeric antibody is comprised of an antigen-binding fragment of an anti-KDR antibody operably linked or otherwise fused to a heterologous Fc portion of a different antibody. In certain embodiments, the heterologous Fc domain is of human origin. In other embodiments, the heterologous Fc domain may be from a different Ig class from the parent antibody, including IgA (including subclasses IgA1 and IgA2), IgD, IgE, IgG (including subclasses IgG1, IgG2, IgG3, and IgG4), and IgM. In further embodiments, the heterologous Fc domain may be comprised of CH2 and CH3 domains from one or more of the different Ig classes. As noted above with regard to humanized antibodies, the anti-KDR antigen-binding fragment of a chimeric antibody may comprise only one or more of the CDRs of the antibodies described herein (e.g., 1, 2, 3, 4, 5, or 6 CDRs of the antibodies described herein), or may comprise an entire variable domain (VL, VH or both).

In certain embodiments, an KDR-binding antibody comprises one or more of the CDRs of the antibodies described herein. In this regard, it has been shown in some cases that the transfer of only the VHCDR3 of an antibody can be performed while still retaining desired specific binding (Barbas *et al.*, *PNAS* (1995) 92: 2529-2533). See also, McLane *et al.*, *PNAS* (1995) 92:5214-5218, Barbas *et al.*, *J. Am. Chem. Soc.* (1994) 116:2161-2162.

Marks *et al* (*Bio/Technology*, 1992, 10:779-783) describe methods of producing repertoires of antibody variable domains in which consensus primers directed at or adjacent to the 5' end of the variable domain area are used in conjunction with consensus primers to the third framework region of human VH genes to provide a repertoire of VH variable domains lacking a CDR3. Marks *et al* further describe how this repertoire may be combined with a CDR3 of a particular antibody. Using analogous techniques, the CDR3-derived sequences of the

presently described antibodies may be shuffled with repertoires of VH or VL domains lacking a CDR3, and the shuffled complete VH or VL domains combined with a cognate VL or VH domain to provide an antibody or antigen-binding fragment thereof that binds KDR. The repertoire may then be displayed in a
5 suitable host system such as the phage display system of WO92/01047 so that suitable antibodies or antigen-binding fragments thereof may be selected. A repertoire may consist of at least from about 10^4 individual members and upwards by several orders of magnitude, for example, to about from 10^6 to 10^8 or 10^{10} or more members. Analogous shuffling or combinatorial techniques are also
10 disclosed by Stemmer (Nature, 1994, 370:389-391), who describes the technique in relation to a β -lactamase gene but observes that the approach may be used for the generation of antibodies.

A further alternative is to generate novel VH or VL regions carrying one or more CDR-derived sequences of the herein described invention
15 embodiments using random mutagenesis of one or more selected VH and/or VL genes to generate mutations within the entire variable domain. Such a technique is described by Gram et al (1992, Proc. Natl. Acad. Sci., USA, 89:3576-3580), who used error-prone PCR. Another method which may be used is to direct mutagenesis to CDR regions of VH or VL genes. Such techniques are disclosed
20 by Barbas et al., (1994, Proc. Natl. Acad. Sci., USA, 91:3809-3813) and Schier et al (1996, J. Mol. Biol. 263:551-567).

In certain embodiments, a specific VH and/or VL of the antibodies described herein may be used to screen a library of the complementary variable domain to identify antibodies with desirable properties, such as increased affinity
25 for KDR. Such methods are described, for example, in Portolano *et al.*, J. Immunol. (1993) 150:880-887; Clarkson *et al.*, Nature (1991) 352:624-628.

Other methods may also be used to mix and match CDRs to identify antibodies having desired binding activity, such as binding to KDR. For example: Klimka *et al.*, *British Journal of Cancer* (2000) 83: 252-260, describe a screening

process using a mouse VL and a human VH library with CDR3 and FR4 retained from the mouse VH. After obtaining antibodies, the VH was screened against a human VL library to obtain antibodies that bound antigen. Beiboer *et al.*, J. Mol. Biol. (2000) 296:833-849 describe a screening process using an entire mouse
5 heavy chain and a human light chain library. After obtaining antibodies, one VL was combined with a human VH library with the CDR3 of the mouse retained. Antibodies capable of binding antigen were obtained. Rader *et al.*, PNAS (1998) 95:8910-8915 describe a process similar to Beiboer et al above.

These just-described techniques are, in and of themselves, known as
10 such in the art. The skilled person will, however, be able to use such techniques to obtain antibodies or antigen-binding fragments thereof according to several embodiments of the invention described herein, using routine methodology in the art.

Also disclosed herein is a method for obtaining an antibody antigen
15 binding domain specific for KDR antigen, the method comprising providing by way of addition, deletion, substitution or insertion of one or more amino acids in the amino acid sequence of a VH domain set out herein a VH domain which is an amino acid sequence variant of the VH domain, optionally combining the VH domain thus provided with one or more VL domains, and testing the VH domain or
20 VH/VL combination or combinations to identify a specific binding member or an antibody antigen binding domain specific for KDR and optionally with one or more desired properties. The VL domains may have an amino acid sequence which is substantially as set out herein. An analogous method may be employed in which one or more sequence variants of a VL domain disclosed herein are combined with
25 one or more VH domains.

An epitope that "specifically binds" or "preferentially binds" (used interchangeably herein) to an antibody or a polypeptide is a term well understood in the art, and methods to determine such specific or preferential binding are also well known in the art. A molecule is said to exhibit "specific binding" or

"preferential binding" if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular cell or substance than it does with alternative cells or substances. An antibody "specifically binds" or "preferentially binds" to a target if it binds with greater affinity, avidity, more readily, 5 and/or with greater duration than it binds to other substances. For example, an antibody that specifically or preferentially binds to a KDR epitope is an antibody that binds one KDR epitope with greater affinity, avidity, more readily, and/or with greater duration than it binds to other KDR epitopes or non-KDR epitopes. It is also understood by reading this definition that, for example, an antibody (or moiety 10 or epitope) that specifically or preferentially binds to a first target may or may not specifically or preferentially bind to a second target. As such, "specific binding" or "preferential binding" does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means preferential binding.

15 Immunological binding generally refers to the non-covalent interactions of the type which occur between an immunoglobulin molecule and an antigen for which the immunoglobulin is specific, for example by way of illustration and not limitation, as a result of electrostatic, ionic, hydrophilic and/or hydrophobic attractions or repulsion, steric forces, hydrogen bonding, van der Waals forces, 20 and other interactions. The strength, or affinity of immunological binding interactions can be expressed in terms of the dissociation constant (K_d) of the interaction, wherein a smaller K_d represents a greater affinity. Immunological binding properties of selected polypeptides can be quantified using methods well known in the art. One such method entails measuring the rates of antigen-binding 25 site/antigen complex formation and dissociation, wherein those rates depend on the concentrations of the complex partners, the affinity of the interaction, and on geometric parameters that equally influence the rate in both directions. Thus, both the "on rate constant" (K_{on}) and the "off rate constant" (K_{off}) can be determined by calculation of the concentrations and the actual rates of association and

dissociation. The ratio of $K_{\text{off}}/K_{\text{on}}$ enables cancellation of all parameters not related to affinity, and is thus equal to the dissociation constant K_d . See, generally, Davies *et al.* (1990) *Annual Rev. Biochem.* 59:439-473.

In certain embodiments, the anti-KDR antibodies described herein
5 have an affinity of about 100, 150, 155, 160, 170, 175, 180, 185, 190, 191, 192, 193, 194, 195, 196, 197, 198 or 199 picomolar, and in some embodiments, the antibodies may have even higher affinity for KDR.

The term "immunologically active", with reference to an epitope being or "remaining immunologically active", refers to the ability of an antibody (e.g., anti-
10 KDR antibody) to bind to the epitope under different conditions, for example, after the epitope has been subjected to reducing and denaturing conditions.

An antibody or antigen-binding fragment thereof according to certain preferred embodiments of the present application may be one that competes for binding to KDR with any antibody described herein which both (i) specifically binds
15 to the antigen and (ii) comprises a VH and/or VL domain disclosed herein, or comprises a VH CDR3 disclosed herein, or a variant of any of these. Competition between antibodies may be assayed easily *in vitro*, for example using ELISA and/or by tagging a specific reporter molecule to one antibody which can be detected in the presence of other untagged antibodies, to enable identification of
20 specific antibodies which bind the same epitope or an overlapping epitope. Thus, there is provided herein a specific antibody or antigen-binding fragment thereof, comprising a human antibody antigen-binding site which competes with an antibody described herein that binds to KDR.

In this regard, as used herein, the terms "competes with", "inhibits
25 binding" and "blocks binding" (e.g., referring to inhibition/blocking of binding of VEGF to KDR or referring to inhibition/blocking of binding of an anti-KDR antibody to KDR) are used interchangeably and encompass both partial and complete inhibition/blocking. The inhibition/blocking of VEGF binding to KDR preferably reduces or alters the normal level or type of cell signaling that occurs when VEGF

binds to KDR without inhibition or blocking. Inhibition and blocking are also intended to include any measurable decrease in the binding of VEGF to KDR when in contact with an anti-KDR antibody as disclosed herein as compared to the ligand not in contact with an anti-KDR antibody, e.g., the blocking of VEGF to KDR
5 by at least about 10%, 20%, 30%, 40%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%.

The constant regions of immunoglobulins show less sequence diversity than the variable regions, and are responsible for binding a number of natural proteins to elicit important biochemical events. In humans there are five
10 different classes of antibodies including IgA (which includes subclasses IgA1 and IgA2), IgD, IgE, IgG (which includes subclasses IgG1, IgG2, IgG3, and IgG4), and IgM. The distinguishing features between these antibody classes are their constant regions, although subtler differences may exist in the V region.

The Fc region of an antibody interacts with a number of Fc receptors
15 and ligands, imparting an array of important functional capabilities referred to as effector functions. For IgG the Fc region comprises Ig domains CH2 and CH3 and the N-terminal hinge leading into CH2. An important family of Fc receptors for the IgG class are the Fc gamma receptors (Fc γ Rs). These receptors mediate communication between antibodies and the cellular arm of the immune system
20 (Raghavan *et al.*, 1996, *Annu Rev Cell Dev Biol* 12:181-220; Ravetch *et al.*, 2001, *Annu Rev Immunol* 19:275-290). In humans this protein family includes Fc γ RI (CD64), including isoforms Fc γ RIa, Fc γ RIb, and Fc γ RIc; Fc γ RII (CD32), including isoforms Fc γ RIIa (including allotypes H131 and R131), Fc γ RIIb (including Fc γ RIIb-1 and Fc γ RIIb-2), and Fc γ RIIc; and Fc γ RIII (CD16), including isoforms Fc γ RIIIa
25 (including allotypes V158 and F158) and Fc γ RIIIb (including allotypes Fc γ RIIIb-NA1 and Fc γ RIIIb-NA2) (Jefferis *et al.*, 2002, *Immunol Lett* 82:57-65). These receptors typically have an extracellular domain that mediates binding to Fc, a membrane spanning region, and an intracellular domain that may mediate some signaling event within the cell. These receptors are expressed in a variety of

immune cells including monocytes, macrophages, neutrophils, dendritic cells, eosinophils, mast cells, platelets, B cells, large granular lymphocytes, Langerhans' cells, natural killer (NK) cells, and T cells. Formation of the Fc/Fc γ R complex recruits these effector cells to sites of bound antigen, typically resulting in signaling
5 events within the cells and important subsequent immune responses such as release of inflammation mediators, B cell activation, endocytosis, phagocytosis, and cytotoxic attack.

The ability to mediate cytotoxic and phagocytic effector functions is a potential mechanism by which antibodies destroy targeted cells. The cell-
10 mediated reaction wherein nonspecific cytotoxic cells that express Fc γ Rs recognize bound antibody on a target cell and subsequently cause lysis of the target cell is referred to as antibody dependent cell-mediated cytotoxicity (ADCC) (Raghavan *et al.*, 1996, *Annu Rev Cell Dev Biol* 12:181-220; Ghetie *et al.*, 2000, *Annu Rev Immunol* 18:739-766; Ravetch *et al.*, 2001, *Annu Rev Immunol* 19:275-
15 290). The cell-mediated reaction wherein nonspecific cytotoxic cells that express Fc γ Rs recognize bound antibody on a target cell and subsequently cause phagocytosis of the target cell is referred to as antibody dependent cell-mediated phagocytosis (ADCP). All Fc γ Rs bind the same region on Fc, at the N-terminal end of the C γ 2 (CH2) domain and the preceding hinge. This interaction is well
20 characterized structurally (Sondermann *et al.*, 2001, *J Mol Biol* 309:737-749), and several structures of the human Fc bound to the extracellular domain of human Fc γ RIIIb have been solved (pdb accession code 1E4K)(Sondermann *et al.*, 2000, *Nature* 406:267-273.) (pdb accession codes 1IIS and 1IIX)(Radaev *et al.*, 2001, *J Biol Chem* 276:16469-16477.)

25 The different IgG subclasses have different affinities for the Fc γ Rs, with IgG1 and IgG3 typically binding substantially better to the receptors than IgG2 and IgG4 (Jefferis *et al.*, 2002, *Immunol Lett* 82:57-65). All Fc γ Rs bind the same region on IgG Fc, yet with different affinities: the high affinity binder Fc γ RI has a Kd for IgG1 of 10⁻⁸ M⁻¹, whereas the low affinity receptors Fc γ RII and Fc γ RIII generally

bind at 10^{-6} and 10^{-5} respectively. The extracellular domains of Fc γ RIIIa and Fc γ RIIIb are 96% identical, however Fc γ RIIIb does not have an intracellular signaling domain. Furthermore, whereas Fc γ RI, Fc γ RIIa/c, and Fc γ RIIIa are positive regulators of immune complex-triggered activation, characterized by
5 having an intracellular domain that has an immunoreceptor tyrosine-based activation motif (ITAM), Fc γ RIIb has an immunoreceptor tyrosine-based inhibition motif (ITIM) and is therefore inhibitory. Thus the former are referred to as activation receptors, and Fc γ RIIb is referred to as an inhibitory receptor. The receptors also differ in expression pattern and levels on different immune cells.
10 Yet another level of complexity is the existence of a number of Fc γ R polymorphisms in the human proteome. A particularly relevant polymorphism with clinical significance is V158/F158 Fc γ RIIIa. Human IgG1 binds with greater affinity to the V158 allotype than to the F158 allotype. This difference in affinity, and presumably its effect on ADCC and/or ADCP, has been shown to be a significant
15 determinant of the efficacy of the anti-CD20 antibody rituximab (Rituxan®, a registered trademark of IDEC Pharmaceuticals Corporation). Patients with the V158 allotype respond favorably to rituximab treatment; however, patients with the lower affinity F158 allotype respond poorly (Cartron *et al.*, 2002, Blood 99:754-758). Approximately 10-20% of humans are V158/V158 homozygous, 45% are
20 V158/F158 heterozygous, and 35-45% of humans are F158/F158 homozygous (Lehrnbecher *et al.*, 1999, Blood 94:4220-4232; Cartron *et al.*, 2002, Blood 99:754-758). Thus 80-90% of humans are poor responders, that is they have at least one allele of the F158 Fc γ RIIIa.

The Fc region is also involved in activation of the complement
25 cascade. In the classical complement pathway, C1 binds with its C1q subunits to Fc fragments of IgG or IgM, which has formed a complex with antigen(s). In certain embodiments of the invention, modifications to the Fc region comprise modifications that alter (either enhance or decrease) the ability of an KDR-specific antibody as described herein to activate the complement system (see e.g., U.S.

Patent 7,740,847). To assess complement activation, a complement-dependent cytotoxicity (CDC) assay may be performed (See, *e.g.*, Gazzano-Santoro *et al.*, J. Immunol. Methods, 202:163 (1996)).

Thus in certain embodiments, the present invention provides anti-
5 KDR antibodies having a modified Fc region with altered functional properties, such as reduced or enhanced CDC, ADCC, or ADCC activity, or enhanced binding affinity for a specific FcγR or increased serum half-life. Other modified Fc regions contemplated herein are described, for example, in issued U.S. patents 7,317,091; 7,657,380; 7,662,925; 6,538,124; 6,528,624; 7,297,775; 7,364,731; Published U.S.
10 Applications US2009092599; US20080131435; US20080138344; and published International Applications WO2006/105338; WO2004/063351; WO2006/088494; WO2007/024249.

Thus, in certain embodiments, antibody variable domains with the desired binding specificities are fused to immunoglobulin constant domain
15 sequences. In certain embodiments, the fusion is with an Ig heavy chain constant domain, comprising at least part of the hinge, C_H2, and C_H3 regions. It is preferred to have the first heavy-chain constant region (C_H1) containing the site necessary for light chain bonding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light
20 chain, are inserted into separate expression vectors, and are co-transfected into a suitable host cell. This provides for greater flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yield of the desired bispecific antibody. It is, however, possible to insert the coding
25 sequences for two or all three polypeptide chains into a single expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios have no significant affect on the yield of the desired chain combination.

Antibodies of the present invention (and antigen-binding fragments and variants thereof) may also be modified to include an epitope tag or label, *e.g.*, for use in purification or diagnostic applications. There are many linking groups known in the art for making antibody conjugates, including, for example, those
5 disclosed in U.S. Pat. No. 5,208,020 or EP Patent 0 425 235 B1, and Chari *et al.*, Cancer Research 52: 127-131 (1992). The linking groups include disulfide groups, thioether groups, acid labile groups, photolabile groups, peptidase labile groups, or esterase labile groups, as disclosed in the above-identified patents, disulfide and thioether groups being preferred.

10 In another contemplated embodiment, a KDR-specific antibody as described herein may be conjugated or operably linked to another therapeutic compound, referred to herein as a conjugate. The conjugate may be a cytotoxic agent, a chemotherapeutic agent, a cytokine, an anti-angiogenic agent, a tyrosine kinase inhibitor, a toxin, a radioisotope, or other therapeutically active agent.
15 Chemotherapeutic agents, cytokines, anti-angiogenic agents, tyrosine kinase inhibitors, and other therapeutic agents have been described above, and all of these aforementioned therapeutic agents may find use as antibody conjugates.

In an alternate embodiment, the antibody is conjugated or operably linked to a toxin, including but not limited to small molecule toxins and
20 enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof. Small molecule toxins include but are not limited to saporin (Kuroda K, *et al.*, The Prostate 70:1286-1294 (2010); Lip, WL. *et al.*, 2007 Molecular Pharmaceutics 4:241-251; Quadros EV., *et al.*, 2010 Mol Cancer Ther; 9(11); 3033-40; Polito L., *et al.* 2009 British Journal of Haematology,
25 147, 710-718), calicheamicin, maytansine (U.S. Pat. No. 5,208,020), trichothene, and CC1065. Toxins include but are not limited to RNase, gelonin, enediynes, ricin, abrin, diphtheria toxin, cholera toxin, gelonin, *Pseudomonas* exotoxin (PE40), *Shigella* toxin, *Clostridium perfringens* toxin, and pokeweed antiviral protein.

In one embodiment, an antibody or antigen-binding fragment thereof of the disclosure is conjugated to one or more maytansinoid molecules.

Maytansinoids are mitototic inhibitors that act by inhibiting tubulin polymerization.

Maytansine was first isolated from the east African shrub *Maytenus serrata* (U.S.

5 Pat. No. 3,896,111). Subsequently, it was discovered that certain microbes also produce maytansinoids, such as maytansinol and C-3 maytansinol esters (U.S. Pat. No. 4,151,042). Synthetic maytansinol and derivatives and analogues thereof are disclosed, for example, in U.S. Pat. Nos. 4,137,230; 4,248,870; 4,256,746; 4,260,608; 4,265,814; 4,294,757; 4,307,016; 4,308,268; 4,308,269; 4,309,428; 10 4,313,946; 4,315,929; 4,317,821; 4,322,348; 4,331,598; 4,361,650; 4,364,866; 4,424,219; 4,450,254; 4,362,663; and 4,371,533. Immunoconjugates containing maytansinoids and their therapeutic use are disclosed, for example, in U.S. Pat. Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235 B1. Liu *et al.*, Proc. Natl. Acad. Sci. USA 93:8618-8623 (1996) described immunoconjugates 15 comprising a maytansinoid designated DM1 linked to the monoclonal antibody C242 directed against human colorectal cancer. The conjugate was found to be highly cytotoxic towards cultured colon cancer cells, and showed antitumor activity in an in vivo tumor growth assay.

Antibody-maytansinoid conjugates are prepared by chemically linking 20 an antibody to a maytansinoid molecule without significantly diminishing the biological activity of either the antibody or the maytansinoid molecule. An average of 3-4 maytansinoid molecules conjugated per antibody molecule has shown efficacy in enhancing cytotoxicity of target cells without negatively affecting the function or solubility of the antibody, although even one molecule of toxin/antibody 25 would be expected to enhance cytotoxicity over the use of naked antibody.

Maytansinoids are well known in the art and can be synthesized by known techniques or isolated from natural sources. Suitable maytansinoids are disclosed, for example, in U.S. Pat. No. 5,208,020 and in the other patents and nonpatent publications referred to hereinabove. Preferred maytansinoids are maytansinol and

maytansinol analogues modified in the aromatic ring or at other positions of the maytansinol molecule, such as various maytansinol esters.

Another conjugate of interest comprises an antibody conjugated to one or more calicheamicin molecules. The calicheamicin family of antibiotics are
5 capable of producing double-stranded DNA breaks at sub-picomolar concentrations. Structural analogues of calicheamicin that may also be used (Hinman *et al.*, 1993, Cancer Research 53:3336-3342; Lode *et al.*, 1998, Cancer Research 58:2925-2928) (U.S. Pat. No. 5,714,586; U.S. Pat. No. 5,712,374; U.S. Pat. No. 5,264,586; U.S. Pat. No. 5,773,001). Dolastatin 10 analogs such as auristatin E (AE) and monomethylauristatin E (MMAE) may find use as conjugates for the presently disclosed antibodies, or variants thereof (Doronina *et al.*, 2003, Nat Biotechnol 21(7):778-84; Francisco *et al.*, 2003 Blood 102(4):1458-65). Useful enzymatically active toxins include but are not limited to diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from
15 *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), *momordica charantia* inhibitor, curcin, crotin, *sapaonaria officinalis* inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. See, for example, PCT WO 93/21232. The
20 present disclosure further contemplates embodiments in which a conjugate or fusion is formed between an KDR-specific antibody as described herein and a compound with nucleolytic activity, for example a ribonuclease or DNA endonuclease such as a deoxyribonuclease (DNase).

In an alternate embodiment, a herein-disclosed antibody may be
25 conjugated or operably linked to a radioisotope to form a radioconjugate. A variety of radioactive isotopes are available for the production of radioconjugate antibodies. Examples include, but are not limited to ^{90}Y , ^{123}I , ^{125}I , ^{131}I , ^{186}Re , ^{188}Re , ^{211}At , and ^{212}Bi .

Antibodies described herein may in certain other embodiments be conjugated to a therapeutic moiety such as a cytotoxin (e.g., a cytostatic or cytotoxic agent), a therapeutic agent or a radioactive element (e.g., alpha-emitters, gamma-emitters, etc.). Cytotoxins or cytotoxic agents include any agent that is detrimental to cells. Examples include paclitaxel/paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, teniposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. One preferred exemplary cytotoxin is saporin (available from Advanced Targeting Systems, San Diego, CA). Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC), and anti-mitotic agents (e.g., vincristine and vinblastine).

Moreover, a KDR-specific antibody (including a functional fragment thereof as provided herein such as an antigen-binding fragment) may in certain embodiments be conjugated to therapeutic moieties such as a radioactive materials or macrocyclic chelators useful for conjugating radiometal ions. In certain embodiments, the macrocyclic chelator is 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA) which can be attached to the antibody via a linker molecule. Such linker molecules are commonly known in the art and described in Denardo *et al.*, 1998, Clin Cancer Res. 4:2483-90; Peterson *et al.*, 1999, Bioconjug. Chem. 10:553; and Zimmerman *et al.*, 1999, Nucl. Med. Biol. 26:943-50.

In yet another embodiment, an antibody may be conjugated to a "receptor" (such as streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then

5 administration of a "ligand" (e.g. avidin) which is conjugated to a cytotoxic agent (e.g. a radionucleotide). In an alternate embodiment, the antibody is conjugated or operably linked to an enzyme in order to employ Antibody Dependent Enzyme Mediated Prodrug Therapy (ADEPT). ADEPT may be used by conjugating or operably linking the antibody to a prodrug-activating enzyme that converts a

10 prodrug (e.g. a peptidyl chemotherapeutic agent, see PCT WO 81/01145) to an active anti-cancer drug. See, for example, PCT WO 88/07378 and U.S. Pat. No. 4,975,278. The enzyme component of the immunoconjugate useful for ADEPT includes any enzyme capable of acting on a prodrug in such a way so as to convert it into its more active, cytotoxic form. Enzymes that are useful in the

15 method of these and related embodiments include but are not limited to alkaline phosphatase useful for converting phosphate-containing prodrugs into free drugs; arylsulfatase useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug, 5-fluorouracil; proteases, such as serratia protease, thermolysin,

20 subtilisin, carboxypeptidases and cathepsins (such as cathepsins B and L), that are useful for converting peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases, useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as β -galactosidase and neuraminidase useful for converting glycosylated prodrugs into free drugs; beta-

25 lactamase useful for converting drugs derivatized with β -lactams into free drugs; and penicillin amidases, such as penicillin V amidase or penicillin G amidase, useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. Alternatively, antibodies with enzymatic activity, also known in the art as "abzymes", may be used to convert

prodrugs into free active drugs (see, for example, Massey, 1987, Nature 328: 457-458). Antibody-abzyme conjugates can be prepared for delivery of the abzyme to a tumor cell population.

Immunoconjugates may be made using a variety of bifunctional
5 protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate, iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-
10 azidobenzoyl)hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). Particular coupling agents include N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP) (Carlsson *et al.*, Biochem. J. 173:723-737 [1978])
15 and N-succinimidyl-4-(2-pyridylthio)pentanoate (SPP) to provide for a disulfide linkage. The linker may be a "cleavable linker" facilitating release of one or more cleavable components. For example, an acid-labile linker may be used (Cancer Research 52: 127-131 (1992); U.S. Pat. No. 5,208,020).

Other modifications of the antibodies (and polypeptides) of the
20 invention are also contemplated herein. For example, the antibody may be linked to one of a variety of nonproteinaceous polymers, *e.g.*, polyethylene glycol, polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol. The antibody also may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization
25 (for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate)microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nanoparticles and nanocapsules), or in macroemulsions. Such techniques are

disclosed in Remington's Pharmaceutical Sciences, 16th edition, Oslo, A., Ed., (1980).

“Carriers” as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers that are nontoxic to the cell or mammal being
5 exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin,
10 gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic
15 surfactants such as polysorbate 20 (TWEEN™) polyethylene glycol (PEG), and poloxamers (PLURONICS™), and the like.

The desired functional properties of anti-KDR antibodies may be assessed using a variety of methods known to the skilled person affinity/binding assays (for example, surface plasmon resonance, competitive inhibition assays);
20 cytotoxicity assays, cell viability assays, cell proliferation or differentiation assays in response to VEGF (*e.g.*, phosphorylation assays), cancer cell and/or tumor growth inhibition using *in vitro* or *in vivo* models. Other assays may test the ability of antibodies described herein to block normal VEGF/KDR-mediated responses, such as, but not limited to, phosphorylation of KDR, angiogenesis, and endothelial
25 cell proliferation. The antibodies described herein may also be tested for effects on KDR receptor internalisation, *in vitro* and *in vivo* efficacy, *etc.* In further embodiments, the antibodies herein may be tested *in vivo* using appropriate animal models. The antibodies described herein may be tested *in vitro* or *in vivo* for their ability to inhibit tumor growth. Such assays may be performed using well-

established protocols known to the skilled person (see *e.g.*, Current Protocols in Molecular Biology (Greene Publ. Assoc. Inc. & John Wiley & Sons, Inc., NY, NY); Current Protocols in Immunology (Edited by: John E. Coligan, Ada M. Kruisbeek, David H. Margulies, Ethan M. Shevach, Warren Strober 2001 John Wiley & Sons, NY, NY); or commercially available kits.

The present invention further provides in certain embodiments an isolated nucleic acid encoding an antibody or antigen-binding fragment thereof as described herein, for instance, a nucleic acid which codes for a CDR or VH or VL domain as described herein. Nucleic acids include DNA and RNA. These and related embodiments may include polynucleotides encoding antibodies that bind KDR as described herein. The term "isolated polynucleotide" as used herein shall mean a polynucleotide of genomic, cDNA, or synthetic origin or some combination thereof, which by virtue of its origin the isolated polynucleotide (1) is not associated with all or a portion of a polynucleotide in which the isolated polynucleotide is found in nature, (2) is linked to a polynucleotide to which it is not linked in nature, or (3) does not occur in nature as part of a larger sequence.

The term "operably linked" means that the components to which the term is applied are in a relationship that allows them to carry out their inherent functions under suitable conditions. For example, a transcription control sequence "operably linked" to a protein coding sequence is ligated thereto so that expression of the protein coding sequence is achieved under conditions compatible with the transcriptional activity of the control sequences.

The term "control sequence" as used herein refers to polynucleotide sequences that can affect expression, processing or intracellular localization of coding sequences to which they are ligated or operably linked. The nature of such control sequences may depend upon the host organism. In particular embodiments, transcription control sequences for prokaryotes may include a promoter, ribosomal binding site, and transcription termination sequence. In other particular embodiments, transcription control sequences for eukaryotes may

include promoters comprising one or a plurality of recognition sites for transcription factors, transcription enhancer sequences, transcription termination sequences and polyadenylation sequences. In certain embodiments, "control sequences" can include leader sequences and/or fusion partner sequences.

5 The term "polynucleotide" as referred to herein means single-stranded or double-stranded nucleic acid polymers. In certain embodiments, the nucleotides comprising the polynucleotide can be ribonucleotides or deoxyribonucleotides or a modified form of either type of nucleotide. Said modifications include base modifications such as bromouridine, ribose
10 modifications such as arabinoside and 2',3'-dideoxyribose and internucleotide linkage modifications such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoraniladate and phosphoroamidate. The term "polynucleotide" specifically includes single and double stranded forms of DNA.

15 The term "naturally occurring nucleotides" includes deoxyribonucleotides and ribonucleotides. The term "modified nucleotides" includes nucleotides with modified or substituted sugar groups and the like. The term "oligonucleotide linkages" includes oligonucleotide linkages such as phosphorothioate, phosphorodithioate, phosphoroselenoate,
20 phosphorodiselenoate, phosphoroanilothioate, phosphoraniladate, phosphoroamidate, and the like. See, *e.g.*, LaPlanche *et al.*, 1986, Nucl. Acids Res., 14:9081; Stec *et al.*, 1984, J. Am. Chem. Soc., 106:6077; Stein *et al.*, 1988, Nucl. Acids Res., 16:3209; Zon *et al.*, 1991, Anti-Cancer Drug Design, 6:539; Zon *et al.*, 1991, OLIGONUCLEOTIDES AND ANALOGUES: A PRACTICAL
25 APPROACH, pp. 87-108 (F. Eckstein, Ed.), Oxford University Press, Oxford England; Stec *et al.*, U.S. Pat. No. 5,151,510; Uhlmann and Peyman, 1990, Chemical Reviews, 90:543, the disclosures of which are hereby incorporated by reference for any purpose. An oligonucleotide can include a detectable label to enable detection of the oligonucleotide or hybridization thereof.

The term "vector" is used to refer to any molecule (e.g., nucleic acid, plasmid, or virus) used to transfer coding information to a host cell. The term "expression vector" refers to a vector that is suitable for transformation of a host cell and contains nucleic acid sequences that direct and/or control expression of
5 inserted heterologous nucleic acid sequences. Expression includes, but is not limited to, processes such as transcription, translation, and RNA splicing, if introns are present.

As will be understood by those skilled in the art, polynucleotides may include genomic sequences, extra-genomic and plasmid-encoded sequences and
10 smaller engineered gene segments that express, or may be adapted to express, proteins, polypeptides, peptides and the like. Such segments may be naturally isolated, or modified synthetically by the skilled person.

As will be also recognized by the skilled artisan, polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be DNA
15 (genomic, cDNA or synthetic) or RNA molecules. RNA molecules may include HnRNA molecules, which contain introns and correspond to a DNA molecule in a one-to-one manner, and mRNA molecules, which do not contain introns. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide according to the present disclosure, and a polynucleotide may, but
20 need not, be linked to other molecules and/or support materials. Polynucleotides may comprise a native sequence or may comprise a sequence that encodes a variant or derivative of such a sequence.

Therefore, according to these and related embodiments, the present disclosure also provides polynucleotides encoding the anti-KDR antibodies
25 described herein. In certain embodiments, polynucleotides are provided that comprise some or all of a polynucleotide sequence encoding an antibody as described herein and complements of such polynucleotides.

In other related embodiments, polynucleotide variants may have substantial identity to a polynucleotide sequence encoding an anti-KDR antibody

described herein. For example, a polynucleotide may be a polynucleotide comprising at least 70% sequence identity, preferably at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% or higher, sequence identity compared to a reference polynucleotide sequence such as a sequence encoding an antibody
5 described herein, using the methods described herein, (e.g., BLAST analysis using standard parameters, as described below). One skilled in this art will recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two nucleotide sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning and the like.

10 Typically, polynucleotide variants will contain one or more substitutions, additions, deletions and/or insertions, preferably such that the binding affinity of the antibody encoded by the variant polynucleotide is not substantially diminished relative to an antibody encoded by a polynucleotide sequence specifically set forth herein.

15 In certain other related embodiments, polynucleotide fragments may comprise or consist essentially of various lengths of contiguous stretches of sequence identical to or complementary to a sequence encoding an antibody as described herein. For example, polynucleotides are provided that comprise or consist essentially of at least about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18,
20 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 200, 300, 400, 500 or 1000 or more contiguous nucleotides of a sequences the encodes an antibody, or antigen-binding fragment thereof, disclosed herein as well as all intermediate lengths there between. It will be readily understood that "intermediate
25 lengths", in this context, means any length between the quoted values, such as 50, 51, 52, 53, *etc.*; 100, 101, 102, 103, *etc.*; 150, 151, 152, 153, *etc.*; including all integers through 200-500; 500-1,000, and the like. A polynucleotide sequence as described here may be extended at one or both ends by additional nucleotides not found in the native sequence. This additional sequence may consist of 1, 2, 3, 4,

5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides at either end of a polynucleotide encoding an antibody described herein or at both ends of a polynucleotide encoding an antibody described herein.

In another embodiment, polynucleotides are provided that are
5 capable of hybridizing under moderate to high stringency conditions to a polynucleotide sequence encoding an antibody, or antigen-binding fragment thereof, provided herein, or a fragment thereof, or a complementary sequence thereof. Hybridization techniques are well known in the art of molecular biology. For purposes of illustration, suitable moderately stringent conditions for testing the
10 hybridization of a polynucleotide as provided herein with other polynucleotides include prewashing in a solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-60°C, 5 X SSC, overnight; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1% SDS. One skilled in the art will understand that the stringency of hybridization can be readily
15 manipulated, such as by altering the salt content of the hybridization solution and/or the temperature at which the hybridization is performed. For example, in another embodiment, suitable highly stringent hybridization conditions include those described above, with the exception that the temperature of hybridization is increased, *e.g.*, to 60°C-65°C or 65°C-70°C.

20 In certain embodiments, the polynucleotides described above, *e.g.*, polynucleotide variants, fragments and hybridizing sequences, encode antibodies that bind KDR, or antigen-binding fragments thereof. In other embodiments, such polynucleotides encode antibodies or antigen-binding fragments, or CDRs thereof, that bind to KDR at least about 50%, at least about 70%, and in certain
25 embodiments, at least about 90% as well as an antibody sequence specifically set forth herein. In further embodiments, such polynucleotides encode antibodies or antigen-binding fragments, or CDRs thereof, that bind to KDR with greater affinity than the antibodies set forth herein, for example, that bind quantitatively at least

about 105%, 106%, 107%, 108%, 109%, or 110% as well as an antibody sequence specifically set forth herein.

As described elsewhere herein, determination of the three-dimensional structures of representative polypeptides (*e.g.*, variant KDR-specific antibodies as provided herein, for instance, an antibody protein having an antigen-binding fragment as provided herein) may be made through routine methodologies such that substitution, addition, deletion or insertion of one or more amino acids with selected natural or non-natural amino acids can be virtually modeled for purposes of determining whether a so derived structural variant retains the space-filling properties of presently disclosed species. A variety of computer programs are known to the skilled artisan for determining appropriate amino acid substitutions (or appropriate polynucleotides encoding the amino acid sequence) within an antibody such that, for example, affinity is maintained or better affinity is achieved.

The polynucleotides described herein, or fragments thereof, regardless of the length of the coding sequence itself, may be combined with other DNA sequences, such as promoters, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like, such that their overall length may vary considerably. It is therefore contemplated that a nucleic acid fragment of almost any length may be employed, with the total length preferably being limited by the ease of preparation and use in the intended recombinant DNA protocol. For example, illustrative polynucleotide segments with total lengths of about 10,000, about 5000, about 3000, about 2,000, about 1,000, about 500, about 200, about 100, about 50 base pairs in length, and the like, (including all intermediate lengths) are contemplated to be useful.

When comparing polynucleotide sequences, two sequences are said to be "identical" if the sequence of nucleotides in the two sequences is the same when aligned for maximum correspondence, as described below. Comparisons between two sequences are typically performed by comparing the sequences over

a comparison window to identify and compare local regions of sequence similarity. A “comparison window” as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous
 5 positions after the two sequences are optimally aligned.

Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O.
 10 (1978) A model of evolutionary change in proteins – Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) *Atlas of Protein Sequence and Structure*, National Biomedical Research Foundation, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J., *Unified Approach to Alignment and Phylogenies*, pp. 626-645 (1990); *Methods in Enzymology* vol. 183, Academic Press, Inc., San Diego, CA;
 15 Higgins, D.G. and Sharp, P.M., *CABIOS* 5:151-153 (1989); Myers, E.W. and Muller W., *CABIOS* 4:11-17 (1988); Robinson, E.D., *Comb. Theor* 11:105 (1971); Santou, N. Nes, M., *Mol. Biol. Evol.* 4:406-425 (1987); Sneath, P.H.A. and Sokal, R.R., *Numerical Taxonomy – the Principles and Practice of Numerical Taxonomy*, Freeman Press, San Francisco, CA (1973); Wilbur, W.J. and Lipman, D.J., *Proc.*
 20 *Natl. Acad., Sci. USA* 80:726-730 (1983).

Alternatively, optimal alignment of sequences for comparison may be conducted by the local identity algorithm of Smith and Waterman, *Add. APL. Math* 2:482 (1981), by the identity alignment algorithm of Needleman and Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity methods of Pearson and
 25 Lipman, *Proc. Natl. Acad. Sci. USA* 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, WI), or by inspection.

One preferred example of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.*, *Nucl. Acids Res.* 25:3389-3402 (1977), and Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990), respectively. BLAST and BLAST 2.0 can be used, for example with the parameters described herein, to determine percent sequence identity among two or more the polynucleotides. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. In one illustrative example, cumulative scores can be calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff and Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)) alignments, (B) of 50, expectation (E) of 10, M=5, N=-4 and a comparison of both strands.

In certain embodiments, the "percentage of sequence identity" is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid bases occurs in both sequences to yield the number of matched positions,

dividing the number of matched positions by the total number of positions in the reference sequence (*i.e.*, the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

It will be appreciated by those of ordinary skill in the art that, as a
5 result of the degeneracy of the genetic code, there are many nucleotide sequences that encode an antibody as described herein. Some of these polynucleotides bear minimal sequence identity to the nucleotide sequence of the native or original polynucleotide sequence that encode antibodies that bind to KDR. Nonetheless, polynucleotides that vary due to differences in codon usage are expressly
10 contemplated by the present disclosure. In certain embodiments, sequences that have been codon-optimized for mammalian expression are specifically contemplated.

Therefore, in another embodiment of the invention, a mutagenesis approach, such as site-specific mutagenesis, may be employed for the preparation
15 of variants and/or derivatives of the antibodies described herein. By this approach, specific modifications in a polypeptide sequence can be made through mutagenesis of the underlying polynucleotides that encode them. These techniques provides a straightforward approach to prepare and test sequence variants, for example, incorporating one or more of the foregoing considerations,
20 by introducing one or more nucleotide sequence changes into the polynucleotide.

Site-specific mutagenesis allows the production of mutants through the use of specific oligonucleotide sequences which encode the DNA sequence of the desired mutation, as well as a sufficient number of adjacent nucleotides, to provide a primer sequence of sufficient size and sequence complexity to form a
25 stable duplex on both sides of the deletion junction being traversed. Mutations may be employed in a selected polynucleotide sequence to improve, alter, decrease, modify, or otherwise change the properties of the polynucleotide itself, and/or alter the properties, activity, composition, stability, or primary sequence of the encoded polypeptide.

In certain embodiments, the inventors contemplate the mutagenesis of the polynucleotide sequences that encode an antibody disclosed herein, or an antigen-binding fragment thereof, to alter one or more properties of the encoded polypeptide, such as the binding affinity of the antibody or the antigen-binding
5 fragment thereof, or the function of a particular Fc region, or the affinity of the Fc region for a particular Fc γ R. The techniques of site-specific mutagenesis are well-known in the art, and are widely used to create variants of both polypeptides and polynucleotides. For example, site-specific mutagenesis is often used to alter a specific portion of a DNA molecule. In such embodiments, a primer comprising
10 typically about 14 to about 25 nucleotides or so in length is employed, with about 5 to about 10 residues on both sides of the junction of the sequence being altered.

As will be appreciated by those of skill in the art, site-specific mutagenesis techniques have often employed a phage vector that exists in both a single stranded and double stranded form. Typical vectors useful in site-directed
15 mutagenesis include vectors such as the M13 phage. These phage are readily commercially-available and their use is generally well-known to those skilled in the art. Double-stranded plasmids are also routinely employed in site directed mutagenesis that eliminates the step of transferring the gene of interest from a plasmid to a phage.

20 In general, site-directed mutagenesis in accordance herewith is performed by first obtaining a single-stranded vector or melting apart of two strands of a double-stranded vector that includes within its sequence a DNA sequence that encodes the desired peptide. An oligonucleotide primer bearing the desired mutated sequence is prepared, generally synthetically. This primer is then
25 annealed with the single-stranded vector, and subjected to DNA polymerizing enzymes such as *E. coli* polymerase I Klenow fragment, in order to complete the synthesis of the mutation-bearing strand. Thus, a heteroduplex is formed wherein one strand encodes the original non-mutated sequence and the second strand bears the desired mutation. This heteroduplex vector is then used to transform

appropriate cells, such as *E. coli* cells, and clones are selected which include recombinant vectors bearing the mutated sequence arrangement.

The preparation of sequence variants of the selected peptide-encoding DNA segments using site-directed mutagenesis provides a means of producing potentially useful species and is not meant to be limiting as there are other ways in which sequence variants of peptides and the DNA sequences encoding them may be obtained. For example, recombinant vectors encoding the desired peptide sequence may be treated with mutagenic agents, such as hydroxylamine, to obtain sequence variants. Specific details regarding these methods and protocols are found in the teachings of Maloy *et al.*, 1994; Segal, 1976; Prokop and Bajpai, 1991; Kuby, 1994; and Maniatis *et al.*, 1982, each incorporated herein by reference, for that purpose.

As used herein, the term “oligonucleotide directed mutagenesis procedure” refers to template-dependent processes and vector-mediated propagation which result in an increase in the concentration of a specific nucleic acid molecule relative to its initial concentration, or in an increase in the concentration of a detectable signal, such as amplification. As used herein, the term “oligonucleotide directed mutagenesis procedure” is intended to refer to a process that involves the template-dependent extension of a primer molecule. The term template dependent process refers to nucleic acid synthesis of an RNA or a DNA molecule wherein the sequence of the newly synthesized strand of nucleic acid is dictated by the well-known rules of complementary base pairing (see, for example, Watson, 1987). Typically, vector mediated methodologies involve the introduction of the nucleic acid fragment into a DNA or RNA vector, the clonal amplification of the vector, and the recovery of the amplified nucleic acid fragment. Examples of such methodologies are provided by U. S. Patent No. 4,237,224, specifically incorporated herein by reference in its entirety.

In another approach for the production of polypeptide variants, recursive sequence recombination, as described in U.S. Patent No. 5,837,458,

may be employed. In this approach, iterative cycles of recombination and screening or selection are performed to “evolve” individual polynucleotide variants having, for example, increased binding affinity. Certain embodiments also provide constructs in the form of plasmids, vectors, transcription or expression cassettes
5 which comprise at least one polynucleotide as described herein.

In many embodiments, the nucleic acids encoding a subject monoclonal antibody are introduced directly into a host cell, and the cell incubated under conditions sufficient to induce expression of the encoded antibody. The antibodies of this disclosure are prepared using standard techniques well known to
10 those of skill in the art in combination with the polypeptide and nucleic acid sequences provided herein. The polypeptide sequences may be used to determine appropriate nucleic acid sequences encoding the particular antibody disclosed thereby. The nucleic acid sequence may be optimized to reflect particular codon “preferences” for various expression systems according to
15 standard methods well known to those of skill in the art.

According to certain related embodiments there is provided a recombinant host cell which comprises one or more constructs as described herein; a nucleic acid encoding any antibody, CDR, VH or VL domain, or antigen-binding fragment thereof; and a method of production of the encoded product,
20 which method comprises expression from encoding nucleic acid therefor. Expression may conveniently be achieved by culturing under appropriate conditions recombinant host cells containing the nucleic acid. Following production by expression, an antibody or antigen-binding fragment thereof, may be isolated and/or purified using any suitable technique, and then used as desired.

25 Antibodies or antigen-binding fragments thereof as provided herein, and encoding nucleic acid molecules and vectors, may be isolated and/or purified, e.g. from their natural environment, in substantially pure or homogeneous form, or, in the case of nucleic acid, free or substantially free of nucleic acid or genes of origin other than the sequence encoding a polypeptide with the desired function.

Nucleic acid may comprise DNA or RNA and may be wholly or partially synthetic. Reference to a nucleotide sequence as set out herein encompasses a DNA molecule with the specified sequence, and encompasses a RNA molecule with the specified sequence in which U is substituted for T, unless context requires
5 otherwise.

Systems for cloning and expression of a polypeptide in a variety of different host cells are well known. Suitable host cells include bacteria, mammalian cells, yeast and baculovirus systems. Mammalian cell lines available in the art for expression of a heterologous polypeptide include Chinese hamster
10 ovary cells, HeLa cells, baby hamster kidney cells, NSO mouse melanoma cells and many others. A common, preferred bacterial host is *E. coli*.

The expression of antibodies and antigen-binding fragments in prokaryotic cells such as *E. coli* is well established in the art. For a review, see for example Pluckthun, A. Bio/Technology 9: 545-551 (1991). Expression in
15 eukaryotic cells in culture is also available to those skilled in the art as an option for production of antibodies or antigen-binding fragments thereof, see recent reviews, for example Ref, M. E. (1993) Curr. Opinion Biotech. 4: 573-576; Trill J. J. *et al.* (1995) Curr. Opinion Biotech 6: 553-560.

Suitable vectors can be chosen or constructed, containing
20 appropriate regulatory sequences, including promoter sequences, terminator sequences, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. Vectors may be plasmids, viral e.g. phage, or phagemid, as appropriate. For further details see, for example, Molecular Cloning: a Laboratory Manual: 2nd edition, Sambrook *et al.*, 1989, Cold Spring Harbor
25 Laboratory Press. Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in Current Protocols in Molecular Biology, Second

Edition, Ausubel *et al.* eds., John Wiley & Sons, 1992, or subsequent updates thereto.

The term "host cell" is used to refer to a cell into which has been introduced, or which is capable of having introduced into it, a nucleic acid
5 sequence encoding one or more of the herein described antibodies, and which further expresses or is capable of expressing a selected gene of interest, such as a gene encoding any herein described antibody. The term includes the progeny of the parent cell, whether or not the progeny are identical in morphology or in genetic make-up to the original parent, so long as the selected gene is present.
10 Accordingly there is also contemplated a method comprising introducing such nucleic acid into a host cell. The introduction may employ any available technique. For eukaryotic cells, suitable techniques may include calcium phosphate transfection, DEAE-Dextran, electroporation, liposome-mediated transfection and transduction using retrovirus or other virus, *e.g.* vaccinia or, for insect cells,
15 baculovirus. For bacterial cells, suitable techniques may include calcium chloride transformation, electroporation and transfection using bacteriophage. The introduction may be followed by causing or allowing expression from the nucleic acid, *e.g.* by culturing host cells under conditions for expression of the gene. In one embodiment, the nucleic acid is integrated into the genome (*e.g.*
20 chromosome) of the host cell. Integration may be promoted by inclusion of sequences which promote recombination with the genome, in accordance-with standard techniques.

The present invention also provides, in certain embodiments, a method which comprises using a construct as stated above in an expression
25 system in order to express a particular polypeptide such as an KDR-specific antibody as described herein. The term "transduction" is used to refer to the transfer of genes from one bacterium to another, usually by a phage. "Transduction" also refers to the acquisition and transfer of eukaryotic cellular sequences by retroviruses. The term "transfection" is used to refer to the uptake

of foreign or exogenous DNA by a cell, and a cell has been "transfected" when the exogenous DNA has been introduced inside the cell membrane. A number of transfection techniques are well known in the art and are disclosed herein. See, e.g., Graham *et al.*, 1973, Virology 52:456; Sambrook *et al.*, 2001, MOLECULAR CLONING, A LABORATORY MANUAL, Cold Spring Harbor Laboratories; Davis *et al.*, 1986, BASIC METHODS IN MOLECULAR BIOLOGY, Elsevier; and Chu *et al.*, 1981, Gene 13:197. Such techniques can be used to introduce one or more exogenous DNA moieties into suitable host cells.

The term "transformation" as used herein refers to a change in a cell's genetic characteristics, and a cell has been transformed when it has been modified to contain a new DNA. For example, a cell is transformed where it is genetically modified from its native state. Following transfection or transduction, the transforming DNA may recombine with that of the cell by physically integrating into a chromosome of the cell, or may be maintained transiently as an episomal element without being replicated, or may replicate independently as a plasmid. A cell is considered to have been stably transformed when the DNA is replicated with the division of the cell. The term "naturally occurring" or "native" when used in connection with biological materials such as nucleic acid molecules, polypeptides, host cells, and the like, refers to materials which are found in nature and are not manipulated by a human. Similarly, "non-naturally occurring" or "non-native" as used herein refers to a material that is not found in nature or that has been structurally modified or synthesized by a human.

The terms "polypeptide" "protein" and "peptide" and "glycoprotein" are used interchangeably and mean a polymer of amino acids not limited to any particular length. The term does not exclude modifications such as myristylation, sulfation, glycosylation, phosphorylation and addition or deletion of signal sequences. The terms "polypeptide" or "protein" means one or more chains of amino acids, wherein each chain comprises amino acids covalently linked by peptide bonds, and wherein said polypeptide or protein can comprise a plurality of

chains non-covalently and/or covalently linked together by peptide bonds, having the sequence of native proteins, that is, proteins produced by naturally-occurring and specifically non-recombinant cells, or genetically-engineered or recombinant cells, and comprise molecules having the amino acid sequence of the native
5 protein, or molecules having deletions from, additions to, and/or substitutions of one or more amino acids of the native sequence. The terms "polypeptide" and "protein" specifically encompass the antibodies that bind to KDR of the present disclosure, or sequences that have deletions from, additions to, and/or substitutions of one or more amino acid of an anti-KDR antibody. Thus, a
10 "polypeptide" or a "protein" can comprise one (termed "a monomer") or a plurality (termed "a multimer") of amino acid chains.

The term "isolated protein" referred to herein means that a subject protein (1) is free of at least some other proteins with which it would typically be found in nature, (2) is essentially free of other proteins from the same source, e.g.,
15 from the same species, (3) is expressed by a cell from a different species, (4) has been separated from at least about 50 percent of polynucleotides, lipids, carbohydrates, or other materials with which it is associated in nature, (5) is not associated (by covalent or noncovalent interaction) with portions of a protein with which the "isolated protein" is associated in nature, (6) is operably associated (by
20 covalent or noncovalent interaction) with a polypeptide with which it is not associated in nature, or (7) does not occur in nature. Such an isolated protein can be encoded by genomic DNA, cDNA, mRNA or other RNA, or may be of synthetic origin, or any combination thereof. In certain embodiments, the isolated protein is substantially free from proteins or polypeptides or other contaminants that are
25 found in its natural environment that would interfere with its use (therapeutic, diagnostic, prophylactic, research or otherwise).

The term "polypeptide fragment" refers to a polypeptide, which can be monomeric or multimeric, that has an amino-terminal deletion, a carboxyl-terminal deletion, and/or an internal deletion or substitution of a naturally-occurring

or recombinantly-produced polypeptide. In certain embodiments, a polypeptide fragment can comprise an amino acid chain at least 5 to about 500 amino acids long. It will be appreciated that in certain embodiments, fragments are at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 150, 200, 250, 300, 350, 400, or 450 amino acids long. Particularly useful polypeptide fragments include functional domains, including antigen-binding domains or fragments of antibodies. In the case of an anti-KDR antibody, useful fragments include, but are not limited to: a CDR region, especially a CDR3 region of the heavy or light chain; a variable region of a heavy or light chain; a portion of an antibody chain or just its variable region including two CDRs; and the like.

Polypeptides may comprise a signal (or leader) sequence at the N-terminal end of the protein, which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be fused in-frame or conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (e.g., poly-His), or to enhance binding of the polypeptide to a solid support.

A peptide linker/spacer sequence may also be employed to separate multiple polypeptide components by a distance sufficient to ensure that each polypeptide folds into its secondary and/or tertiary structures, if desired. Such a peptide linker sequence can be incorporated into a fusion polypeptide using standard techniques well known in the art.

Certain peptide spacer sequences may be chosen, for example, based on: (1) their ability to adopt a flexible extended conformation; (2) their inability to adopt a secondary structure that could interact with functional epitopes on the first and second polypeptides; and/or (3) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes.

In one illustrative embodiment, peptide spacer sequences contain, for example, Gly, Asn and Ser residues. Other near neutral amino acids, such as Thr and Ala, may also be included in the spacer sequence.

Other amino acid sequences which may be usefully employed as
5 spacers include those disclosed in Maratea *et al.*, *Gene* 40:39 46 (1985); Murphy *et al.*, *Proc. Natl. Acad. Sci. USA* 83:8258 8262 (1986); U.S. Pat. No. 4,935,233 and U.S. Pat. No. 4,751,180.

Other illustrative spacers may include, for example, Glu-Gly-Lys-Ser-Ser-Gly-Ser-Gly-Ser-Glu-Ser-Lys-Val-Asp (Chaudhary *et al.*, 1990, *Proc. Natl.*
10 *Acad. Sci. U.S.A.* 87:1066-1070) and Lys-Glu-Ser-Gly-Ser-Val-Ser-Ser-Glu-Gln-Leu-Ala-Gln-Phe-Arg-Ser-Leu-Asp (Bird *et al.*, 1988, *Science* 242:423-426).

In some embodiments, spacer sequences are not required when the first and second polypeptides have non-essential N-terminal amino acid regions that can be used to separate the functional domains and prevent steric
15 interference. Two coding sequences can be fused directly without any spacer or by using a flexible polylinker composed, for example, of the pentamer Gly-Gly-Gly-Gly-Ser repeated 1 to 3 times. Such a spacer has been used in constructing single chain antibodies (scFv) by being inserted between VH and VL (Bird *et al.*, 1988, *Science* 242:423-426; Huston *et al.*, 1988, *Proc. Natl. Acad. Sci. U.S.A.*
20 85:5979-5883).

A peptide spacer, in certain embodiments, is designed to enable the correct interaction between two beta-sheets forming the variable region of the single chain antibody.

In certain illustrative embodiments, a peptide spacer is between 1 to
25 5 amino acids, between 5 to 10 amino acids, between 5 to 25 amino acids, between 5 to 50 amino acids, between 10 to 25 amino acids, between 10 to 50 amino acids, between 10 to 100 amino acids, or any intervening range of amino acids.

In other illustrative embodiments, a peptide spacer comprises about 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 or more amino acids in length.

Amino acid sequence modification(s) of the antibodies described herein are contemplated. For example, it may be desirable to improve the binding
5 affinity and/or other biological properties of the antibody. For example, amino acid sequence variants of an antibody may be prepared by introducing appropriate nucleotide changes into a polynucleotide that encodes the antibody, or a chain thereof, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the
10 amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution may be made to arrive at the final antibody, provided that the final construct possesses the desired characteristics (e.g., high affinity binding to KDR). The amino acid changes also may alter post-translational processes of the antibody, such as changing the number or position of glycosylation sites. Any of
15 the variations and modifications described above for polypeptides of the present invention may be included in antibodies of the present invention.

The present disclosure provides variants of the antibodies disclosed herein. In certain embodiments, such variant antibodies or antigen-binding fragments, or CDRs thereof, bind to KDR at least about 50%, at least about 70%,
20 and in certain embodiments, at least about 90% as well as an antibody sequence specifically set forth herein. In further embodiments, such variant antibodies or antigen-binding fragments, or CDRs thereof, bind to KDR with greater affinity than the antibodies set forth herein, for example, that bind quantitatively at least about 105%, 106%, 107%, 108%, 109%, or 110% as well as an antibody sequence
25 specifically set forth herein.

In particular embodiments, a subject antibody may have: a) a heavy chain variable region having an amino acid sequence that is at least 80% identical, at least 95% identical, at least 90%, at least 95% or at least 98% or 99% identical, to the heavy chain variable region of an anti-KDR antibody described herein; and

b) a light chain variable region having an amino acid sequence that is at least 80% identical, at least 85%, at least 90%, at least 95% or at least 98% or 99% identical, to the light chain variable region of an anti-KDR antibody described herein. The amino acid sequence of illustrative heavy and light chain regions are set forth in
5 SEQ ID NOs:1, 2, 9 and 10).

In particular embodiments, the antibody may comprise: a) a heavy chain variable region comprising: i. a CDR1 region that is identical in amino acid sequence to the heavy chain CDR1 region of a selected antibody described herein; ii. a CDR2 region that is identical in amino acid sequence to the heavy
10 chain CDR2 region of the selected antibody; and iii. a CDR3 region that is identical in amino acid sequence to the heavy chain CDR3 region of the selected antibody; and b) a light chain variable domain comprising: i. a CDR1 region that is identical in amino acid sequence to the light chain CDR1 region of the selected antibody; ii. a CDR2 region that is identical in amino acid sequence to the light chain CDR2
15 region of the selected antibody; and iii. a CDR3 region that is identical in amino acid sequence to the light chain CDR3 region of the selected antibody; wherein the antibody specifically binds a selected target (e.g., KDR). In a further embodiment, the antibody, or antigen-binding fragment thereof, is a variant antibody wherein the variant comprises a heavy and light chain identical to the selected antibody except
20 for up to 8, 9, 10, 11, 12, 13, 14, 15, or more amino acid substitutions in the CDR regions of the VH and VL regions. In this regard, there may be 1, 2, 3, 4, 5, 6, 7, 8, or in certain embodiments, 9, 10, 11, 12, 13, 14, 15 more amino acid substitutions in the CDR regions of the selected antibody. Substitutions may be in CDRs either in the VH and/or the VL regions. (See e.g., Muller, 1998, Structure 6:1153-1167).

25 Determination of the three-dimensional structures of representative polypeptides (e.g., variant KDR-specific antibodies as provided herein, for instance, an antibody protein having an antigen-binding fragment as provided herein) may be made through routine methodologies such that substitution, addition, deletion or insertion of one or more amino acids with selected natural or

non-natural amino acids can be virtually modeled for purposes of determining whether a so derived structural variant retains the space-filling properties of presently disclosed species. See, for instance, Donate et al., 1994 *Prot. Sci.* 3:2378; Bradley et al., *Science* 309: 1868-1871 (2005); Schueler-Furman et al.,
5 *Science* 310:638 (2005); Dietz et al., *Proc. Nat. Acad. Sci. USA* 103:1244 (2006); Dodson et al., *Nature* 450:176 (2007); Qian et al., *Nature* 450:259 (2007); Raman et al. *Science* 327:1014-1018 (2010). Some additional non-limiting examples of computer algorithms that may be used for these and related embodiments, such as for rational design of KDR-specific antibodies antigen-binding domains thereof
10 as provided herein, include VMD which is a molecular visualization program for displaying, animating, and analyzing large biomolecular systems using 3-D graphics and built-in scripting (see the website for the Theoretical and Computational Biophysics Group, University of Illinois at Urbana-Champaign, at ks.uiuc.edu/Research/vmd/). Many other computer programs are known in the art
15 and available to the skilled person and which allow for determining atomic dimensions from space-filling models (van der Waals radii) of energy-minimized conformations; GRID, which seeks to determine regions of high affinity for different chemical groups, thereby enhancing binding, Monte Carlo searches, which calculate mathematical alignment, and CHARMM (Brooks et al. (1983) *J. Comput.*
20 *Chem.* 4:187-217) and AMBER (Weiner et al (1981) *J. Comput. Chem.* 106: 765), which assess force field calculations, and analysis (see also, Eisenfield et al. (1991) *Am. J. Physiol.* 261:C376-386; Lybrand (1991) *J. Pharm. Belg.* 46:49-54; Froimowitz (1990) *Biotechniques* 8:640-644; Burbam et al. (1990) *Proteins* 7:99-111; Pedersen (1985) *Environ. Health Perspect.* 61:185-190; and Kini et al. (1991)
25 *J. Biomol. Struct. Dyn.* 9:475-488). A variety of appropriate computational computer programs are also commercially available, such as from Schrödinger (Munich, Germany).

In another embodiment of invention, the anti-KDR antibodies and humanized versions thereof are derived from rabbit monoclonal antibodies, and in

particular are generated using RabMAb® technology. These antibodies are advantageous as they require minimal sequence modifications, thereby facilitating retention of functional properties after humanization using mutational lineage guided (MLG) humanization technology (see e.g., U.S. Patent No. 7,462,697).

5 Thus, illustrative methods for making the anti-KDR antibodies of the present disclosure include the RabMab® rabbit monoclonal antibody technology described, for example, in U.S. Patents 5,675,063 and 7,429,487. In this regard, in certain embodiments, the anti-KDR antibodies of the disclosure are produced in rabbits. In particular embodiments, a rabbit-derived immortal B-lymphocyte capable of fusion
10 with a rabbit splenocyte is used to produce a hybrid cell that produces an antibody. The immortal B-lymphocyte does not detectably express endogenous immunoglobulin heavy chain and may contain, in certain embodiments, an altered immunoglobulin heavy chain-encoding gene.

15 Compositions and Methods of Use

The present disclosure provides compositions comprising the KDR-specific antibodies, antigen-binding fragments thereof and administration of such composition in a variety of therapeutic settings.

Administration of the KDR-specific antibodies described herein, in
20 pure form or in an appropriate pharmaceutical composition, can be carried out via any of the accepted modes of administration of agents for serving similar utilities. The pharmaceutical compositions can be prepared by combining an antibody or antibody-containing composition with an appropriate physiologically acceptable carrier, diluent or excipient, and may be formulated into preparations in solid,
25 semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants, gels, microspheres, and aerosols. In addition, other pharmaceutically active ingredients (including other anti-cancer agents as described elsewhere herein) and/or suitable excipients such as salts, buffers and stabilizers may, but need not, be present within the

composition. Administration may be achieved by a variety of different routes, including oral, parenteral, nasal, intravenous, intradermal, subcutaneous or topical. Preferred modes of administration depend upon the nature of the condition to be treated or prevented. An amount that, following administration, reduces, inhibits, prevents or delays the progression and/or metastasis of a cancer is considered effective.

In certain embodiments, the amount administered is sufficient to result in tumor regression, as indicated by a statistically significant decrease in the amount of viable tumor, for example, at least a 50% decrease in tumor mass, or by altered (*e.g.*, decreased with statistical significance) scan dimensions. In other embodiments, the amount administered is sufficient to result in clinically relevant reduction in symptoms of disorders associated with VEGF or KDR expression and/or activity, including but not limited to any of a variety of oncological diseases, inflammatory diseases, and angiogenesis-related diseases. Thus, a therapeutically effective amount of one or more of the antibodies described herein is administered to result in clinically relevant reduction in symptoms of disorders including, but not limited to, rheumatoid arthritis, diabetes and ischemic retinopathies, age-related macular degeneration, psoriasis and glomerular hypertrophy associate with proteinuria and a variety of oncological diseases including angiosarcoma, renal cell carcinoma, gastrointestinal cancer, metastatic gastric or gastro-esophageal junction adenocarcinoma, breast cancer, bladder cancer, hepatocellular carcinoma, colorectal cancer, prostate cancer, non-small cell lung cancer, neuroblastoma, ovarian cancers, melanoma, and recurrent glioblastoma multiforme, leukemias and solid tumors. Such clinically relevant symptoms are known to the skilled clinician and will vary depending on the disease indication being treated.

The precise dosage and duration of treatment is a function of the disease being treated and may be determined empirically using known testing protocols or by testing the compositions in model systems known in the art and

extrapolating therefrom. Controlled clinical trials may also be performed. Dosages may also vary with the severity of the condition to be alleviated. A pharmaceutical composition is generally formulated and administered to exert a therapeutically useful effect while minimizing undesirable side effects. The composition may be
5 administered one time, or may be divided into a number of smaller doses to be administered at intervals of time. For any particular subject, specific dosage regimens may be adjusted over time according to the individual need.

The KDR-specific antibody-containing compositions may be administered alone or in combination with other known cancer treatments, such as
10 radiation therapy, chemotherapy, transplantation, immunotherapy, hormone therapy, photodynamic therapy, *etc.* The compositions may also be administered in combination with antibiotics.

Typical routes of administering these and related pharmaceutical compositions thus include, without limitation, oral, topical, transdermal, inhalation,
15 parenteral, sublingual, buccal, rectal, vaginal, and intranasal. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques. Pharmaceutical compositions according to certain embodiments of the present invention are formulated so as to allow the active ingredients contained therein to be bioavailable upon
20 administration of the composition to a patient. Compositions that will be administered to a subject or patient may take the form of one or more dosage units, where for example, a tablet may be a single dosage unit, and a container of a herein described KDR-specific antibody in aerosol form may hold a plurality of dosage units. Actual methods of preparing such dosage forms are known, or will
25 be apparent, to those skilled in this art; for example, see *Remington: The Science and Practice of Pharmacy*, 20th Edition (Philadelphia College of Pharmacy and Science, 2000). The composition to be administered will, in any event, contain a therapeutically effective amount of an antibody of the present disclosure, for treatment of a disease or condition of interest in accordance with teachings herein.

A pharmaceutical composition may be in the form of a solid or liquid. In one embodiment, the carrier(s) are particulate, so that the compositions are, for example, in tablet or powder form. The carrier(s) may be liquid, with the compositions being, for example, an oral oil, injectable liquid or an aerosol, which
5 is useful in, for example, inhalatory administration. When intended for oral administration, the pharmaceutical composition is preferably in either solid or liquid form, where semi-solid, semi-liquid, suspension and gel forms are included within the forms considered herein as either solid or liquid.

As a solid composition for oral administration, the pharmaceutical
10 composition may be formulated into a powder, granule, compressed tablet, pill, capsule, chewing gum, wafer or the like. Such a solid composition will typically contain one or more inert diluents or edible carriers. In addition, one or more of the following may be present: binders such as carboxymethylcellulose, ethyl cellulose, microcrystalline cellulose, gum tragacanth or gelatin; excipients such as
15 starch, lactose or dextrans, disintegrating agents such as alginic acid, sodium alginate, Primogel, corn starch and the like; lubricants such as magnesium stearate or Sterotex; glidants such as colloidal silicon dioxide; sweetening agents such as sucrose or saccharin; a flavoring agent such as peppermint, methyl salicylate or orange flavoring; and a coloring agent. When the pharmaceutical
20 composition is in the form of a capsule, for example, a gelatin capsule, it may contain, in addition to materials of the above type, a liquid carrier such as polyethylene glycol or oil.

The pharmaceutical composition may be in the form of a liquid, for example, an elixir, syrup, solution, emulsion or suspension. The liquid may be for
25 oral administration or for delivery by injection, as two examples. When intended for oral administration, preferred composition contain, in addition to the present compounds, one or more of a sweetening agent, preservatives, dye/colorant and flavor enhancer. In a composition intended to be administered by injection, one or

more of a surfactant, preservative, wetting agent, dispersing agent, suspending agent, buffer, stabilizer and isotonic agent may be included.

The liquid pharmaceutical compositions, whether they be solutions, suspensions or other like form, may include one or more of the following adjuvants:

- 5 sterile diluents such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or diglycerides which may serve as the solvent or suspending medium, polyethylene glycols, glycerin, propylene glycol or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic
10 acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. Physiological saline is a preferred adjuvant. An injectable pharmaceutical
15 composition is preferably sterile.

- A liquid pharmaceutical composition intended for either parenteral or oral administration should contain an amount of an KDR-specific antibody as herein disclosed such that a suitable dosage will be obtained. Typically, this amount is at least 0.01% of the antibody in the composition. When intended for
20 oral administration, this amount may be varied to be between 0.1 and about 70% of the weight of the composition. Certain oral pharmaceutical compositions contain between about 4% and about 75% of the antibody. In certain embodiments, pharmaceutical compositions and preparations according to the present invention are prepared so that a parenteral dosage unit contains between
25 0.01 to 10% by weight of the antibody prior to dilution.

The pharmaceutical composition may be intended for topical administration, in which case the carrier may suitably comprise a solution, emulsion, ointment or gel base. The base, for example, may comprise one or more of the following: petrolatum, lanolin, polyethylene glycols, bee wax, mineral

oil, diluents such as water and alcohol, and emulsifiers and stabilizers. Thickening agents may be present in a pharmaceutical composition for topical administration. If intended for transdermal administration, the composition may include a transdermal patch or iontophoresis device. The pharmaceutical composition may be intended for rectal administration, in the form, for example, of a suppository, which will melt in the rectum and release the drug. The composition for rectal administration may contain an oleaginous base as a suitable nonirritating excipient. Such bases include, without limitation, lanolin, cocoa butter and polyethylene glycol.

10 The pharmaceutical composition may include various materials, which modify the physical form of a solid or liquid dosage unit. For example, the composition may include materials that form a coating shell around the active ingredients. The materials that form the coating shell are typically inert, and may be selected from, for example, sugar, shellac, and other enteric coating agents.

15 Alternatively, the active ingredients may be encased in a gelatin capsule. The pharmaceutical composition in solid or liquid form may include an agent that binds to the antibody of the invention and thereby assists in the delivery of the compound. Suitable agents that may act in this capacity include other monoclonal or polyclonal antibodies, one or more proteins or a liposome. The pharmaceutical

20 composition may consist essentially of dosage units that can be administered as an aerosol. The term aerosol is used to denote a variety of systems ranging from those of colloidal nature to systems consisting of pressurized packages. Delivery may be by a liquefied or compressed gas or by a suitable pump system that dispenses the active ingredients. Aerosols may be delivered in single phase,

25 bi-phasic, or tri-phasic systems in order to deliver the active ingredient(s). Delivery of the aerosol includes the necessary container, activators, valves, subcontainers, and the like, which together may form a kit. One of ordinary skill in the art, without undue experimentation may determine preferred aerosols.

The pharmaceutical compositions may be prepared by methodology well known in the pharmaceutical art. For example, a pharmaceutical composition intended to be administered by injection can be prepared by combining a composition that comprises an KDR-specific antibody as described herein and
5 optionally, one or more of salts, buffers and/or stabilizers, with sterile, distilled water so as to form a solution. A surfactant may be added to facilitate the formation of a homogeneous solution or suspension. Surfactants are compounds that non-covalently interact with the antibody composition so as to facilitate dissolution or homogeneous suspension of the antibody in the aqueous delivery
10 system.

The compositions may be administered in a therapeutically effective amount, which will vary depending upon a variety of factors including the activity of the specific compound (*e.g.*, KDR-specific antibody) employed; the metabolic stability and length of action of the compound; the age, body weight, general
15 health, sex, and diet of the patient; the mode and time of administration; the rate of excretion; the drug combination; the severity of the particular disorder or condition; and the subject undergoing therapy. Generally, a therapeutically effective daily dose is (for a 70 kg mammal) from about 0.001 mg/kg (*i.e.*, 0.07 mg) to about 100 mg/kg (*i.e.*, 7.0 g); preferably a therapeutically effective dose is (for a 70 kg
20 mammal) from about 0.01 mg/kg (*i.e.*, 0.7 mg) to about 50 mg/kg (*i.e.*, 3.5 g); more preferably a therapeutically effective dose is (for a 70 kg mammal) from about 1 mg/kg (*i.e.*, 70 mg) to about 25 mg/kg (*i.e.*, 1.75 g). As would be recognized by the skilled person, in certain embodiments, it may be preferable to use doses expressed as milligrams per meter squared (*i.e.*, mg/m²). For example, to express
25 a mg/kg dose in any given species as the equivalent mg/m² dose, multiply the dose by the appropriate km factor. In adult humans, 100 mg/kg is equivalent to 100 mg/kg x 37 kg/m² = 3700 mg/m². See *e.g.*, FDA guidelines for Industry and Reviewers; see also Freireich, EJ, *et al.* Quantitative comparison of toxicity of

anticancer agents in mouse, rat, dog, monkey and man. *Cancer Chemother Rep.*1966;50(4):219-244.

Compositions comprising the KDR-specific antibodies of the present disclosure may also be administered simultaneously with, prior to, or after
5 administration of one or more other therapeutic agents. Such combination therapy may include administration of a single pharmaceutical dosage formulation which contains a compound of the invention and one or more additional active agents, as well as administration of compositions comprising antibodies of the invention and each active agent in its own separate pharmaceutical dosage formulation. For
10 example, an antibody as described herein and the other active agent can be administered to the patient together in a single oral dosage composition such as a tablet or capsule, or each agent administered in separate oral dosage formulations. Similarly, an antibody as described herein and the other active agent can be administered to the patient together in a single parenteral dosage
15 composition such as in a saline solution or other physiologically acceptable solution, or each agent administered in separate parenteral dosage formulations. Where separate dosage formulations are used, the compositions comprising antibodies and one or more additional active agents can be administered at essentially the same time, *i.e.*, concurrently, or at separately staggered times, *i.e.*,
20 sequentially and in any order; combination therapy is understood to include all these regimens.

Thus, in certain embodiments, also contemplated is the administration of anti-KDR antibody compositions of this disclosure in combination with one or more other therapeutic agents. Such therapeutic agents may be
25 accepted in the art as a standard treatment for a particular disease state as described herein, such as inflammatory disease (e.g., rheumatoid arthritis, or other inflammatory disorder), any of a variety of oncological diseases, and angiogenesis-mediated diseases (such as, but not limited to age-related macular degeneration). Exemplary therapeutic agents contemplated include cytokines, growth factors,

steroids, NSAIDs, DMARDs, anti-inflammatories, chemotherapeutics, radiotherapeutics, or other active and ancillary agents.

In certain embodiments, the anti-KDR antibodies disclosed herein may be administered in conjunction with any number of chemotherapeutic agents.

- 5 Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclophosphamide (CYTOXANTM); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide,
- 10 triethylenethiophosphoramide and trimethylolomelamine; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine;
- 15 antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabycin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin,
- 20 potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-
- 25 azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiothane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil;

bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK.RTM.;

5 razoxane; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2, 2',2"-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g. paclitaxel (TAXOL®, Bristol-Myers Squibb Oncology, Princeton, N.J.) and doxorubicin (TAXOTERE®, Rhne-Poulenc

10 Rorer, Antony, France); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylomithine

15 (DMFO); retinoic acid derivatives such as Targretin™ (bexarotene), Panretin™ (alitretinoin) ; ONTAK™ (denileukin diftitox) ; esperamicins; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens including for example tamoxifen,

20 raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (Fareston); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

A variety of other therapeutic agents may be used in conjunction with

25 the anti-KDR antibodies described herein. In one embodiment, the antibody is administered with an anti-inflammatory agent. Anti-inflammatory agents or drugs include, but are not limited to, steroids and glucocorticoids (including betamethasone, budesonide, dexamethasone, hydrocortisone acetate, hydrocortisone, hydrocortisone, methylprednisolone, prednisolone, prednisone,

triamcinolone), nonsteroidal anti-inflammatory drugs (NSAIDs) including aspirin, ibuprofen, naproxen, methotrexate, sulfasalazine, leflunomide, anti-TNF medications, cyclophosphamide and mycophenolate.

The compositions comprising herein described KDR-specific
5 antibodies may be administered to an individual afflicted with a disease as described herein, including, but not limited to disorders associated with VEGF or KDR expression and/or activity, including but not limited to, rheumatoid arthritis, diabetes and ischemic retinopathies, age-related macular degeneration, psoriasis and glomerular hypertrophy associate with proteinuria and a variety of oncological
10 diseases including angiosarcoma, renal cell carcinoma, gastrointestinal cancer, metastatic gastric or gastro-esophageal junction adenocarcinoma, breast cancer, bladder cancer, hepatocellular carcinoma, colorectal cancer, prostate cancer, non-small cell lung cancer, neuroblastoma, ovarian cancers, melanoma, and recurrent glioblastoma multiforme, leukemias and solid tumors. For *in vivo* use for the
15 treatment of human disease, the antibodies described herein are generally incorporated into a pharmaceutical composition prior to administration. A pharmaceutical composition comprises one or more of the antibodies described herein in combination with a physiologically acceptable carrier or excipient as described elsewhere herein. To prepare a pharmaceutical composition, an
20 effective amount of one or more of the compounds is mixed with any pharmaceutical carrier(s) or excipient known to those skilled in the art to be suitable for the particular mode of administration. A pharmaceutical carrier may be liquid, semi-liquid or solid. Solutions or suspensions used for parenteral, intradermal, subcutaneous or topical application may include, for example, a sterile
25 diluent (such as water), saline solution, fixed oil, polyethylene glycol, glycerin, propylene glycol or other synthetic solvent; antimicrobial agents (such as benzyl alcohol and methyl parabens); antioxidants (such as ascorbic acid and sodium bisulfite) and chelating agents (such as ethylenediaminetetraacetic acid (EDTA)); buffers (such as acetates, citrates and phosphates). If administered intravenously,

suitable carriers include physiological saline or phosphate buffered saline (PBS), and solutions containing thickening and solubilizing agents, such as glucose, polyethylene glycol, polypropylene glycol and mixtures thereof.

The compositions comprising KDR-specific antibodies as described
5 herein may be prepared with carriers that protect the antibody against rapid elimination from the body, such as time release formulations or coatings. Such carriers include controlled release formulations, such as, but not limited to, implants and microencapsulated delivery systems, and biodegradable, biocompatible polymers, such as ethylene vinyl acetate, polyanhydrides,
10 polyglycolic acid, polyorthoesters, polylactic acid and others known to those of ordinary skill in the art.

Provided herein are methods of treatment using the antibodies that bind KDR. In one embodiment, an antibody of the present invention is administered to a patient having a disease involving inappropriate expression of
15 KDR, which is meant in the context of the present disclosure to include diseases and disorders characterized by aberrant KDR expression or activity, due for example to alterations (*e.g.*, statistically significant increases or decreases) in the amount of a protein present, or the presence of a mutant protein, or both. An overabundance may be due to any cause, including but not limited to
20 overexpression at the molecular level, prolonged or accumulated appearance at the site of action, or increased (*e.g.*, in a statistically significant manner) activity of KDR relative to that which is normally detectable. Such an overabundance of KDR can be measured relative to normal expression, appearance, or activity of KDR signalling events, and said measurement may play an important role in the
25 development and/or clinical testing of the antibodies described herein.

In particular, the present antibodies are useful for the treatment of a variety of cancers associated with the expression of KDR. For example, one embodiment of the invention provides a method for the treatment of a cancer including, but not limited to, disorders associated with VEGF or KDR expression

and/or activity, including but not limited to, a variety of oncological diseases including angiosarcoma, renal cell carcinoma, gastrointestinal cancer, metastatic gastric or gastro-esophageal junction adenocarcinoma, breast cancer, bladder cancer, hepatocellular carcinoma, colorectal cancer, prostate cancer, non-small
5 cell lung cancer, neuroblastoma, ovarian cancers, melanoma, and recurrent glioblastoma multiforme, leukemias and solid tumors, by administering to a cancer patient a therapeutically effective amount of a herein disclosed KDR-specific antibody. An amount that, following administration, inhibits, prevents or delays the progression and/or metastasis of a cancer in a statistically significant manner (*i.e.*,
10 relative to an appropriate control as will be known to those skilled in the art) is considered effective.

Another embodiment provides a method for preventing metastasis of a cancer including, but not limited to, cancers including, but not limited to, a variety of oncological diseases including angiosarcoma, renal cell carcinoma,
15 gastrointestinal cancer, metastatic gastric or gastro-esophageal junction adenocarcinoma, breast cancer, bladder cancer, hepatocellular carcinoma, colorectal cancer, prostate cancer, non-small cell lung cancer, neuroblastoma, ovarian cancers, melanoma, and recurrent glioblastoma multiforme, leukemias and solid tumors, by administering to a cancer patient a therapeutically effective
20 amount of a herein disclosed KDR-specific antibody (*e.g.*, an amount that, following administration, inhibits, prevents or delays metastasis of a cancer in a statistically significant manner, *i.e.*, relative to an appropriate control as will be known to those skilled in the art).

Another embodiment provides a method for preventing a cancer
25 including, but not limited to, cancers including, but not limited to, a variety of oncological diseases including angiosarcoma, renal cell carcinoma, gastrointestinal cancer, metastatic gastric or gastro-esophageal junction adenocarcinoma, breast cancer, bladder cancer, hepatocellular carcinoma, colorectal cancer, prostate cancer, non-small cell lung cancer, neuroblastoma,

ovarian cancers, melanoma, and recurrent glioblastoma multiforme, leukemias and solid tumors, by administering to a cancer patient a therapeutically effective amount of a herein disclosed KDR-specific antibody.

Another embodiment provides a method for treating, inhibiting the
5 progression of or prevention of cancers including, but not limited to, a variety of
oncological diseases such as angiosarcoma, renal cell carcinoma, gastrointestinal
cancer, metastatic gastric or gastro-esophageal junction adenocarcinoma, breast
cancer, bladder cancer, hepatocellular carcinoma, colorectal cancer, prostate
cancer, non-small cell lung cancer, neuroblastoma, ovarian cancers, melanoma,
10 and recurrent glioblastoma multiforme, leukemias and solid tumors, and other
disorders associated with VEGF or KDR expression and/or activity, including but
not limited to, rheumatoid arthritis, diabetes and ischemic retinopathies, age-
related macular degeneration, psoriasis and glomerular hypertrophy associate with
proteinuria, by administering to a patient afflicted by one or more of these diseases
15 a therapeutically effective amount of a herein disclosed KDR-specific antibody.

In further embodiments, the present antibodies are useful for the
treatment of a variety of inflammatory diseases. For example, one embodiment of
the invention provides a method for the treatment of an inflammatory disease
including, but not limited to, inflammatory disorders associated with VEGF or KDR
20 expression and/or activity, including but not limited to, rheumatoid arthritis,
diabetes, gout, cryopyrin-associated periodic syndrome, chronic obstructive
pulmonary disorder and various cardiovascular diseases such as atherosclerosis
and vasculitis. The present antibodies are useful for the treatment of inflammatory
syndromes characterized by attacks of sterile inflammation of joints, serositis,
25 fever, and skin lesions. Inflammatory diseases include, but are not limited to,
Crohn's disease, colitis, dermatitis, psoriasis, diverticulitis, hepatitis, irritable bowel
syndrom (IBS), lupus erythematosus, nephritis, Parkinson's disease, ulcerative
colitis, multiple sclerosis (MS), Alzheimer's disease, arthritis, rheumatoid arthritis,
asthma, and various cardiovascular diseases such as atherosclerosis and

vasculitis. In one embodiment, the present disclosure provides a method of treating, reducing the severity of or preventing inflammation or an inflammatory disease by administering to a patient in need thereof a therapeutically effective amount of a herein disclosed compositions.

5 In another embodiment, anti-KDR antibodies of the present invention are used to determine the structure of bound antigen, *e.g.*, conformational epitopes, which structure may then be used to develop compounds having or mimicking this structure, *e.g.*, through chemical modeling and SAR methods.

Various other embodiments of the present invention relate, in part, to
10 diagnostic applications for detecting the presence of cells or tissues expressing KDR. Thus, the present disclosure provides methods of detecting KDR in a sample, such as detection of cells or tissues expressing KDR. Such methods can be applied in a variety of known detection formats, including, but not limited to immunohistochemistry (IHC), immunocytochemistry (ICC), *in situ* hybridization
15 (ISH), whole-mount *in situ* hybridization (WISH), fluorescent DNA *in situ* hybridization (FISH), flow cytometry, enzyme immuno-assay (EIA), and enzyme linked immuno-assay (ELISA).

ISH is a type of hybridization that uses a labeled complementary DNA or RNA strand (*i.e.*, primary binding agent) to localize a specific DNA or RNA
20 sequence in a portion or section of a cell or tissue (*in situ*), or if the tissue is small enough, the entire tissue (whole mount ISH). One having ordinary skill in the art would appreciate that this is distinct from immunohistochemistry, which localizes proteins in tissue sections using an antibody as a primary binding agent. DNA ISH can be used on genomic DNA to determine the structure of chromosomes.
25 Fluorescent DNA ISH (FISH) can, for example, be used in medical diagnostics to assess chromosomal integrity. RNA ISH (hybridization histochemistry) is used to measure and localize mRNAs and other transcripts within tissue sections or whole mounts.

In various embodiments, the antibodies described herein are conjugated to a detectable label that may be detected directly or indirectly. In this regard, an antibody "conjugate" refers to an anti-KDR antibody that is covalently linked to a detectable label. In the present invention, DNA probes, RNA probes, 5 monoclonal antibodies, antigen-binding fragments thereof, and antibody derivatives thereof, such as a single-chain-variable-fragment antibody or an epitope tagged antibody, may all be covalently linked to a detectable label. In "direct detection", only one detectable antibody is used, *i.e.*, a primary detectable antibody. Thus, direct detection means that the antibody that is conjugated to a 10 detectable label may be detected, *per se*, without the need for the addition of a second antibody (secondary antibody).

A "detectable label" is a molecule or material that can produce a detectable (such as visually, electronically or otherwise) signal that indicates the presence and/or concentration of the label in a sample. When conjugated to a 15 antibody, the detectable label can be used to locate and/or quantify the target to which the specific antibody is directed. Thereby, the presence and/or concentration of the target in a sample can be detected by detecting the signal produced by the detectable label. A detectable label can be detected directly or indirectly, and several different detectable labels conjugated to different specific- 20 antibodies can be used in combination to detect one or more targets.

Examples of detectable labels, which may be detected directly, include fluorescent dyes and radioactive substances and metal particles. In contrast, indirect detection requires the application of one or more additional antibodies, *i.e.*, secondary antibodies, after application of the primary antibody. 25 Thus, the detection is performed by the detection of the binding of the secondary antibody or binding agent to the primary detectable antibody. Examples of primary detectable binding agents or antibodies requiring addition of a secondary binding agent or antibody include enzymatic detectable binding agents and hapten detectable binding agents or antibodies.

In some embodiments, the detectable label is conjugated to a nucleic acid polymer which comprises the first binding agent (*e.g.*, in an ISH, WISH, or FISH process). In other embodiments, the detectable label is conjugated to an antibody which comprises the first binding agent (*e.g.*, in an IHC process).

5 Examples of detectable labels which may be conjugated to antibodies used in the methods of the present disclosure include fluorescent labels, enzyme labels, radioisotopes, chemiluminescent labels, electrochemiluminescent labels, bioluminescent labels, polymers, polymer particles, metal particles, haptens, and dyes.

10 Examples of fluorescent labels include 5-(and 6)-carboxyfluorescein, 5- or 6-carboxyfluorescein, 6-(fluorescein)-5-(and 6)-carboxamido hexanoic acid, fluorescein isothiocyanate, rhodamine, tetramethylrhodamine, and dyes such as Cy2, Cy3, and Cy5, optionally substituted coumarin including AMCA, PerCP, phycobiliproteins including R-phycoerythrin (RPE) and allophycoerythrin (APC),
15 Texas Red, Princeton Red, green fluorescent protein (GFP) and analogues thereof, and conjugates of R-phycoerythrin or allophycoerythrin, inorganic fluorescent labels such as particles based on semiconductor material like coated CdSe nanocrystallites.

 Examples of polymer particle labels include micro particles or latex
20 particles of polystyrene, PMMA or silica, which can be embedded with fluorescent dyes, or polymer micelles or capsules which contain dyes, enzymes or substrates.

 Examples of metal particle labels include gold particles and coated gold particles, which can be converted by silver stains. Examples of haptens include DNP, fluorescein isothiocyanate (FITC), biotin, and digoxigenin. Examples
25 of enzymatic labels include horseradish peroxidase (HRP), alkaline phosphatase (ALP or AP), β -galactosidase (GAL), glucose-6-phosphate dehydrogenase, β -N-acetylglucosaminidase, β -glucuronidase, invertase, Xanthine Oxidase, firefly luciferase and glucose oxidase (GO). Examples of commonly used substrates for horseradishperoxidase include 3,3'-diaminobenzidine (DAB), diaminobenzidine

with nickel enhancement, 3-amino-9-ethylcarbazole (AEC), Benzidine dihydrochloride (BDHC), Harker-Yates reagent (HYR), Indophane blue (IB), tetramethylbenzidine (TMB), 4-chloro-1-naphthol (CN), .alpha.-naphthol pyronin (.alpha.-NP), o-dianisidine (OD), 5-bromo-4-chloro-3-indolylphosphate (BCIP),
 5 Nitro blue tetrazolium (NBT), 2-(p-iodophenyl)-3-p-nitrophenyl-5-phenyl tetrazolium chloride (INT), tetranitro blue tetrazolium (TNBT), 5-bromo-4-chloro-3-indoxyl-beta-D-galactoside/ferro-ferricyanide (BCIG/FF).

Examples of commonly used substrates for Alkaline Phosphatase include Naphthol-AS-B 1-phosphate/fast red TR (NABP/FR), Naphthol-AS-MX-phosphate/fast red TR (NAMP/FR), Naphthol-AS-B1-phosphate/- fast red TR (NABP/FR), Naphthol-AS-MX-phosphate/fast red TR (NAMP/FR), Naphthol-AS-B1-phosphate/new fuschin (NABP/NF), bromochloroindolyl phosphate/nitroblue tetrazolium (BCIP/NBT), 5-Bromo-4-chloro-3-indolyl-beta-D-galactopyranoside (BCIG).

15 Examples of luminescent labels include luminol, isoluminol, acridinium esters, 1,2-dioxetanes and pyridopyridazines. Examples of electrochemiluminescent labels include ruthenium derivatives. Examples of radioactive labels include radioactive isotopes of iodide, cobalt, selenium, tritium, carbon, sulfur and phosphorous.

20 Detectable labels may be linked to the antibodies described herein or to any other molecule that specifically binds to a biological marker of interest, *e.g.*, an antibody, a nucleic acid probe, or a polymer. Furthermore, one of ordinary skill in the art would appreciate that detectable labels can also be conjugated to second, and/or third, and/or fourth, and/or fifth binding agents or antibodies, etc.
 25 Moreover, the skilled artisan would appreciate that each additional binding agent or antibody used to characterize a biological marker of interest may serve as a signal amplification step. The biological marker may be detected visually using, *e.g.*, light microscopy, fluorescent microscopy, electron microscopy where the detectable substance is for example a dye, a colloidal gold particle, a luminescent

reagent. Visually detectable substances bound to a biological marker may also be detected using a spectrophotometer. Where the detectable substance is a radioactive isotope detection can be visually by autoradiography, or non-visually using a scintillation counter. See, e.g., Larsson, 1988, Immunocytochemistry:

- 5 Theory and Practice, (CRC Press, Boca Raton, Fla.); Methods in Molecular Biology, vol. 80 1998, John D. Pount (ed.) (Humana Press, Totowa, N.J.).

The invention further provides kits for detecting KDR or cells or tissues expressing KDR in a sample, wherein the kits contain at least one antibody, polypeptide, polynucleotide, vector or host cell as described herein. . In
10 certain embodiments, a kit may comprise buffers, enzymes, labels, substrates, beads or other surfaces to which the antibodies of the invention are attached, and the like, and instructions for use.

EXAMPLES

EXAMPLE 1

PRODUCTION AND HUMANIZATION OF ANTI-KDR ANTIBODIES

5 Anti-KDR Antibody Generation

New Zealand white rabbits were immunized with recombinant KDR-rabbit Fc fusion protein. The rabbit with the highest serum titers of specific binding to human KDR was chosen for hybridoma generation. A total of 230 hybridomas were identified as positive for binding to soluble KDR-Fc. Of these 230, 100 clones
10 were also found positive for binding to cell surface KDR through the use of KDR-transfectant 293 cells. The double positive hybridomas were selected for further functional characterization.

Functional Screening of Hybridomas

Screening for functional antibodies that block binding of KDR to

15 **VEGF:** The double positive anti-KDR antibodies (100 clones) that bind both soluble KDR and cell surface KDR were assessed for their ability to inhibit the binding of KDR to VEGF. Out of 100 anti-KDR clones, 41 were found to exhibit inhibition and were recombinantly expressed and purified for further
20 characterization. The potency of the top 10 anti-KDR antibodies that inhibited the binding of KDR to VEGF is summarized in Table 2 below and is shown in Figure 1.

Table 2: IC₅₀ of Anti-KDR Antibodies

Antibody	IC ₅₀ (nM)
15	17.48
23	21.37
24	19.83
27	5.95
36	6.25

Antibody	IC50 (nM)
43	7.54
71	9.94
81	4.56
83	9.77
92	32.94

Screening for antibodies that inhibit KDR phosphorylation: The top 10 anti-KDR antibodies selected based on the ligand receptor binding assay were further tested in a cell-based KDR phosphorylation assay using HUVEC cells.

5 Cultured HUVEC cells were treated with anti-KDR antibodies at 5 μ g/ml (a relatively saturated dose) for 1 hour at 37°C before adding 20 ng/ml (an optimal stimulation dose) of human VEGF (R&D System). The harvested cell lysates were quantified by protein concentration. The phosphorylation of KDR was determined using a phosphor ELISA assay using a rabbit anti-KDR YK-5 (a non-functional

10 anti-KDR clone) as the capture antibody and a mouse anti- phosphotyrosine, P-Tyr-100 (Cell Signaling Technology) as the detection antibody. The top 6 clones that inhibit KDR phosphorylation are shown in Figure 2. Of the top 6 clones, clone 36 exhibited the strongest inhibition of VEGF-stimulated KDR phosphorylation (Figure 2A). The phosphor ELISA result of clone 36 was further confirmed by

15 Western blot (Figure 2B) using anti-phosphor-KDR Y996 polyclonal antibody (Epitomics, Burlingame, CA).

Screening for cross species anti-KDR antibodies: In order to evaluate the efficacy of blocking KDR therapy in *in vivo* animal studies, in which

20 KDR is only expressed on endothelial cells of the host, the top 6 anti-KDR antibodies were screened for cross reactivity with mouse KDR. Antibodies #27, #36, #43, #68, #83 were found to be cross-reactive with both human and mouse KDR (data not shown). Table 3 below summarizes three groups of rabbit antibodies identified in the screen as follows: 1) Anti-human KDR antibodies that

25 block KDR phosphorylation, and also cross react with mouse KDR, including

clones 4, 6, 14, 27, 30, 36, 68, 69, 81, 83, 91 and 95; 2) Anti-human KDR antibodies that block KDR phosphorylation but do not cross-react with mouse KDR, including clones 5, 13, 15, 17, 23, 24, 25, 43, 71, 77, 92 and 93; 3) Anti-human KDR antibodies that cross-react with mouse KDR but do not block KDR phosphorylation, including clones 40, 42 and 50.

Table 3: Anti-KDR Rabbit Antibodies Summary

Anti-KDR clone	Mouse reactive	Blocking KDR phosphorylation	VH amino acid SEQ ID NO	VL amino acid SEQ ID NO
4	+	+	17	43
5	-	++	18	44
6	+	+	19	45
13	-	+++	20	46
14	+	++	21	47
15	-	+	22	48
17	-	+	23	49
23	-	++	24	50
24	-	+++	25	51
25	-	+++	26	52
27	+	++	27	53
30	+	++	28	54
36	+	+++	1	2
40	+	-	29	55
42	+	-	30	56
43	-	+++	31	57
50	+	-	32	58

Anti-KDR clone	Mouse reactive	Blocking KDR phosphorylation	VH amino acid SEQ ID NO	VL amino acid SEQ ID NO
68	+	++	33	59
69	+	+++	34	60
71	-	+	35	61
77	-	++	36	62
81	+	+	37	63
83	+	++	38	64
91	+	+	39	65
92	-	+++	40	66
93	-	+++	41	67
95	+	++	42	68

Figure 11 shows an alignment of the VH and VL regions of the anti-KDR antibodies. The CDRs are underlined. The VHCDR1 amino acid sequences are provided in SEQ ID Nos:69-95; the VHCDR2 amino acid sequences are provided in SEQ ID Nos:96-122; the VHCDR3 amino acid sequences are provided in SEQ ID Nos:123-149; the VLCDR1 amino acid sequences are provided in SEQ ID Nos:150-176; the VLCDR2 amino acid sequences are provided in SEQ ID Nos:177-203; the VLCDR3 amino acid sequences are provided in SEQ ID Nos:204-230.

10

Based on the potency of anti-phosphorylation of human KDR and cross reactivity to mouse KDR, clone 36 was selected as the first candidate anti-KDR antibody.

Recombinant Anti- KDR Antibody Clone 36

DNA fragments of L chains and the variable region (VH) of H chains of rabbit IgG from clone 36 were amplified by PCR. The L chain fragment was cloned into pTT5 vector at Hind III and Not I sites and the VH fragment into the
5 constant region of H chain built-in pTT5 vector at Hind III and Kpn I sites. For each hybridoma, three DNA clones of L or H chain were sequenced and the plasmid with a consensus sequence was identified and used for recombinant expression. To express the recombinant antibody, the L and H chain plasmids were co-transfected into 293-6E cells (National Research Council Canada). The
10 supernatants were harvested 5 days later and quantified using an ELISA assay to measure the IgG concentration before functional assays.

Humanization Design

Mutational lineage guided (MLG) humanization technology was used to humanize the lead clone 36. First, the heavy chain (VH) and light chain (VK) variable region sequences of clone 36 were blasted against the human germline
15 VH and VK database. The closest human germline sequences were identified as the template for humanization. Secondly, the rabbit residues in the framework regions potentially involved in CDR contacts or inter-chain contacts were identified based on knowledge from human and rabbit antibodies. Residues considered not
20 critical to the structural activity of the antibodies were identified based on knowledge from previous humanized rabbit antibodies.

The light and heavy chain frameworks of the humanized 36 (APX004) are 93% identical to the human germline sequences. In addition to humanization of the frameworks, the MLG method allowed us to further humanize
25 both CDR1 and CDR2 of the heavy chain from 47% to 58% homology to human germline sequences. The binding potency of APX004 to KDR was found to be similar to its parental rabbit monoclonal antibody 36 (See Figure 4). The amino acid sequences of the humanized VH and VL regions for clone 36 are set forth in

SEQ ID NOs:9 and 10, respectively. The amino acid sequence of the VHCDR1 and VHCDR2 of the humanized clone 36 are set forth in SEQ ID NOs:11 and 12. The amino acid sequence of the VHCDR3 of the humanized clone 36 anti-KDR antibody (APX004) is the same as the parental VHCDR3 as set forth in SEQ ID NO:5. The amino acid sequences of the VLCDR1, VLCDR2 and VLCDR3 of the humanized clone 36 anti-KDR antibody (APX004) are the same as the rabbit parental VLCDR sequences as set forth in SEQ ID NOs:6-8.

Expression of Humanized Clone(s)

10 DNA encoding humanized VK and VH of clone R-36 was synthesized by MCLab (South San Francisco, CA, USA). The DNA fragments include signal peptide and a Kozak sequence at the 5' end. To express the humanized version of clone 36, the humanized VK fragment was cloned into human CK built-in pTT5 vector at Hind III and Nhe I. The humanized VH was cloned into human IgG1 CH1 built-in pTT5 vector at Hind III and BsiW I site. DNA and amino acid sequences of human CK (SEQ ID NOs:13 and 14, respectively) and IgG1 CH1 (SEQ ID NOs:15 and 16, respectively) were chosen for the constant region. Humanized versions of clone 36 were expressed in 293-6E cells, purified through a protein A column and quantified by UV280 after dialyzing against PBS buffer.

20

EXAMPLE 2

BINDING SELECTIVITY OF APX004

APX004 is a humanized IgG1 antibody against KDR (VEGFR2). It binds with high affinity ($K_d = 5.3 \times 10^{-11}$ M) and specificity to human KDR. APX004 cross reacts with monkey and mouse KDR. APX004 blocks the binding of KDR to VEGF and inhibits KDR phosphorylation, resulting in inhibition KDR downstream

signaling and biologic functions, such as endothelial cell proliferation and angiogenesis.

Binding selectivity of APX004 was assessed by direct ELISA to VEGFR family proteins. A total of 1µg/ml of rabbit Fc-fusion protein of human KDR, mouse KDR, human VEGFR1, human VEGFR3, human OX40L and human VEGF were coated on ELISA plates followed by incubation with 1µg/ml of APX004. Bound APX004 was detected using goat anti-human HRP-conjugated IgG.

As shown in Figure 3, APX004 binds selectively to human and mouse KDR but not to other human VEGFR family proteins or VEGF. Thus APX004 can be tested in mouse tumor models for *in vivo* efficacy and PK studies. Since monkey KDR shares 99.9% sequence identity (only a conserved amino acid difference in the non-ligand binding domain) to human KDR in the extra cellular domain, APX004 should be able to recognize monkey KDR.

15

EXAMPLE 3

APX004 BLOCKS BINDING OF KDR TO VEGF

An ELISA-based KDR-VEGF binding assay was developed and used to assess the potency of APX004 at blocking KDR binding to VEGF. A total of 2µg/ml VEGF was coated on ELISA plates. APX004, the parental rabbit antibody R-36 or IgG1 isotype control antibody were pre-incubated with 5µg/ml recombinant human KDR before being transferred to the VEGF-A coated ELISA plates. KDR bound to immobilized VEGF was detected by a mouse anti-KDR monoclonal antibody, followed by the addition of goat anti-mouse IgG conjugated with alkaline phosphatase (Fisher Scientific/Pierce Biotechnology, Rockford, IL). ELISA plates

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were developed with p-nitrophenyl phosphate substrate and absorbance at 405 nm was recorded. All experiments were performed in triplicate.

As shown in Figure 4, APX004 potently blocks the binding of KDR to VEGF with IC₅₀ of 2.6 nM. Humanization of R-36 did not affect its potency to
5 inhibit KDR binding to VEGF.

EXAMPLE 3

INHIBITION OF KDR PHOSPHORYLATION BY APX004

10

To assess the inhibitory effect of APX004 on VEGF-induced KDR phosphorylation, antibodies at various concentrations were pre-incubated with cultured HUVEC cells for 1h before adding 5 nM of human VEGF (R&D System). The KDR phosphorylation was detected by Western blot. Cell lysates from treated
15 HUVEC cells containing equal amounts of protein were resolved on 4–20% SDS–PAGE gel and the proteins were transferred to a PVDF membrane (Millipore, Billerica, MA). The blots were probed sequentially with anti-phosphor-KDR antibody, total KDR antibody and anti-alpha-tubulin antibody (Epitomics). Primary antibody was stripped by washing in glycine buffer before re-probing with another
20 primary antibody. The specific signals were visualized on X-ray film after incubation of blotted membrane with appropriate horseradish peroxidase–conjugated secondary antibodies (Fisher Scientific/Pierce Biotechnology), followed by ECL reagent development (GE Healthcare Bio-Sciences). As shown in Figure 5, APX004 inhibited the KDR phosphorylation induced by VEGF.

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EXAMPLE 4

INHIBITION OF HUVEC PROLIFERATION BY APX004

To determine the inhibitory effect of APX004 on VEGF-induced
5 HUVEC proliferation, various concentrations of APX004 were added to HUVEC
cultures at 4,000 cells / well in a 96-well plate and incubated for 1 hour before
adding 15 ng/ml of VEGF (final concentration). HUVEC cells were further
incubated at 37°C for 72 hours before 10% AlamarBlue® was added to each well.
After 24 hour incubation, HUVEC cell viability was measured by reading the
10 fluorescence intensity using Wallac Victor V 1420 Multilabel HTS Counter
(PerkinElmer) with excitation at 530 nm and emission at 590 nm. All assays were
done in triplicate. As shown in Figure 6, APX004 inhibits HUVEC proliferation in a
dose-dependent manner.

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EXAMPLE 5

INHIBITION OF TUMOR GROWTH IN HUMAN LUNG CANCER H460 MODEL

One of the advantages of APX004 over Ramucirumab is that
20 APX004 cross reacts with mouse KDR. Thus, APX004 can be directly evaluated in
human tumor xenograft models in mice. The *in vivo* anti-angiogenesis and anti-
tumor efficacy of APX004 was shown in multiple tumor xenograft models.

To evaluate the *in vivo* efficacy of APX004 in the human H460
xenograft model, human NSCLC tumor H460 (KDR negative) xenografts were
25 established by subcutaneous inoculation of human NSCLC cell line H460 into the
dorsal flanks of female BALB/c nude mice. When tumor sizes reached an average
volume of 200 mm³ at day 22, the tumor bearing mice were randomized into 3
treatment groups as indicated (n=8-10). Then the randomized groups received ip

injections of a 5 mg/kg/dose of APX004 or Avastin at 3 times/week for a total of 8 doses. Tumor volumes and body weights were calculated according to the following equation: $\text{Volume} = (\text{width})^2 \times \text{length} / 2$ every 2 to 3 days (Figure 7A). Photomicrographs of immunohistochemistry staining with an anti-CD34 mAb (x400) was performed to illustrate microvasculature density (brown color)(Figure 7B).

APX004 exhibited more potent anti-tumor activity (77% inhibition) than Avastin (69% inhibition) at the termination of study (Day 41). APX004 treatment resulted in reduction of tumor microvasculature (CD34⁺ EC staining). APX004 did not show significant toxicity (*i.e.*, there was no change in body weight relative to the control group).

EXAMPLE 6

DOSE RESPONSE INHIBITION IN HUMAN LUNG CANCER H460 MODEL

To determine the effective dose of APX004 in the human H460 xenograft model, human NSCLC tumor H460 xenografts were established by subcutaneous inoculation of human NSCLC cell line H460 into the dorsal flanks of female BALB/c nude mice. When tumors reached an average volume of 160 mm³, the tumor bearing mice were randomized into 3 treatment groups as indicated (n=8-10). APX004 was administered intraperitoneally at 2.5 mg/kg or 0.6 mg/kg 3 times/week for a total of 8 doses. Tumor size and body weight were recorded every 2 to 3 days. Tumor volumes were calculated according to the following equation: $\text{Volume} = (\text{width})^2 \times \text{length} / 2$. As shown in Figure 8, APX004 demonstrated a significant anti-tumor activity at a dose of 2.5 mg/kg (p<0.01) in the H460 tumor model. APX004 did not show significant toxicity (no change of body weight relative to the control group).

EXAMPLE 7

INHIBITION OF TUMOR GROWTH IN HUMAN MELANOMA A375 MODEL

5 To determine the effect of APX004 on tumor growth in the human A375 xenograft model, human melanoma tumor xenografts were established by subcutaneous inoculation of human A375 cells into the dorsal flanks of female BALB/c nude mice. When tumors reached an average volume of 100 mm³, the tumor bearing mice were randomized into 6 treatment groups as indicated. Various
10 doses of APX004 or 3 mg/kg of Avastin were administered intraperitoneally twice weekly for 3 weeks for a total of 6 doses. Tumor size and body weights were recorded every 3 days. Perpendicular dimensions of the tumor were measured using a Vernier scale caliper. Tumor volumes were calculated according to the following equation: Volume = (width)² x length/2. Symbols and bars mean +
15 standard deviation. As shown in Figure 9, APX004 significantly inhibits A375 tumor growth at 3 mg/kg. At this dose, APX004 exhibits anti-tumor activity similar to that of Avastin. The data suggested that the A375 model is less dependent on VEGF-KDR pathways. Therefore, no apparent dose-dependent anti-tumor effect was observed.

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EXAMPLE 8

INHIBITION OF TUMOR GROWTH IN HUMAN COLON CANCER HT29 MODEL

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To determine the dose and efficacy relationship of APX004 in the human colorectal cancer HT29 xenograft model, human colorectal cancer

xenografts were established by subcutaneous inoculation of human HT29 cells into the dorsal flanks of female BALB/c nude mice. When tumors reached an average volume of 100 mm³, the tumor bearing mice were randomized into 4 treatment groups as indicated. Various doses of APX004 were administered intraperitoneally 5 3 times/week for 3 weeks. Tumor size was measured every 3 days. Perpendicular dimensions of the tumor were measured using a Vernier scale caliper. Tumor volumes were calculated according to the following equation: Volume = (width)² x length/2. Symbols and bars, mean + standard deviation. As shown in Figure 10, APX004 significantly inhibits HT29 tumor growth at 5 mg/kg and 10 mg/kg. A dose- 10 dependent anti-tumor activity of APX004 was observed in this tumor model.

In summary, as shown in the above Examples, APX004 is a humanized IgG1 antibody with high KDR binding affinity which potently inhibits angiogenesis. During the humanization process, both the frameworks and the 15 CDRs were humanized to maximally reduce potential immunogenicity.

APX004 is a neutralizing antibody that blocks the binding of KDR to its ligands and inhibits VEGF-induced KDR phosphorylation and endothelial cell proliferation. Because APX004 cross reacts with mouse KDR, it was possible to evaluate APX004 directly in *in vivo* mouse models of human tumor xenografts 20 without the need to generate a surrogate antibody. APX004 inhibited the tumor-induced microvasculature formation and tumor growth in multiple human tumor xenografts with anti-tumor effect similar to Avastin. APX004 seems to be more potent than the ramucirumab's surrogate DC-101 in *in vivo* tumor models, where a much higher dose of DC-101 (>40 mg) was required to inhibit tumor growth. In 25 contrast to TKIs, APX004 is a more specific KDR targeting agent (does not inhibit other VEGF receptors). As such, it is expected the APX004 will have fewer off target side effects.

In contrast to Avastin, which binds only one of the VEGF family ligands (VEGF-A only), APX004 potentially blocks all known VEGFs from binding

to KDR. This may have a more profound inhibitory effect on tumor angiogenesis than just blocking VEGF-A, and may also be able to contribute to reverse Avastin resistance caused by ligand redundancy. Thus, APX004 has the potential to improve the treatment of patients with tumors that produce abundant VEGF family
 5 ligands in the tumor environment and may overcome resistance to anti-VEGF therapy.

Furthermore, two thirds of the tumor models used in the above studies (A375 and HT-27) express KDR and may use the VEGF-KDR pathway as an autocrine loop to grow. Thus, the anti-tumor effect mediated by the antibodies
 10 described herein may include at least 2 modes of action (i) anti-angiogenesis and (ii) direct inhibition of tumor growth (46, 47).

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The various embodiments described above can be combined to provide further embodiments. All of the U.S. patents, U.S. patent application publications, U.S. patent application, foreign patents, foreign patent application

20 and non-patent publications referred to in this specification and/or listed in the Application Data Sheet are incorporated herein by reference, in their entirety. Aspects of the embodiments can be modified, if necessary to employ concepts of the various patents, application and publications to provide yet further embodiments.

25 These and other changes can be made to the embodiments in light of the above-detailed description. In general, in the following claims, the terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible embodiments along with the full scope of equivalents to which such

30 claims are entitled. Accordingly, the claims are not limited by the disclosure.

CLAIMS

What is claimed is:

1. An isolated antibody, or an antigen-binding fragment thereof, that binds to KDR, comprising (i) a heavy chain variable region comprising the VHCDR1 region set forth in SEQ ID NO:3 or 11, the VHCDR2 region set forth in SEQ ID NO:4 or 12, and the VHCDR3 region set forth in SEQ ID NO:5; and (ii) a light chain variable region comprising the VLCDR1 region set forth in SEQ ID NO:6, the VLCDR2 region set forth in SEQ ID NO:7, and the VLCDR3 region set forth in SEQ ID NO: 8;

or a variant of said antibody, or an antigen-binding fragment thereof, comprising heavy and light chain variable regions identical to the heavy and light chain variable regions of (i) and (ii) except for up to 8 amino acid substitutions in said CDR regions.

2. The isolated antibody, or antigen-binding fragment thereof, of claim 1 wherein the heavy chain variable region comprises the amino acid sequence set forth in SEQ ID NO:1.

3. The isolated antibody, or antigen-binding fragment thereof, of claim 1 wherein the light chain variable region comprises the amino acid sequence set forth in SEQ ID NO:2.

4. An isolated antibody, or an antigen-binding fragment thereof, that binds to KDR, comprising (i) a heavy chain variable region comprising a VHCDR1, a VHCDR2, and a VHCDR3 of an antibody as set forth in Figure 11; and (ii) a corresponding light chain variable region comprising a VLCDR1, a VLCDR2 and a VLCDR3 of an antibody as set forth in Figure 11;

or a variant of said antibody, or an antigen-binding fragment thereof, comprising heavy and light chain variable regions identical to the heavy and light chain variable regions of (i) and (ii) except for up to 8 amino acid substitutions in said CDR regions.

5. The isolated antibody, or antigen-binding fragment thereof, of claim 4 wherein the heavy chain variable region comprises any one of the amino acid sequences set forth in SEQ ID NOs:17-42.

6. The isolated antibody, or antigen-binding fragment thereof, of claim 4 wherein the light chain variable region comprises any one of the amino acid sequences set forth in SEQ ID NO:43-68.

7. An isolated antibody, or an antigen-binding fragment thereof, that binds to KDR, comprising a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:1.

8. The isolated antibody, or antigen-binding fragment thereof, of claim 7 comprising a light chain variable region which comprises an amino acid sequence having at least 90% identity to the amino acid sequence set forth in SEQ ID NO:2.

9. The isolated antibody, or an antigen-binding fragment thereof, of claim 7 comprising a light chain variable region which comprises the amino acid sequence set forth in SEQ ID NO:2.

10. An isolated antibody, or an antigen-binding fragment thereof, that binds to KDR, comprising a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:2.

11. The isolated antibody, or antigen binding fragment thereof, of claim 10 comprising a heavy chain variable region which comprises an amino acid sequence having at least 90% identity to the amino acid sequence set forth in SEQ ID NO:1.

12. The isolated antibody of claim 1, wherein the antibody is humanized.

13. The isolated antibody of claim 12, wherein the VH region comprises the amino acid sequence set forth in SEQ ID NO:9 and the VL region comprises the amino acid sequence set forth in SEQ ID NO:10.

14. The isolated antibody of claim 1 wherein the antibody is selected from the group consisting of a single chain antibody, a ScFv, a univalent antibody lacking a hinge region, and a minibody.

15. The isolated antibody of claim 1 wherein the antibody is a Fab or a Fab' fragment.

16. The isolated antibody of claim 1 wherein the antibody is a F(ab')₂ fragment.

17. The isolated antibody of claim 1 wherein the antibody is a whole antibody.

18. The isolated antibody of claim 1 comprising a human IgG constant domain.

19. The isolated antibody of claim 18 wherein the IgG constant domain comprises an IgG1 CH1 domain.

20. The isolated antibody of claim 18 wherein the IgG constant domain comprises an IgG1 Fc region.

21. An isolated antibody, or an antigen-binding fragment thereof, that competes with the antibody of claim 1 for binding to human KDR.

22. An isolated antibody, or antigen-binding fragment thereof, that binds KDR with a KD of 5.3×10^{-11} M or lower.

23. An isolated antibody, or antigen-binding fragment thereof, wherein the isolated antibody, or antigen-binding fragment thereof:

- a. blocks VEGF binding to KDR;
- b. inhibits KDR signaling;
- c. inhibits endothelial cell proliferation;
- d. inhibits tumor angiogenesis;
- e. inhibits tumor cell growth;
- f. a combination of any one or more of a.-e.

24. The isolated antibody or antigen-binding fragment thereof of claim 23, wherein the isolated antibody or antigen-binding fragment thereof, blocks VEGF binding to KDR, inhibits KDR signaling, inhibits endothelial cell proliferation, inhibits tumor angiogenesis, and inhibits tumor cell growth.

25. An isolated antibody or antigen-binding fragment thereof, that directly inhibits tumor growth.

26. The isolated antibody of any one of claims 1, 4, or 21, 22-25, wherein the antibody crossreacts with murine KDR or non-human primate KDR.

27. An isolated polynucleotide encoding the isolated antibody, or antigen-binding fragment thereof, according to claim 1 or claim 13.
28. An expression vector comprising the isolated polynucleotide of claim 27.
29. An isolated host cell comprising the vector of claim 28.
30. A composition comprising a physiologically acceptable carrier and a therapeutically effective amount of the isolated antibody or antigen-binding fragment thereof according to any one of claims 1, 13, or 21- 26.
31. The composition of claim 30 for use in treating cancer.
32. The composition of claim 30 for use in treating a cancer associated with aberrant VEGF or KDR expression.
33. The composition of claim 32 wherein the cancer is selected from the group consisting of angiosarcoma, renal cell carcinoma, gastrointestinal cancer, metastatic gastric or gastro-esophageal junction adenocarcinoma, breast cancer, bladder cancer, hepatocellular carcinoma, colorectal cancer, prostate cancer, non-small cell lung cancer, neuroblastoma, ovarian cancer, melanoma, recurrent glioblastoma multiforme, and leukemia.
34. The composition of claim 30 for use in treating a patient afflicted by an inflammatory disease.
35. The composition of claim 30 for use in treating rheumatoid arthritis.

36. The composition of claim 30 for use in treating psoriasis.
37. The composition of claim 30 for use in treating an angiogenesis-mediated disease.
38. The composition of claim 37, wherein the angiogenesis-mediated disease is age-related macular degeneration.

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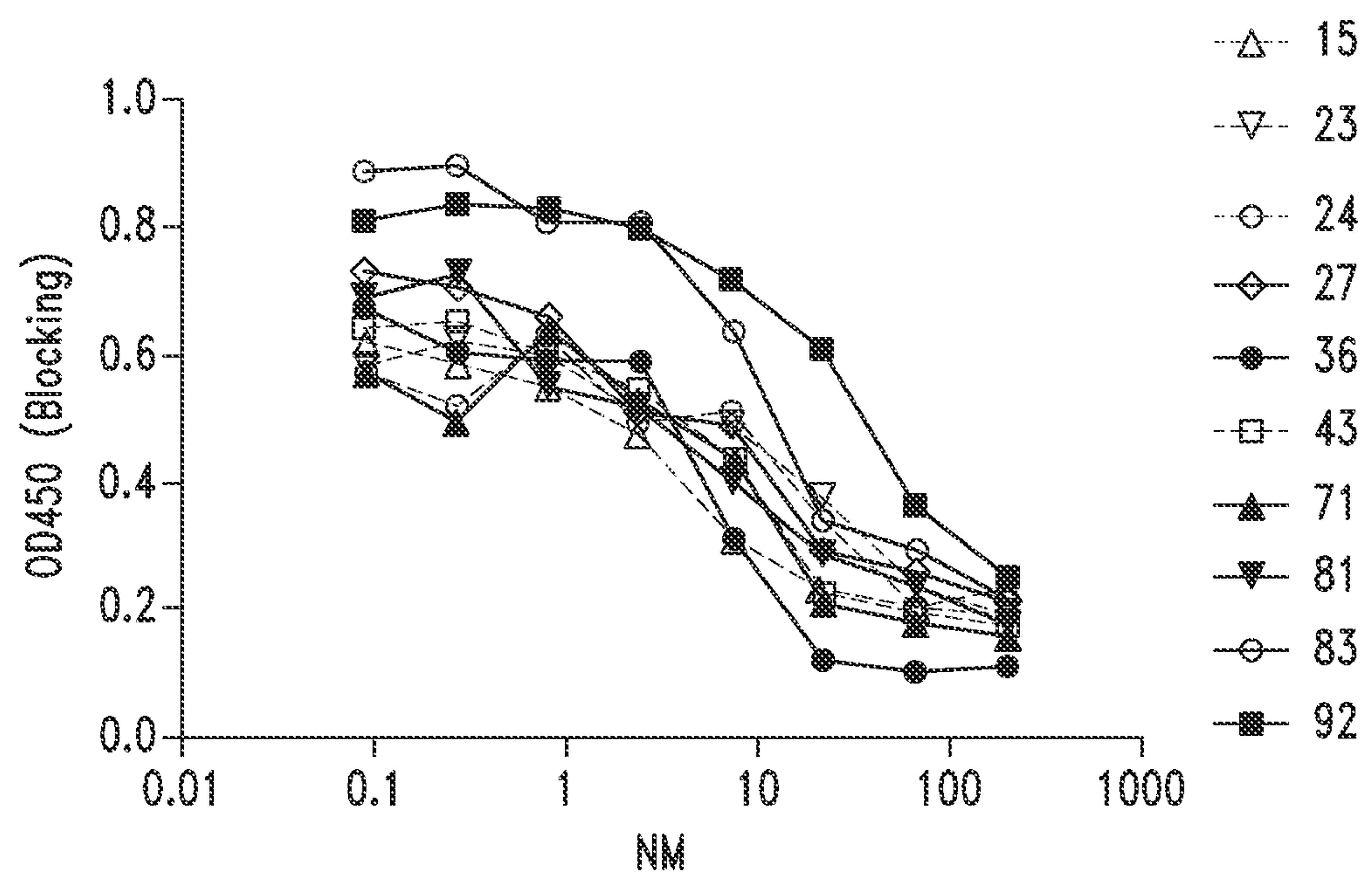
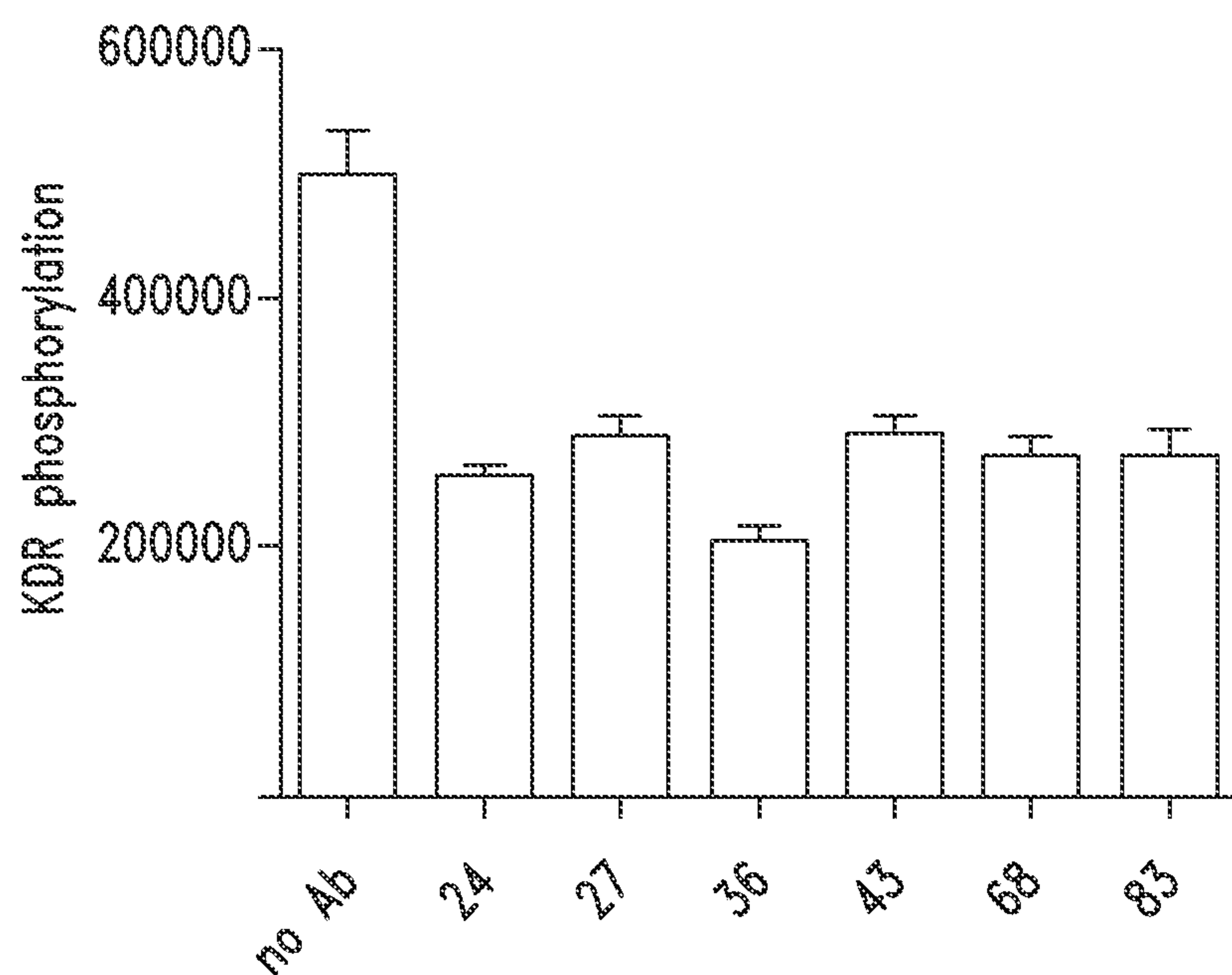
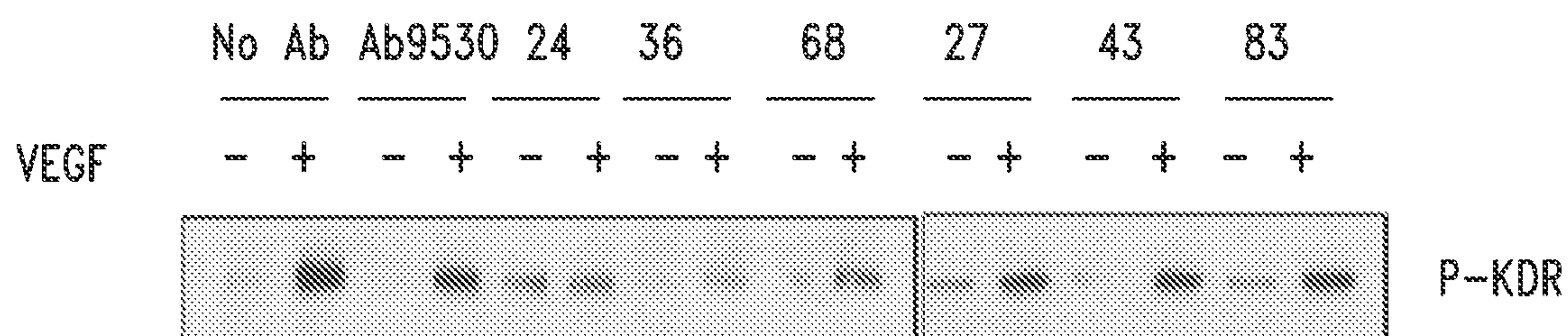
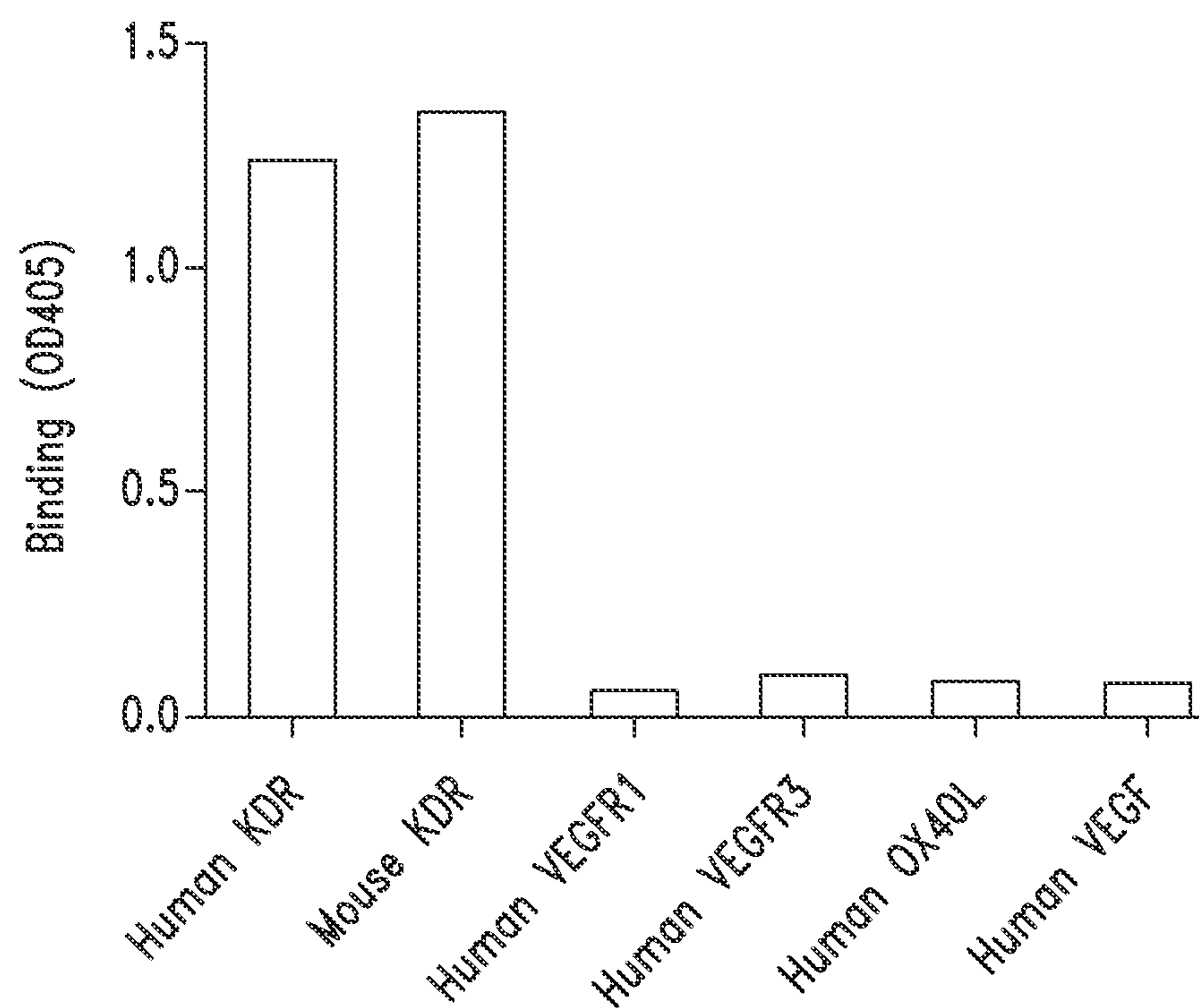
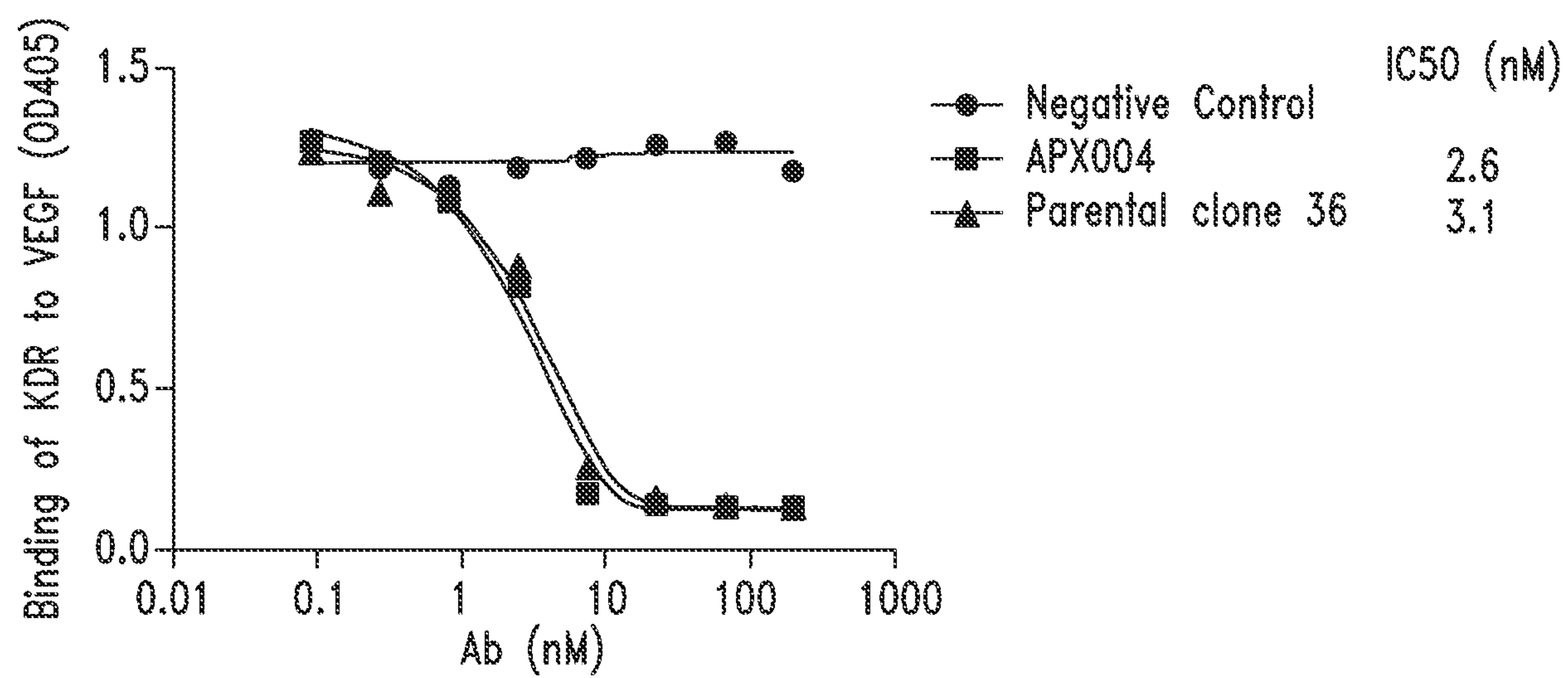
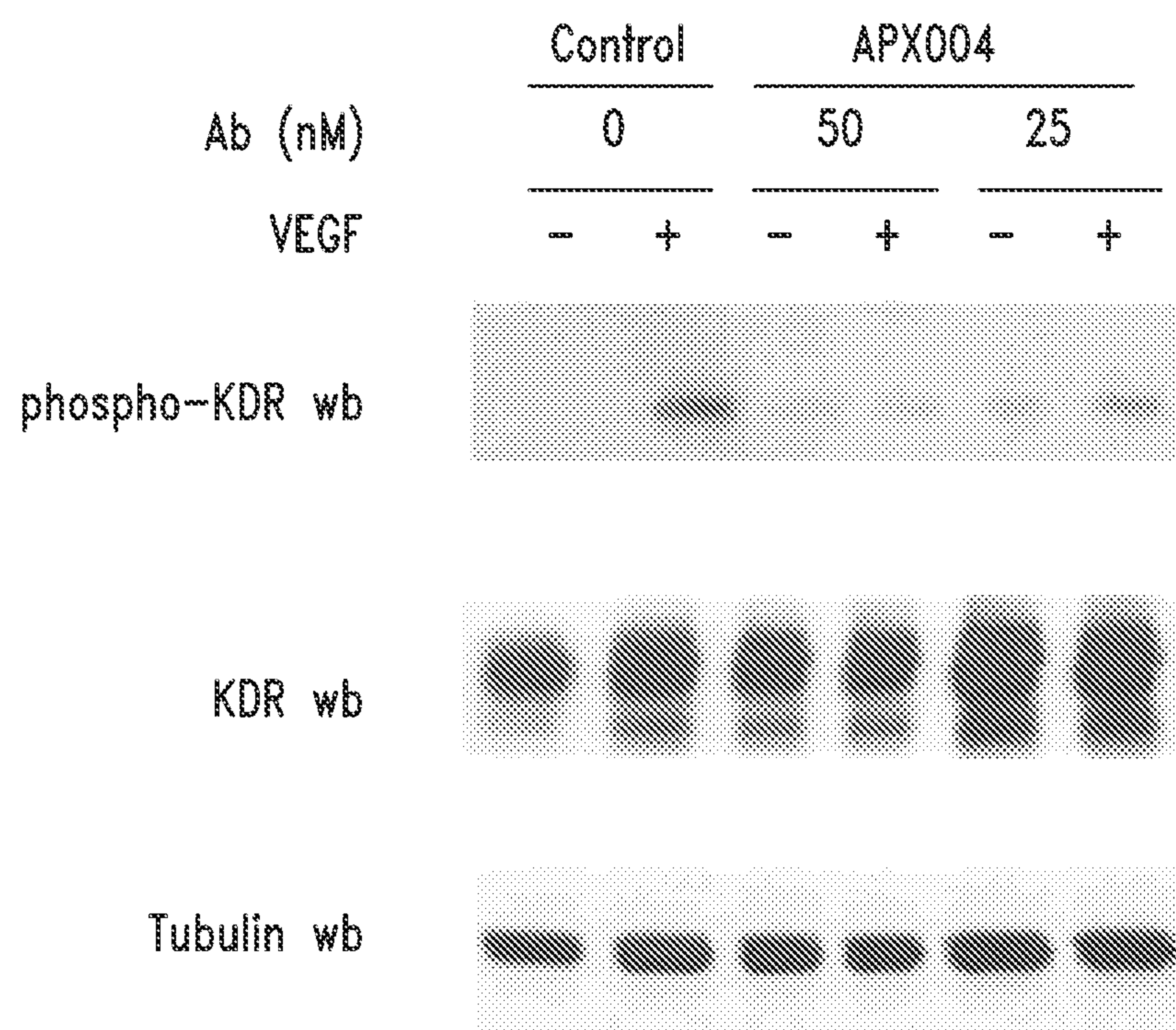
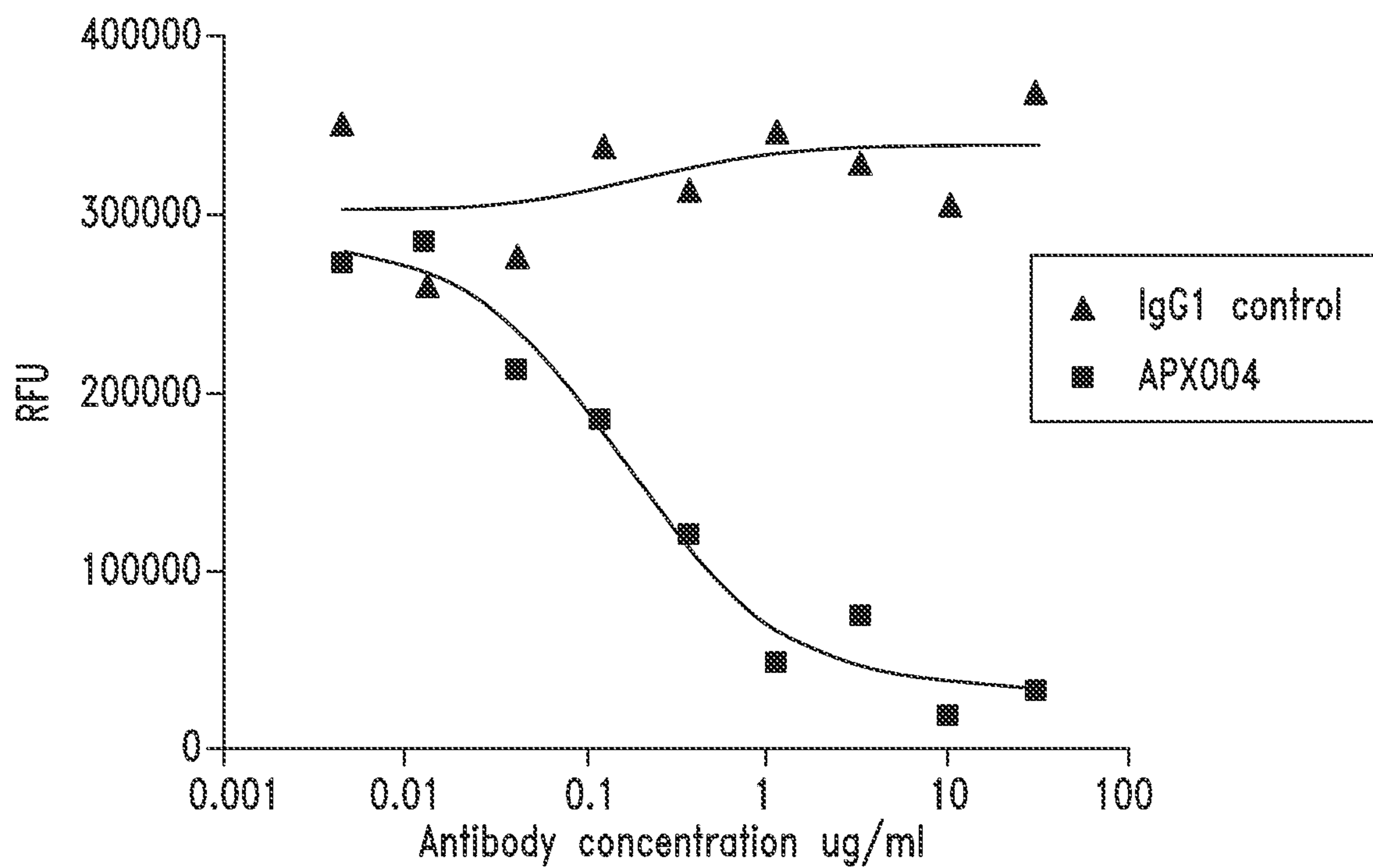


FIG. 1

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*FIG. 2A**FIG. 2B*

*FIG. 3**FIG. 4*

*FIG. 5**FIG. 6*

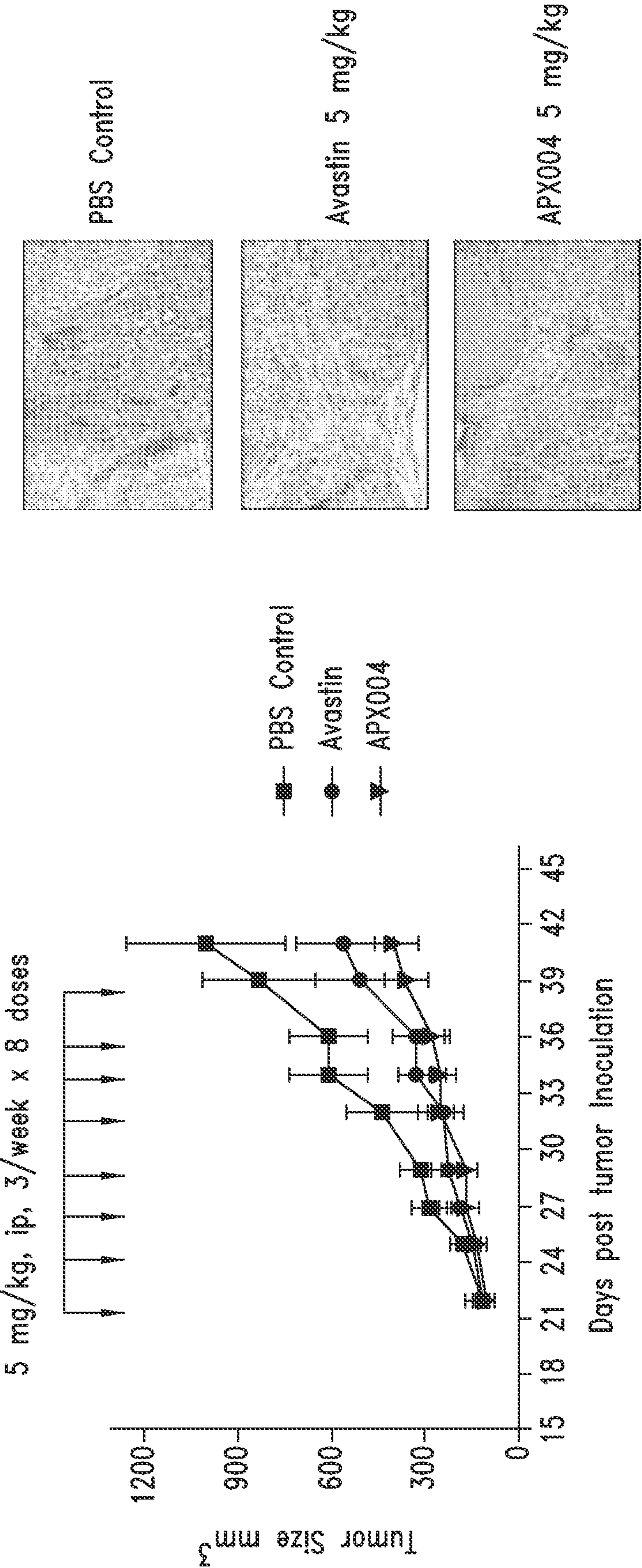


FIG. 7B

FIG. 7A

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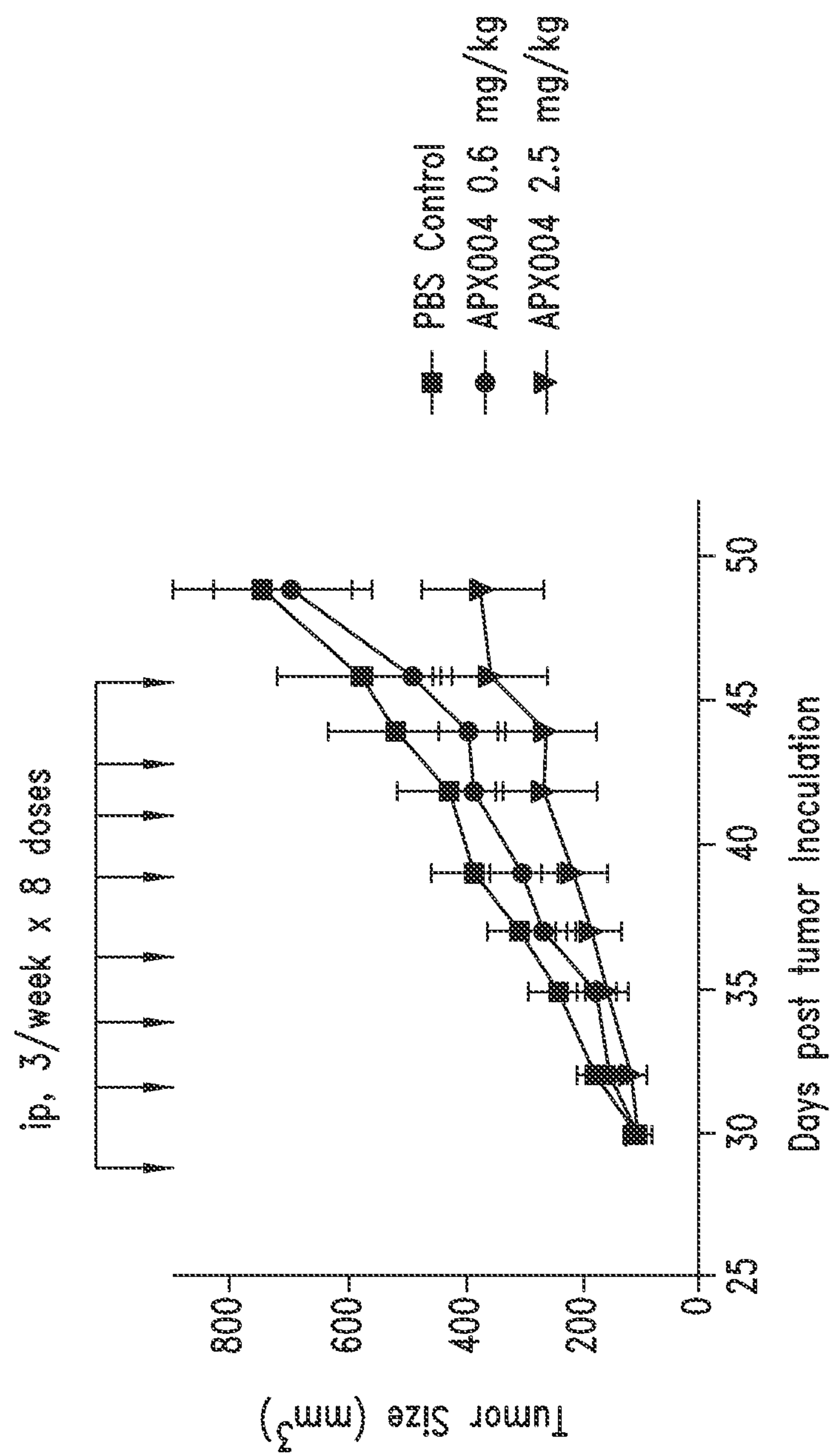


FIG. 8

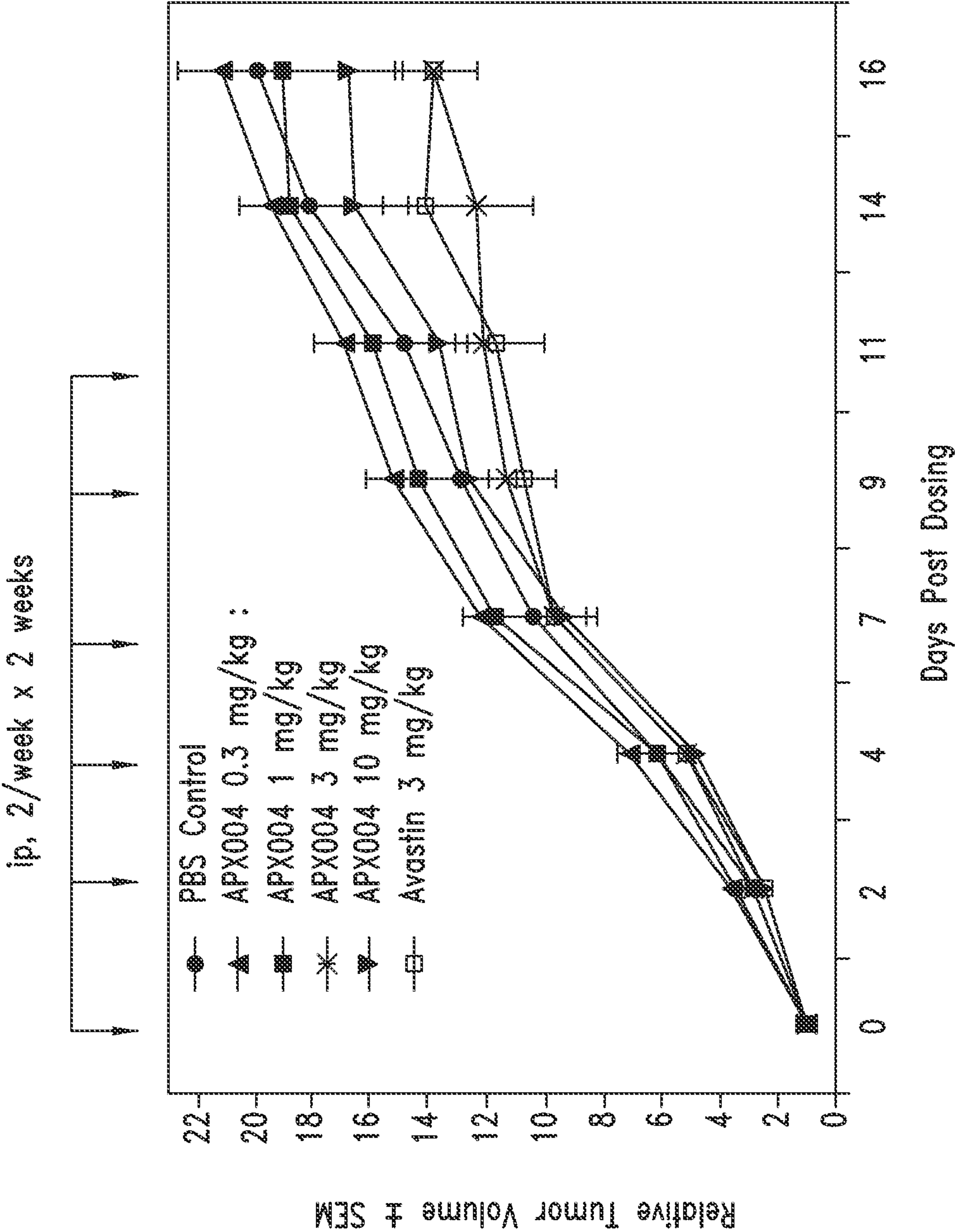


FIG. 9

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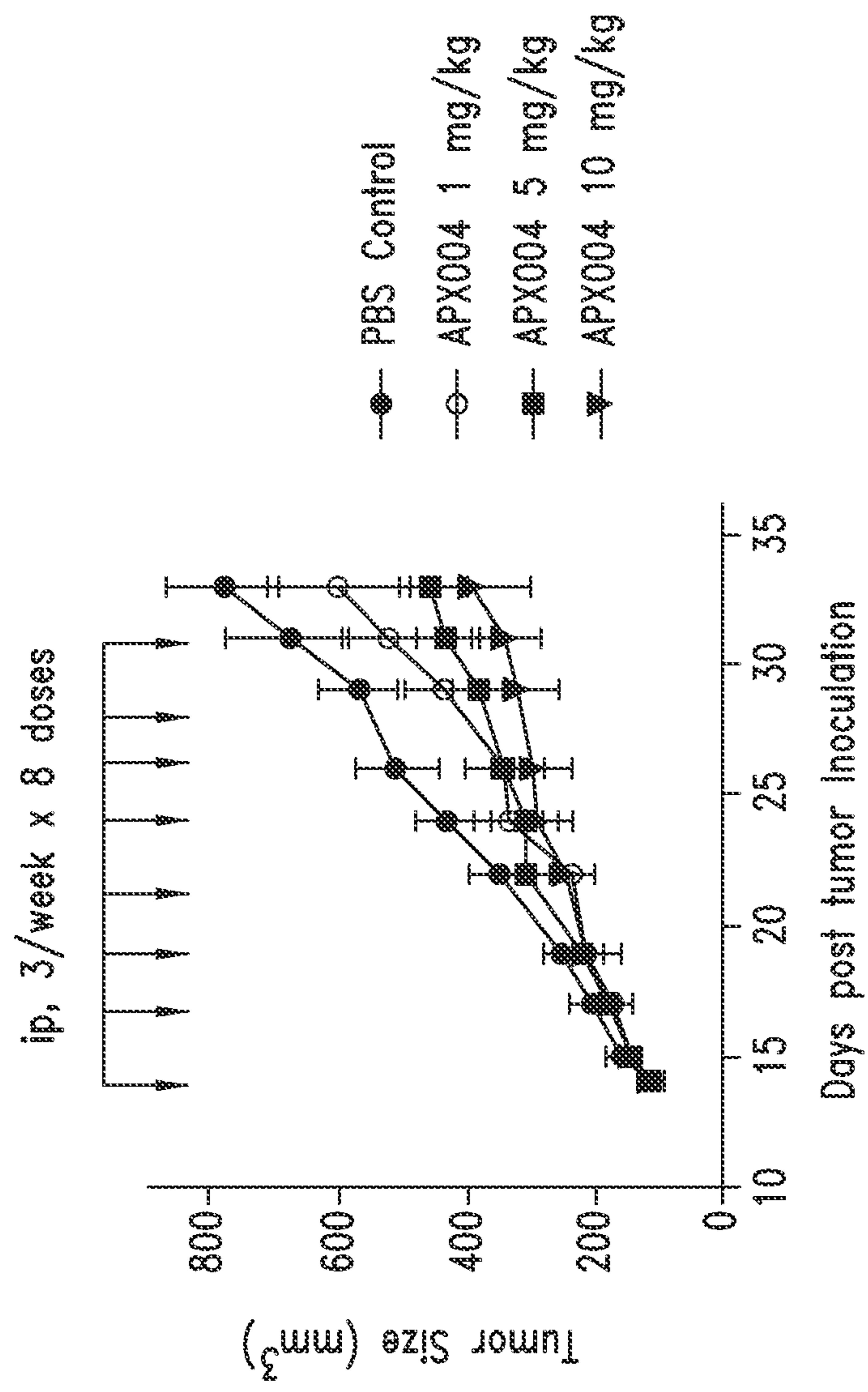


FIG. 10

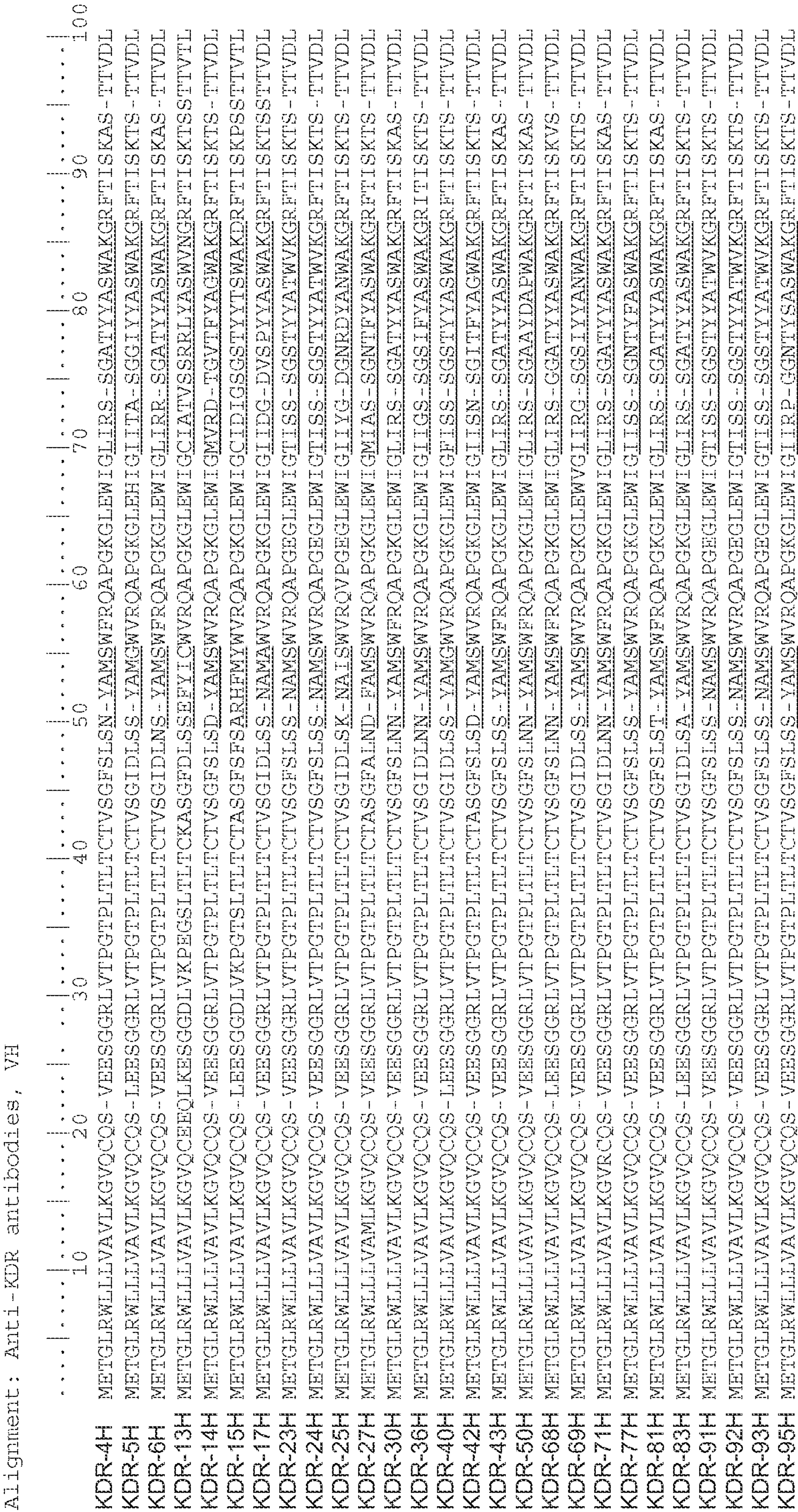


FIG. 11A1

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Alignment: Anti-KDR antibodies, VH

	110	120	130	140
KDR-4H	KFTSPTT	EDTATYFCAR--	<u>VLWPGDIAAYAHNI</u>	WGPGSLVTVSSGQ
KDR-5H	RIPSPTT	EDTGTYFCAR--	<u>TENSY---</u>	<u>FLYFTI</u> WGPGTLVTVSSGQ
KDR-6H	KFTSPTT	EDTATYFCAR--	<u>VLWPGDIAAYAHNI</u>	WGPGTLVTVSSGQ
KDR-13H	QMPSLTA	ADTATYFCAR--	<u>D--DSARNWFYFY</u>	<u>LWGP</u> TLVTVSSGQ
KDR-14H	KFTSPTT	EDTATYFCAR--	<u>VLWAGYVAYAHNI</u>	WGPGTLVTVSSGQ
KDR-15H	QMTSLTA	ADTATYFCAR--	<u>-----SSGY</u>	<u>PYYFTL</u> WGPGTLVTVSSGQ
KDR-17H	KMTSLTT	EDTATYFCAR--	<u>-----PFNI</u>	WGPGTLVTVSSGQ
KDR-23H	KMTSLTA	ADTATYFCAR--	<u>GDDDVSDYFY</u>	<u>YFPI</u> WGPGTLVTVSSGQ
KDR-24H	KMTSLTA	ADTATYFCAR--	<u>GDDDVSDYFY</u>	<u>YFPI</u> WGPGTLVTVSSGQ
KDR-25H	KVTSPTT	EDTATYFCAR--	<u>GTTIWS--</u>	<u>DYLDI</u> WGPGTLVTISSGQ
KDR-27H	KFTSPTT	EDTATYFCAR--	<u>VLWPGYIAYAHNI</u>	WGPGTLVTVSSGQ
KDR-30H	KFTSPTT	EDTATYFCAR--	<u>VLWPGDIAAYAHNI</u>	WGPGTLVTVSSGQ
KDR-36H	KFTSLTT	EDTATYFCAR--	<u>VLWAGSVAYAHNI</u>	WGPGTLVTVSSGQ
KDR-40H	KITSPTT	EDTATYFCAN--	<u>NYDDYGDFLHY</u>	<u>FNI</u> WGPGTLVTVSSGQ
KDR-42H	KFTSPTT	EDTATYFCAR--	<u>ALWAGYIAYVYHNI</u>	WGPGTLVTVSSGQ
KDR-43H	KFTSPTT	EDTATYFCAR--	<u>VLWPGDIAAYAHNI</u>	WGPGTLVTVSSGQ
KDR-50H	KFTSPTT	EDTATYFCAR--	<u>VLWPGDIAAYAHNI</u>	WGPGTLVTVSSGQ
KDR-68H	KFTSPTT	EDTATYFCAR--	<u>VLWPGDIAAYAHNI</u>	WGPGTLVTVSSGQ
KDR-69H	RFASPTT	EDTATYFCAR--	<u>VLWPGSVAYAHNI</u>	WGPGTLVTVSSGQ
KDR-71H	KFTSPTT	EDTATYFCAR--	<u>VLWPGDIAAYAHNI</u>	WGPGTLVTVSAGQ
KDR-77H	KFTSPTT	EDTATYFCAR--	<u>VLWAGSVAYAHNI</u>	WGPGTLVTVSSGQ
KDR-81H	KFTSPTT	EDTATYFCAR--	<u>VLWPGDIAAYAHNI</u>	WGPGTLVTVSSGQ
KDR-83H	KMTSPTT	EDTATYFCAR--	<u>VLWPGEIAYAHNI</u>	WGPGTLVTVSSGQ
KDR-91H	KMTSLTA	ADTATYFCAR--	<u>GDDDVSDYFY</u>	<u>YFPI</u> WGPGTLVTVSSGQ
KDR-92H	KMTSLTA	ADTATYFCAR--	<u>GDDDVSDYFY</u>	<u>YFPI</u> WGPGTLVTVSSGQ
KDR-93H	KMTSLTA	ADTATYFCAR--	<u>GDDDVSDYFY</u>	<u>YFPI</u> WGPGTLVTVSSGQ
KDR-95H	KFTSPTP	EDTATYFCAR--	<u>VLWAGDVAYAHNI</u>	WGPGTLVTVSSGQ

FIG. 11A2

Alignment: Anti-KDR antibodies, VL

.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|

10203040506070

KDR-4L MDTRAPTQLLGLLLWLPGARCDLVMT--QTPSPVSAAVGGTVTINCAASEEIGG--NLAWYQKPGQPP
KDR-5L MDTRAPTQLLGLLLWLPGARCAVVLVLT--QTPSSVSAAVGGTVTINCAASENIYS--NLAWYQKPGQPP
KDR-6L MDTRAPTQLLGLLLWLPGARCDLVMT--QTPSPVSAAVGGTVTINCAASEEIGG--NLAWYQKPGQPP
KDR-13L MDTRAPTQLLGLLLWLPGARCAVVLVLT--QTASPVSAGVGGTVTINCAASONIYS--NLAWYQHKEPGQPP
KDR-14L MDTRAPTQLLGLLLWLPGVICGPVMT--QTPASVSEPVGGTVTIKCAASEDIGS--NLAWYQKPGQPP
KDR-15L MDTRAPTQLLGLLLWLPGARCAVVLVLT--QTTSSVSADVGGTVTIKCAASENIYS--LLAWYQKPGQPP
KDR-17L MDTRAPTQLLGLLLWLPGAIFAQVLVLT--QSPSPVSAAVGGTVTINCAASQSVYNGDWLGWYQKPGQPP
KDR-23L MDTRAPTQLLGLLLWLPGARCAVVLVLT--QTASPVSAGVGGTVTIKCAASONIYN--NLAWYRQKPGQPP
KDR-24L MDTRAPTQLLGLLLWLPGARCAVVLVLT--QTASPVSAGVGGTVTIKCAASONIYN--NLAWYRQKPGQPP
KDR-25L MDTRAPTQLLGLLLWLPGARCALVMT--QTPASVSAAVGGTVTISCAASQSIHS--WVSWYQKPGQPP
KDR-27L MDTRAPTQLLGLLLWLPGARCDPVLT--QTPASVSAVVG GTVTINCAQTSEDIAS--NLAWYQKSGQRP
KDR-30L MDTRAPTQLLGLLLWLPGARCDLVMT--QTPSPVSAAVGGTVTINCAASEEIGG--NLAWYQKPGQPP
KDR-36L MDTRAPTQLLGLLLWLPGVICDPVMT--QTPASVSEPVGGTVTINCAASEELGS--NLAWYQKPGQPP
KDR-40L MDTRAPTQLLGLLLWLPGARCAVVLVLT--QTASPVSAGVGGTVTIKCAASOHIYS--NLAWYQKPGQRP
KDR-42L MDTRAPTQLLGLLLWLPGVICGPVMT--QTPASVSEPVGGTVTMKCAASEDIGS--NLAWYQKPGQPP
KDR-43L MDTRAPTQLLGLLLWLPGARCDLVMT--QTPSPVSAAVGGTVTINCAASEEIGG--NLAWYQKPGQPP
KDR-50L MDTRAPTQLLGLLLWLPGARCDLVMT--QTPSPVSAAVGGTVTINCAASEEIGG--NLAWYLQKPGQPP
KDR-68L MDTRAPTQLLGLLLWLPGARCDLVMT--QTPSPVSAAVGGTVTINCAASEEIGD--NLAWYQKPGQPP
KDR-69L MDTRAPTQLLGLLLWLPGARCDPVLT--QTPASVSEPVGGTVTIKCAASEDLGS--NLAWYQKPGQPP
KDR-71L MDTRAPTQLLGLLLWLPGARCDLVMT--QTPSPVSAVVG GTVTINCAASEEIGG--NLAWYQKPGQPP
KDR-77L MDTRAPTQLLGLLLWLPGARCDVMT--QTPASVSEPVGGTVTINCAASEEIGS--NLAWYQKPGQPP
KDR-81L MDTRAPTQLLGLLLWLPGARCDLVMT--QTPSPVSAAVGGTVTINCAASEEIGG--NLAWYQKPGQPP
KDR-83L MDTRAPTQLLGLLLWLPGARCDVMT--QTPASVSEPVGGTVTINCAASEEIGG--NLAWYQKPGQPP
KDR-91L MDTRAPTQLLGLLLWLPGARCAVVLVLT--QTASPVSAGVGGTVTIKCAASONIYN--NLAWYRQKPGQPP
KDR-92L MDTRAPTQLLGLLLWLPGARCAVVLVLT--QTASPVSAGVGGTVTIKCAASONIYN--NLAWYRQKPGQPP
KDR-93L MDTRAPTQLLGLLLWLPGARCDVME^{TT}QTPASVSEPVGGTVTINCAASEEIAS--NLAWYQKPGQPP
KDR-95L MDTRAPTQLLGLLLWLPGARCAVVLVLT--QTASPVSAGVGGTVTIKCAASONIYN--NLAWYRQKPGQPP

FIG. 11B1

Alignment: Anti-KDR antibodies, VL

	60	70	80	90	100	110				
KKDR-4L	KLIIYSA	TLTSGVPSR	FKSGGTEY	TLTISD	LECD	DAATYYCQYTY	-GFT- YVGP	FCGGTEV		
KKDR-5L	KLIIYG	ASALP	SGVPSR	FSGSR	SGTQ	FTLTISD	LECAD	ATYYCQSYFYSSND	DN-PFCGGTEV	
KKDR-6L	KLIIYSA	STLAS	GVPSR	FKSGGTEY	SLISD	LECAD	ATYYCQYTY	-GFS- YVGP	FCGGTEV	
KKDR-13L	KLIIYKA	STLAS	GVPSR	FKSGGTEY	ILTISD	LECAD	ATYYCQTNYY	-SINGGE	VTFCGGTEV	
KKDR-14L	NLLVYSA	STL	SGVPSR	FSGSGGTEY	TLTISD	LECAD	ATYYCQDTYY	-GNT- YLGA	FCGGTEV	
KKDR-15L	KLIIYSA	SDLAS	GVPSR	FSGSGGTEY	FTLTISD	LECAD	ATYYHCQSY	YSGSSAD	TGAFGGTEV	
KKDR-17L	KLIIYDA	STLAS	GVSS	LINC	SGTQ	WTLTIS	GVQCDD	ATYYCQGEFS	-CSSAD	CVAFGGTEV
KKDR-23L	KLIIYAA	SKLAS	GVPSR	FSGSR	SGTQ	FTLTISD	LECAD	ATYYCQSYF	SSSTDA-AFCGGTEV	
KKDR-24L	KLIIYAA	SKLAS	GVPSR	FSGSR	SGTQ	FTLTISD	LECAD	ATYYCQSYF	SSSTDA-AFCGGTEV	
KKDR-25L	KLIIYAA	ATLAS	GVPSR	FKSGGTEY	TKQFTLTISD	LECAD	ATYYCQQDFG	-GSD- VDNT	FCGGTEV	
KKDR-27L	KLIIYEA	SKLAS	GVPSR	FKSGGTEY	TLTISD	LECAD	ATYYCQYTY	-GSS- YLGA	FCGGTEV	
KKDR-30L	QLLIYSA	STL	SGVPSR	FKSGGTEY	TLTISD	LECAD	ATYYCQYTY	-GFT- YVGP	FCGGTEV	
KKDR-36L	KLIIYSA	STLAS	GVPSR	FKSGGTEY	TLTISD	LECAD	ATYYCQTYF	-GSS- YLGA	FCGGTEV	
KKDR-40L	KLIIYAA	SKLAS	GVPSR	FSGSR	SGTEY	TLTISD	LECAD	ATYYCQSY	YSSDSTDN-TFCGGTEV	
KKDR-42L	NLLVFA	STLAS	GVPSR	FAGSGGTEY	TLTISD	LECAD	ATYYCQDSYY	-GNS- YLGA	FCGGTEV	
KKDR-43L	KLIIYSA	STL	SGVPSR	FKSGGTEY	TLTISD	LECAD	ATYYCQYTY	-GFS- YVGP	FCGGTEV	
KKDR-50L	QLLIYSA	STL	SGVPSR	FKSGGTEY	TLTISD	LECAD	ATYYCQYTY	-GFS- YVGP	FCGGTEV	
KKDR-68L	KLIIYSA	STL	SGVPSR	FKSGGTEY	TLTISD	LECAD	ATYYCQYTY	-GFS- YVGP	FCGGTEV	
KKDR-69L	KLIIYSA	STL	SGVPSR	FKSGGTEY	TLTISG	VQCDD	ATYYCQDTYY	-GNT- YLGA	FCGGTEV	
KKDR-71L	KLIIYSA	STL	SGVPSR	FKSGGTEY	TLTISG	VQCDD	ATYYCQYTY	-GFS- YVGP	FCGGTEV	
KKDR-77L	KLIIYSA	STL	SGVPSR	FKSGGTEY	TLTISD	LECAD	ATYYCQYTY	-GFS- YVGP	FCGGTEV	
KKDR-81L	KLIIYSA	STL	SGVPSR	FKSGGTEY	TLTIN	DLECAD	ATYYCQPYIY	-GFS- YVGP	FCGGTEV	
KKDR-83L	KLIIYSA	SS	LTSGVPSR	FKSGGTEY	TLTISD	LECAD	ATYYCQSYIY	-GFN- YVGP	FCGGTEV	
KKDR-91L	KLIIYAA	SKLAS	GVPSR	FSGSR	SGTQ	FTLTISD	LECAD	ATYYCQSYF	SSSTDA-AFCGGTEV	
KKDR-92L	KLIIYAA	SKLAS	GVPSR	FSGSR	SGTQ	FTLTISD	LECAD	ATYYCQSYF	SSSTDA-AFCGGTEV	
KKDR-93L	KLIIYSA	STL	SGVPSR	FKSGGTEY	TLTISD	LECAD	ATYYCQYTY	-GFN- YVGP	FCGGTEV	
KKDR-95L	KLIIYAA	SKLAS	GVPSR	FSGSR	SGTQ	FTLTISD	LECAD	ATYYCQSYF	SSSTDA-AFCGGTEV	

FIG. 11B2

5 mg/kg, ip, 3/week x 8 doses

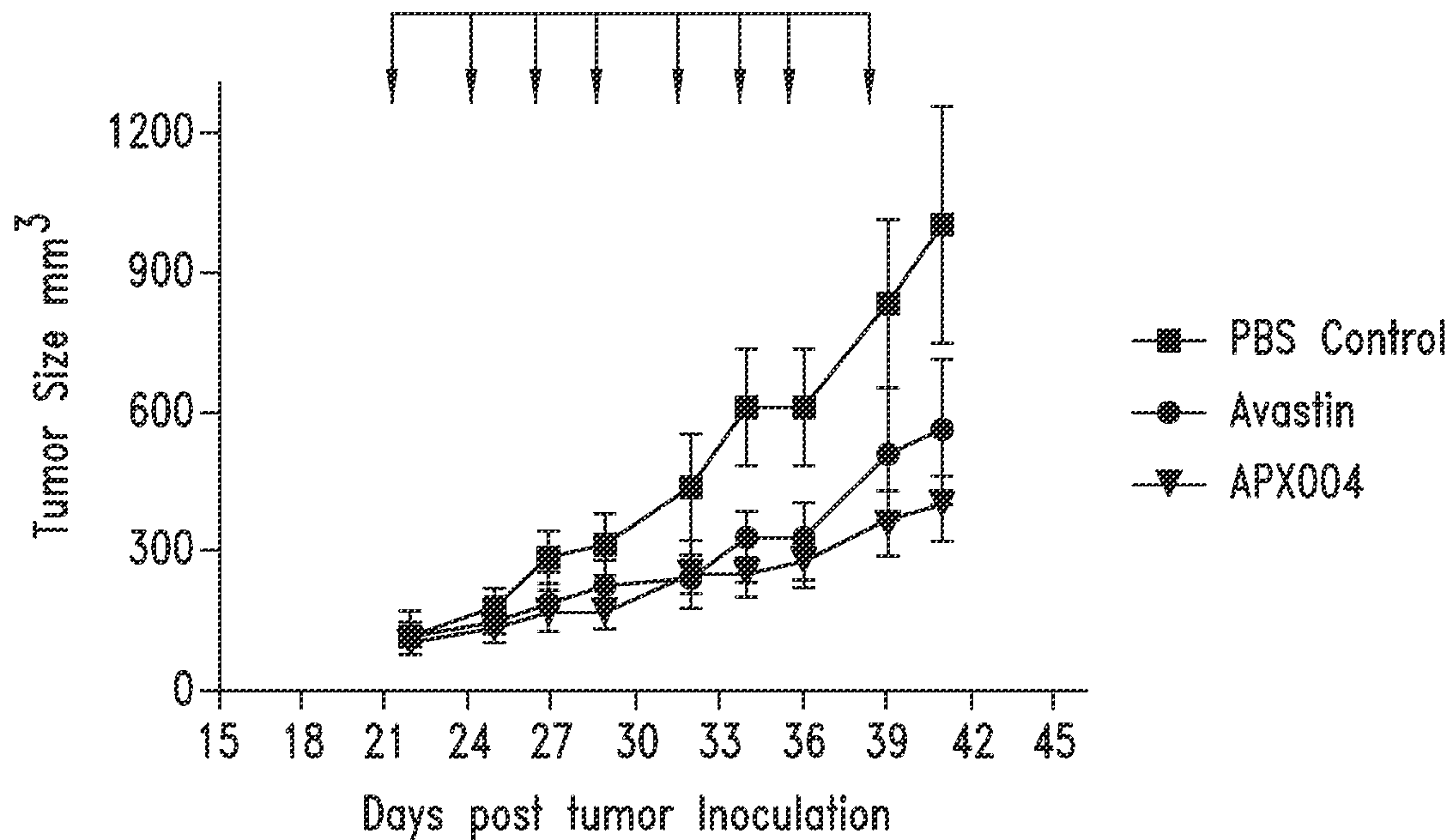


FIG. 7A