(12) STANDARD PATENT (11) Application No. **AU 2012347972 B2**
(19) AUSTRALIAN PATENT OFFICE

(54) Title
PDGF receptor beta binding polypeptides

(51) International Patent Classification(s)
C07K 16/28 (2006.01) ***C07K 14/705*** (2006.01)
A61K 39/395 (2006.01) ***C12N 15/13*** (2006.01)
A61P 35/00 (2006.01) ***C40B 40/08*** (2006.01)
C07K 14/49 (2006.01)

(21) Application No: **2012347972** (22) Date of Filing: **2012.12.05**

(87) WIPO No: **WO13/085972**

(30) Priority Data

(31) Number	(32) Date	(33) Country
61/566,778	**2011.12.05**	**US**
61/610,905	**2012.03.14**	**US**

(43) Publication Date: **2013.06.13**
(44) Accepted Journal Date: **2018.05.10**

(71) Applicant(s)
X-Body, Inc.

(72) Inventor(s)
Chen, Yan;Wagner, Richard W.;Pazmany, Csaba

(74) Agent / Attorney
FB Rice Pty Ltd, L 23 44 Market St, Sydney, NSW, 2000, AU

(56) Related Art
WO 2008/103704 A2

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
13 June 2013 (13.06.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/085972 A1

(51) **International Patent Classification**:
C07K 16/28 (2006.01) ***A61K 39/395*** (2006.01)
C07K 14/49 (2006.01) ***C40B 40/08*** (2006.01)
C07K 14/705 (2006.01) ***A61P 35/00*** (2006.01)
C12N 15/13 (2006.01)

(21) **International Application Number**:
PCT/US2012/067909

(22) **International Filing Date**:
5 December 2012 (05.12.2012)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:
61/566,778 5 December 2011 (05.12.2011) US
61/610,905 14 March 2012 (14.03.2012) US

(71) **Applicant**: **X-BODY, INC.** [US/US]; 100 Beaver Street, Suite 101, Waltham, MA 02453 (US).

(72) **Inventors**: **CHEN, Yan**; 179 Grove Street, Lexington, MA 02420 (US). **WAGNER, Richard, W.**; 24 Coolidge Avenue, Cambridge, MA 02138 (US). **PAZMANY, Csaba**; 452 Windsor Street, #2, Cambridge, MA 02141 (US).

(74) **Agents**: **VELEMA, James, H.** et al.; Lathrop & Gage LLP, 28 State Street, Suite 0700, Boston, MA 02109 (US).

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

WO 2013/085972 A1

(54) **Title**: PDGF RECEPTOR BETA BINDING POLYPEPTIDES

(57) **Abstract**: The present invention provides binding polypeptides (e.g., antibodies or fragments thereof) that specifically bind to a target antigen (e.g., a human antigen, e.g., human PDGFRβ) with high affinity. The invention also provides, libraries of binding polypeptides, pharmaceutical compositions, as well as nucleic acids encoding binding polypeptides, recombinant expression vectors and host cells for making such binding polypeptides. Methods of using binding polypeptide of the invention to diagnose and treat disease are also encompassed by the invention.

PDGF RECEPTOR BETA BINDING POLYPEPTIDES

RELATED APPLICATIONS

This application claims priority to U.S. provisional application 61/566,778 filed on December 5, 2011, and U.S. provisional application 61/610,905 filed on March 14, 2012, both of which are hereby incorporated by reference in their entirety.

INTRODUCTION

Platelet derived growth factor (PDGF) is a potent mitogen and chemoattractant in cells of mesenchymal origin and is involved in the pathologies of many diseases. PDGF exists as a disulfide-linked dimer consisting of two homologous chains, A or B, that can combine to form three distinct PDGF isoforms, AA, BB or AB. All isoforms of PDGF mediate their mitogenic effect by binding to a cell surface PDGF receptor (PDGFR).

PDGF receptors belong to the tyrosine kinase family and consist of two receptor isoforms, alpha and beta. The alpha and beta isoforms can be distinguished by their distinct ligand binding specificities. PDGF beta receptor can bind to only B-chain (isoforms BB and AB), while PDGF alpha receptor can bind to all isoforms of PDGF.

Binding of PDGF to a cell surface PDGFR causes receptor dimerization and trans-autophosphorylation which, in turn, results in intracellular signalling events that cause, *inter alia*, cell proliferation and cell migration. Accordingly, PDGFR antagonists that block PDGF binding and/or receptor dimerization can be used to treat or prevent diseases associated with PDGFR activation.

There is a therefore a need in the art for novel PDGFR antagonists that can be used to treat diseases associated with PDGFR activation.

SUMMARY OF THE INVENTION

The present invention provides binding polypeptides (e.g., antibodies or fragments thereof) that specifically bind to a target antigen (e.g., a human antigen, e.g., human PDGF) with high affinity. In a preferred embodiment, the invention provides binding polypeptides that bind to PDGFRβ (e.g., human PDGFRβ) with high affinity and antagonize PDGFRβ activation. Such binding polypeptides are particularly useful for treating PDGFRβ-associated diseases or disorders (e.g., age-related macular degeneration (AMD)). The invention also provides, libraries of binding polypeptides, pharmaceutical compositions, as well as nucleic acids encoding binding polypeptides,

recombinant expression vectors and host cells for making such binding polypeptides. Methods of using binding polypeptides of the invention to detect PDGFRβ and to modulate PDGFRβ activity are also encompassed by the invention.

Accordingly, in one aspect the invention provides an isolated binding polypeptide comprising a VH domain, wherein, as an isolated domain, the VH domain binds to an antigen with a Kd of less than 100pM.

In another aspect, the invention provides an isolated binding polypeptide that specifically binds to PDGFRβ, comprising the CDR3 sequence set forth in SEQ ID NO: 1.

In certain embodiments, the binding polypeptide comprises a VH domain comprising the HCDR3 amino acid sequence set forth in SEQ ID NO: 1. The VH domain may further comprise a HCDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 2-32, and/or a HCDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 33-62.

In certain embodiments, the polypeptide comprises a VH domain comprising an amino acid sequence sharing at least 80 % (e.g., 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) amino acid identity with a VH domain amino acid sequence selected from the group consisting of SEQ ID NOs: 318-368.

In certain embodiments, the polypeptide comprises a VH domain comprising an am amino acid sequence selected from the group consisting of SEQ ID NOs: 318-368

In certain embodiments, the binding polypeptide comprises a VL domain. The VH domain may further comprise a LCDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 63-147, a LCDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 148-232, and/or a LCDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 233-317.

In certain embodiments, the polypeptide comprises a VL domain comprising an amino acid sequence sharing at least 80 % (e.g., 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) amino acid identity with a VL domain amino acid sequence selected from the group consisting of SEQ ID NOs: 369-453.

In certain embodiments, the polypeptide comprises a VL domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 369-453.

In further aspect, the invention provides a binding polypeptide that binds to the same epitope on PDGFRβ as a binding polypeptide comprising the VH domain amino acid sequence set forth in SEQ ID No: 318. In a preferred embodiment, the binding polypeptide comprises a VH domain amino acid sequence sharing at least 80 % amino acid identity (e.g., 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) with a VH domain amino acid sequence selected from the group consisting of SEQ ID NOs: 318-368.

In a further aspect, the invention provides a binding polypeptide that competes for binding to PDGFRβ with a binding polypeptide comprising the VH domain amino acid sequence set forth in SEQ ID No: 318. In a preferred embodiment, the binding polypeptide comprises a VH domain amino acid sequence sharing at least 80 % amino acid identity with a VH domain amino acid sequence selected from the group consisting of SEQ ID NOs: 318-368.

In certain embodiments, the binding polypeptides of the invention inhibit the activity of PDGFRβ. In one embodiment, the activity of PDGFRβ is inhibited by antagonizing PDGF binding to PDGFRβ. In another embodiment, the activity of PDGFRβ is inhibited by antagonizing PDGFRβ dimerization.

In certain embodiments, the binding polypeptides of the invention bind to PDGFRβ with a Kd of less than 100pM and/or with an off-rate of less than 10^{-3} s^{-1}.

In certain embodiments, the binding polypeptides of the invention bind specifically to mouse and human PDGFRβ.

In certain embodiments, the binding polypeptides of the invention antagonize PDGF binding to the PDGFRβ with an IC50 of less than 5nM, inhibit ligand induced tyrosine phosphorylation of PDGFRβ with an IC50 of less than 4nM, inhibit retinal pericyte migration with an IC50 of less than 6nM, and/or have a melting temperature (Tm) of at least 68°C.

In a further aspect, the invention provides an isolated nucleic acid encoding a binding polypeptide of the invention.

In a further aspect, the invention provides a recombinant expression vector comprising an isolated nucleic acid encoding a binding polypeptide of the invention.

In a further aspect, the invention provides a host cell expressing a binding polypeptide of the invention.

In a further aspect, the invention provides a method of producing a binding polypeptide that binds specifically to human PDGFRβ, comprising culturing a host cell capable of expressing a binding polypeptide of the invention under conditions such that the binding polypeptide is produced by the host cell.

In a further aspect, the invention provides a pharmaceutical composition comprising a binding polypeptide of the invention and one or more pharmaceutically acceptable carrier.

In a further aspect, the invention provides a method for treating a disease or disorder PDGFRβ-associated disease or disorder (e.g., age-related macular degeneration (AMD) or cancer), the method comprising administering to a subject in need of thereof a pharmaceutical composition of the invention.

In a further aspect, the invention provides a diverse library of unpaired VH domains wherein each member of the library binds to human PDGFRβ. In one preferred embodiment, each member of the library comprises the CDR3 amino acid sequence set forth in SEQ ID NO: 1 and diversity lies in the FR1-FR3 regions. In one preferred embodiment, the library is a nucleic acid display library (e.g., a DNA display library).

In a further aspect, the invention provides a diverse library of stable VH/VL pairs wherein each member of the library binds to human PDGFRβ. In one preferred embodiment, each member of the library comprises a VH domain comprising the CDR3 amino acid sequence set forth in SEQ ID NO: 1. In one preferred embodiment, the VL domains are human VL domains. In one preferred embodiment, the library is a nucleic acid display library (e.g., a DNA display library).

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 is a schematic representation of the construction of exemplary VH domain nucleic acid display libraries for use in the disclosed methods.

Figure 2 depicts the results of *in vitro* binding assays measuring the binding to human or mouse PDGFRβ of the XB1511VH domain, an unselected XB1511 CDR3/framework shuffled DNA display library (R0), and an XB1511 CDR3/framework shuffled DNA display library pool after four rounds of selection (R4).

Figure 3 depicts the results of surface Plasmon resonance binding studies measuring the binding kinetics of XB1511 and the framework shuffled derivatives XB2202 and XB2708 to human PDGFRβ.

Figure 4 depicts the results of surface plasmon resonance binding assays measuring the binding kinetics of XB2202 to human (A) and mouse (B) PDGFRβ.

Figure 5 depicts the results of surface plasmon resonance binding assays measuring the binding kinetics of XB2708 to human (A) and mouse (B) PDGFRβ.

Figure 6 is a schematic representation of the construction of exemplary VL nucleic acid display libraries for use in the disclosed methods.

Figure 7 depicts the results of ELISA assays measuring the binding to human PDGFRβ of XB1511/VL scFv comprising VL domains isolated from the second round screening pool of a VH/VL pairing DNA display screen.

Figure 8 depicts the results of ELISA assays measuring the binding to human PDGFRβ of XB1511/VL scFv comprising VL domains isolated from the third round screening pool of a VH/VL pairing DNA display screen.

Figure 9 depicts the results of ELISA assays measuring the binding to human PDGFRβ of XB2202/VL scFv comprising VL domains isolated from the second round screening pool of a VH/VL pairing DNA display screen.

Figure 10 depicts the results of ELISA assays measuring the binding to human PDGFRβ of unpaired VL domains from the XB1511/VL scFv set forth in Figure 9.

Figure 11 depicts the results of solution binding affinity studies measuring the binding to human PDGFRβ of ^{35}S Met labeled XB1511 VH domain and XB1511-containing scFV obtained from VH/VL pairing DNA display screens.

Figure 12 depicts the results of solution binding affinity studies measuring the binding to human PDGFRβ of ^{35}S Met labeled XB2202 VH domain and XB2202-containing scFV obtained from VH/VL pairing DNA display screens.

Figure 13 depicts the results of surface plasmon resonance competition binding assays measuring the kinetics of binding of PDGF-BB to PDGFRβ at various concentrations of XB2202.

Figure 14 depicts the results of *in vitro* cell migration assays measuring inhibition of pericyte migration by XB2708.

Figure 15 depicts the results of label-free migration assays measuring the ability of an XB1511-containing IgG1 to inhibit the migration of human foreskin fibroblasts.

Figure 16 depicts the results of ELISA assays measuring the binding to human PDGFRβ of XB2202 VH domain and XB2202/A4 scFv after incubation at various temperatures.

DETAILED DESCRIPTION

The present invention provides binding polypeptides (e.g., antibodies or fragments thereof) that specifically bind to a target antigen (e.g., a human antigen) with high affinity. In a preferred embodiment, the binding polypeptides of the invention bind to PDGFRβ (e.g., human PDGFRβ) with high affinity and inhibit the activity of PDGFRβ. Such binding polypeptides are particularly useful for treating PDGFRβ-associated diseases or disorders (e.g., age-related macular degeneration (AMD)). The invention also provides, libraries of binding polypeptides, pharmaceutical compositions, as well as nucleic acids encoding binding polypeptides, recombinant expression vectors and host cells for making such binding polypeptides. Methods of using binding polypeptides of the invention to detect PDGFRβ and to modulate PDGFRβ activity are also encompassed by the invention.

I. Definitions

In order that the present invention may be more readily understood, certain terms are first defined.

As used herein, the term "PDGFRβ" refers to platelet-derived growth factor receptor beta. PDGFRβ nucleotide and polypeptide sequences are well known in the art. An exemplary human PDGFRβ amino sequence is set forth in GenBank deposit GI:4505683 and an exemplary mouse PDGFRβ amino sequence is set forth in GenBank deposit GI: 226371752.

As used herein, the term "PDGF" refers to platelet-derived growth factor. PDGF nucleotide and polypeptide sequences are well known in the art. An exemplary human PDGF amino sequence is set forth in GenBank deposit GI:4505681 and an exemplary mouse PDGF amino sequence is set forth in GenBank deposit GI:400744.

As used herein, the term "binding polypeptide" refers to a polypeptide that contains all or a part of the antigen binding site of an antibody (e.g., all or part of the heavy and/or light chain variable domain, e.g., at least HCDR3 of the heavy chain variable domain) such that the binding polypeptide specifically recognizes a target

antigen. Non-limiting examples of binding polypeptides include antibodies, or fragments thereof, and immunoglobulin-like domains (e.g., fibronectin domains) that have been altered to comprise all or a part of the antigen binding site of an antibody.

As used herein, the term "antibody" refers to immunoglobulin molecules comprising four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, as well as multimers thereof (e.g., IgM). Each heavy chain comprises a heavy chain variable region (abbreviated VH) and a heavy chain constant region. The heavy chain constant region comprises three domains, CH1, CH2 and CH3. Each light chain comprises a light chain variable region (abbreviated VL) and a light chain constant region. The light chain constant region comprises one domain (CL1). The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR).

As used herein, the term "antigen-binding portion" of an antibody includes any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. Antigen-binding fragments of an antibody may be derived, e.g., from full antibody molecules using any suitable standard techniques such as proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of DNA encoding antibody variable and optionally constant domains. Non-limiting examples of antigen-binding portions include: (i) Fab fragments; (ii) $F(ab')_2$ fragments; (iii) Fd fragments; (iv) Fv fragments; (v) single-chain Fv (scFv) molecules; (vi) dAb fragments; and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (e.g., an isolated complementarity determining region (CDR)). Other engineered molecules, such as diabodies, triabodies, tetrabodies and minibodies, are also encompassed within the expression "antigen-binding portion."

As used herein, the terms "VH domain" and "VL domain" refer to single antibody variable heavy and light domains, respectively, comprising FR (Framework Regions) 1, 2, 3 and 4 and CDR (Complementary Determinant Regions) 1, 2 and 3 (see Kabat et al. (1991) Sequences of Proteins of Immunological Interest. (NIH Publication No. 91-3242, Bethesda).

As used herein, the term "FR1-FR3" refers to the region of a VH encompassing FR1, CDR2, FR2, CDR2 and FR3, but excluding the CDR3 and FR4 regions.

As used herein, the term "unpaired" refers to VH or VL that are not linked (either covalently or non-covalently) to a complementary VL or VH domain, respectively.

As used herein, the term "complementary VL or VH domain" refers to a VL or VH domain that associates with a VH or VL domain, respectively, to form a VH/VL pair.

As used herein, the term "VH/VL pair" refers to a non-covalent dimer of a single VH and a single VL domain, wherein the VL and VH domain are associated in a similar manner to that observed in a complete, tetrameric immunogobulin molecule, and the dimer can bind specifically to at least one target antigen. A "stable VH/VL pair" is a VH/VL pair that does not exhibit significant dissociation of the substituent VH and VL domains under physiological conditions.

As used herein, the term "CDR" or "complementarity determining region" means the noncontiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. These particular regions have been described by Kabat *et al.*, J. Biol. Chem. 252, 6609-6616 (1977) and Kabat *et al.*, Sequences of protein of immunological interest. (1991), and by Chothia *et al.*, J. Mol. Biol. 196:901-917 (1987) and by MacCallum *et al.*, J. Mol. Biol. 262:732-745 (1996) where the definitions include overlapping or subsets of amino acid residues when compared against each other. The amino acid residues which encompass the CDRs as defined by each of the above cited references are set forth for comparison. Preferably, the term "CDR" is a CDR as defined by Kabat, based on sequence comparisons.

As used herein the term "framework (FR) amino acid residues" refers to those amino acids in the framework region of an immunogobulin chain. The term "framework region" or "FR region" as used herein, includes the amino acid residues that are part of the variable region, but are not part of the CDRs (e.g., using the Kabat definition of CDRs).

As used herein, the term "specifically binds to" refers to the ability of a binding polypeptide to bind to an antigen with an Kd of at least about 1 x 10^{-6} M, 1 x 10^{-7} M, 1 x 10^{-8} M, 1 x 10^{-9} M, 1 x 10^{-10} M, 1 x 10^{-11} M, 1 x 10^{-12} M, or more, and/or bind to an antigen with an affinity that is at least two-fold greater than its affinity for a nonspecific antigen. It shall be understood, however, that the binding polypeptide are capable of

specifically binding to two or more antigens which are related in sequence. For example, the binding polypeptides of the invention can specifically bind to both human and a non-human (e.g., mouse or non-human primate) PDGFRβ.

As used herein, the term "antigen" refers to the binding site or epitope recognized by a binding polypeptide.

As used herein, the term "nucleic acid display library" refers to any art recognized *in vitro* cell-free phenotype-genotype linked display, including, without limitation those set forth in, for example, U.S. Patent Nos. 7,195,880; 6,951,725; 7,078,197; 7,022,479; 6,518,018; 7,125,669; 6,846,655; 6,281,344; 6,207,446; 6,214,553; 6,258,558; 6,261,804; 6,429,300; 6,489,116; 6,436,665; 6,537,749; 6,602,685; 6,623,926; 6,416,950; 6,660,473; 6,312,927; 5,922,545; and 6,348,315, and in WO2010/011944, which are all hereby incorporated by reference in their entirety.

As used herein, the term "vector" is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid," which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. The terms, "plasmid" and "vector" may be used interchangeably. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

As used herein, the term "host cell" is intended to refer to a cell into which a recombinant expression vector has been introduced. It should be understood that this term is intended to refer not only to the particular subject cell but to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either

mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein.

As used herein, the term "treat," "treating," and "treatment" refer to therapeutic or preventative measures described herein. The methods of "treatment" employ administration to a subject, an antibody or antigen binding portion of the present invention, for example, a subject having a PDGFRβ-associated disease or disorder (e.g. AMD) or predisposed to having such a disease or disorder, in order to prevent, cure, delay, reduce the severity of, or ameliorate one or more symptoms of the disease or disorder or recurring disease or disorder, or in order to prolong the survival of a subject beyond that expected in the absence of such treatment.

As used herein, the term "PDGFRβ-associated disease or disorder" includes disease states and/or symptoms associated with PDGFRβ activity. Exemplary PDGFRβ-associated diseases or disorders include, but are not limited to, age-related macular degeneration (AMD) and cancer.

As used herein, the term "effective amount" refers to that amount of a binding polypeptide that is sufficient to effect treatment, prognosis or diagnosis of a PDGFRβ-associated disease or disorder, as described herein, when administered to a subject. A therapeutically effective amount will vary depending upon the subject and disease condition being treated, the weight and age of the subject, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art. The dosages for administration can range from, for example, about 1 ng to about 10,000 mg, about about 1 ug to about 5,000 mg, about 1 mg to about 1,000 mg, about 10 mg to about 100 mg, of a binding polypeptide according to the invention. Dosage regiments may be adjusted to provide the optimum therapeutic response. An effective amount is also one in which any toxic or detrimental effects (i.e., side effects) of a binding polypeptide are minimized and/or outweighed by the beneficial effects.

As used herein, the term "subject" includes any human or non-human animal.

As used herein, the term "surface plasmon resonance" refers to an optical phenomenon that allows for the analysis of real-time interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIAcore™ system (Biacore Life Sciences division of GE Healthcare, Piscataway, NJ).

As used herein, the term "K_D" refers to the equilibrium dissociation constant of a particular binding polypeptide/antigen interaction.

As used herein, the term "off-rate" is refers to the dissociation rate (K_{off}) for a particular binding polypeptide/antigen interaction.

As used herein, the term "epitope" refers to an antigenic determinant that interacts with the specific antigen binding site in a binding molecule of the invention. A single antigen may have more than one epitope. Thus, different antibodies may bind to different areas on an antigen and may have different biological effects. Epitopes may be either conformational or linear. A conformational epitope is produced by spatially juxtaposed amino acids from different segments of the linear polypeptide chain. A linear epitope is one produced by adjacent amino acid residues in a polypeptide chain.

II. Binding Polypeptides

In one aspect, the invention provides binding polypeptides comprising a VH domain, wherein, as an isolated domain, the VH domain binds to an antigen with a Kd of less than about 200pM (e.g., about 200, 190, 180, 175, 170, 160, 150, 140, 130, 120, 110, 100, 95, 90, 80, 75, 70, 65, 60, 55, 50, 40, 30, 20, 10, 5, or 1 pM or less).

In another aspect, the invention provides binding polypeptides (e.g., antibodies, or antigen binding fragments thereof) that specifically bind to PDGFRβ and inhibit PDGFRβ activity. Such binding polypeptides are particularly useful for treating PDGFRβ-associated disease or disorders (e.g., age-related macular degeneration or AMD).

In general, PDGFRβ binding polypeptides of the invention comprise a heavy chain CDR3 (HCDR3) amino acid sequence that specifically binds to PDGFRβ. One, non-limiting, HCDR3 sequence suitable for use in the binding polypeptides of the invention is the heavy chain CDR3 amino acid sequence set forth in SEQ ID NO: 1 (HGGDRSY)). In other embodiments, the heavy chain CDR3 sequence is a variant of SEQ ID NO:1 which comprises at least one (e.g., one, two, or three) conservative amino acid substitutions relative to SEQ ID NO:1.

Any binding polypeptide that can incorporate a heavy chain CDR3 amino acid sequence that specifically binds to PDGFRβ (e.g., the CDR3 amino acid sequence set forth in SEQ ID NO: 1 (HGGDRSY)) can be used in the binding polypeptides of the invention including, without limitation antibodies, or fragments thereof, and

immunoglobulin-like domains. Suitable immunoglobulin-like domains include, without limitation, fibronectin domains (see, for example, Koide et al. (2007), *Methods Mol. Biol.* 352: 95–109, which is incorporated by reference herein in its entirety), DARPin (see, for example, Stumpp et al. (2008) *Drug Discov. Today* 13 (15–16): 695–701, which is incorporated by reference herein in its entirety), Z domains of protein A (see, Nygren et al. (2008) *FEBS J.* 275 (11): 2668–76, which is incorporated by reference herein in its entirety), Lipocalins (see, for example, Skerra et al. (2008) *FEBS J.* 275 (11): 2677–83, which is incorporated by reference herein in its entirety), Affilins (see, for example, Ebersbach et al. (2007) *J. Mol. Biol.* 372 (1): 172–85, which is incorporated by reference herein in its entirety), Affitins (see, for example, Krehenbrink et al. (2008). *J. Mol. Biol.* 383 (5): 1058–68 , which is incorporated by reference herein in its entirety), Avimers (see, for example, Silverman et al. (2005) *Nat. Biotechnol.* 23 (12): 1556–61 , which is incorporated by reference herein in its entirety), Fynomers, (see, for example, Grabulovski et al. (2007) *J Biol Chem* 282 (5): 3196–3204 , which is incorporated by reference herein in its entirety), and Kunitz domain peptides (see, for example, Nixon et al. (2006) *Curr Opin Drug Discov Devel* 9 (2): 261–8, which is incorporated by reference herein in its entirety).

In a preferred embodiment, the PDGFRβ binding polypeptides are antibodies, or antigen binding fragment thereof, comprising a VH domain and/or a VL domain. Exemplary CDR, VH, and VL amino acid sequences suitable for use in the invention are set forth in Tables 1-4. Accordingly, in certain embodiments, the binding polypeptides may comprise HCDR3 (SEQ ID NO:1) together with HCDR2 and/or HCDR1 sequences which are independently selected from any one of the heavy chain HCDR2 or HCDR1 sequences set forth in Table 1. In certain embodiments, the binding polypeptides of the invention may further comprise light chain CDRs which are independently selected from any one of the light chain CDR1, CDR2 or CDR3 sequences set forth in Table 2. For example, the binding polypeptide of the invention may comprise any one of the heavy chain variable (VH) domains set forth in Table 3, optionally paired with any one of the light chain variable (VL) domains set forth in Table 4.

Table 1. Heavy chain CDR amino acid sequences of exemplary anti-PDGFRβ antibodies.

Clone name	CDR3	SEQ ID NO.	CDR2	SEQ ID NO.	CDR1	SEQ ID NO.
XB1511	HGGDRSY	1	GIIPIFGTANYAQKFQG	2	SYAIS	33
G2	HGGDRSY	1	GIIPIFGTANYAQKFQG	2	GYAIS	34
XB2202	HGGDRSY	1	GILPILKTPNYAQRFQG	3	RHAIS	35
C05.	HGGDRSY	1	GVLPILKTPNYAQRFQG	4	RHAIS	35
E2.	HGGDRSY	1	WINPNSGNTGYAQKFQG	5	DYYIQ	36
A3.	HGGDRSY	1	WINPNSGGTYFAQKFQG	6	DYYIQ	36
C3.	HGGDRSY	1	GILPILKTPNYAQRFQG	3	DYYIQ	36
F10.	HGGDRSY	1	WINPDSGGTYFAQKFQG	7	DYYIQ	36
C12.	HGGDRSY	1	WMNPDSGGTIYAQKFQG	8	DYYIQ	36
H2.	HGGDRSY	1	WLNPNSGDTHSAQKFQG	9	AYYIQ	37
B1.	HGGDRSY	1	WINPNNGNTTYAQKFQG	10	DYYIH	38
E11.	HGGDRSY	1	GIIPIFGTANYAQKFQG	2	DYYIH	38
H1.	HGGDRSY	1	WINPNSGGTNSAPKFQG	11	DYHLH	39
E6.	HGGDRSY	1	WINPNSGGTNYAQKFQG	12	DYYLH	40
A1.	HGGDRSY	1	WIVVGSGNTNYAQKFQE	13	SSAVQ	41
H7.	HGGDRSY	1	WIVVGSGNTNYAQKFQE	13	SSAMQ	42
G04.	HGGDRSY	1	WIVVGSGNTNYAQKFQE	13	SYAIS	33
B2.	HGGDRSY	1	VINTGVGSTNYAQKFQG	14	NYQVQ	43
A7.	HGGDRSY	1	VINTGVGSTNYAQKFQG	14	NYPVQ	44
H3.	HGGDRSY	1	LSNPSGDYTVYAPKFQG	15	NSFMQ	45
B4.	HGGDRSY	1	LSNPSGDYTVYAPKLQG	16	NSFMQ	45
D06.	HGGDRSY	1	VISYDGSNKYYADSVKG	17	SYGMH	46
F3.	HGGDRSY	1	WISADNGNTNYAQKFQE	18	SHGMS	47
A12.	HGGDRSY	1	WISADNGNTKYAQKFQD	19	SHGMS	47
G3.	HGGDRSY	1	GFDPEDGETIYAQKFQG	20	ELSMH	48
H12.	HGGDRSY	1	GIIPIFGTANYAQKFQG	2	DNYVH	49
G12.	HGGDRSY	1	GIIPVSGTPNYAQKFQG	21	AYPIS	50
C06.	HGGDRSY	1	GIIPIFGTANYAQKFQG	2	GHYIH	51
C11.	HGGDRSY	1	GIIPIFGTANYAQKFQG	2	NDYIH	52
F08.	HGGDRSY	1	GIIPIFGTANYAQKFQG	2	SSYIH	53
E9.	HGGDRSY	1	ITYPADSTTVYSPSFQG	22	NYWIG	54
E11.	HGGDRSY	1	RINNDGSSTSYADSVKG	23	SYWMH	55
C08.	HGGDRSY	1	RISIDGTTTYADSVQG	24	AFWMH	56
XB2708	HGGDRSY	1	FILFDGNNKYYADSVKG	25	SYGMH	46
D03.	HGGDRSY	1	RINADGTSTAYAESVKG	26	NDWMH	57
A10.	HGGDRSY	1	LIYSDGSTYYADSVKG	27	DYAMN	58
C09.	HGGDRSY	1	AIDGSGGTTYYAGSVKG	28	NNAMS	59
A06.	HGGDRSY	1	HISNDGSITRYADSVKG	29	GHWMH	60
C05.	HGGDRSY	1	RIKTDGSSTSYADSVKG	30	SNWMH	61
H01.	HGGDRSY	1	RISSDGSTTAYADSVRG	31	SDWMH	62
G07.	HGGDRSY	1	RISSDGSSTAYADSVKG	32	SDWMH	62

Table 2. Light chain CDR amino acid sequences of exemplary anti-PDGFRβ antibodies.

Clone name	CDR3	SEQ ID NO.	CDR2	SEQ ID NO.	CDR1	SEQ ID NO.
B10.	HQSSSLPWT	63	AYQSVS	148	RASQTIGSTLH	233
H10.	HQSSSLPHT	64	SSQSFS	149	RASQSIGSSLH	234
F10.	RASQSIGSGLH	65	ASQSMS	150	HQSSSLPWT	235
B12.	HQTSSLPLT	66	ASQPFS	151	RASQSIGIKLH	236
B11.	QQYGSSPWT	67	GASSRAS	152	RASQSVSSNYLA	237
E7.	QQYGSSPQT	68	GASSRAT	153	RASQSVSSSYLA	238
E8.	QQYGSSPPYT	69	GASSRAT	154	RASQSVSSSYLA	239
H8.	QQYAGSPFT	70	GASSRAT	155	RASQSVSSNYLA	240
H12.	QQFGSSPWT	71	GASRRAT	156	RASQSVRSSYVA	241

F8.	QQYGSSPLT	72	VASRRVT	157	SGGRSNIGGNAVN	242
D11.	QQYGASPRT	73	GASSRAT	158	RASQNITSNFFA	243
G8.	QQYGSALLT	74	DASNRAA	159	RASQSLSGTYLA	244
H9.	QQYGNSWT	75	RASTRAT	160	RASEDIYNNYLA	245
H11.	HQSRNLPFT	76	ASQSFS	161	RASQSIGSSLH	246
G12.	HQSRSFPLT	77	SSQSIS	162	RASESIGTALH	247
E11.	QQYETSWT	78	RASTRAT	163	RTSQILHSQYLA	248
F12.	RDGLNHLV	79	GENNRPS	164	QGDTLRTCYAS	249
C8.	GIWDSSLSVVI	80	YDNYQRFS	165	SGSTSNIGKNFVS	250
A8.	HQTGSFPYT	81	LASQSFS	166	RASRYIGSNLH	251
B8.	LLSYSGPRVV	82	DTSNKQS	167	GSSTGAVTSGHSPF	252
F7.	QQSYRTPFS	83	WASTRES	168	KSSXSLLYRSNNKNYL A	253
B7.	QVWDSSSVI	84	RDSNRPS	169	GGANIANKNVH	254
G9.	KSRDSSAMRWV	85	GKDNRPS	170	QGDSLRTYYAS	255
A9.	LLYFNPTRV	86	DTHNRHS	171	GSSTGAVTSGHYPY	256
A11.	GADHGRV	87	GIVGSKGD	172	TLSSGYSNYKVD	257
E12.	QVWHSGVI	88	FDSDRPS	173	GGNNIGSKSVH	258
H7.	HQSRSSHT	89	YASQSFS	174	RASQNIGNSLH	259
A10.	QSFDVYSHEVV	90	GNNQRPS	175	TRCTGNIASHFVQ	260
C11.	MQSTHFPFT	91	EVSKRFS	176	KSSQSLLNSDDGKTYL Y	261
D10.	QQYDSPPWT	92	DASHLEA	177	QASHDISNYLN	262
D12.	QQHDTSQWT	93	GASSRAA	178	RASQSVSRTYLA	263
C7.	MQGLHIPHT	94	EVSGRFS	179	KSSQSLLHSDGKTHLF	264
D7.	MQSTHQWT	95	SVSKRDS	180	RSSHSLVHSDGNIYLN	265
C9.	QQYDSYSRT	96	EASRLES	181	RASQSISSWLA	266
C12.	QQSFSMRT	97	GASGLQS	182	RTSQGIRNYLS	267
D8.	QQYVNSRT	98	DASNRAT	183	RASQSVTSNYLA	268
D9.	QQYNDFFT	99	GASTRAT	184	RASQSVSSKLA	269
G7.	MQATQFPS	100	KISNRMS	185	RSSESPVHSDGNIYLS	270
G11.	QQYGDSVFT	101	GGSIRAS	186	RASQSVSSRNLA	271
F9.	QQSYSTPRT	102	AASTLHY	187	RASDNIGNYLN	272
E9.	QESYSTLLYT	103	AASRLQS	188	RASESISNYLN	273
B1.	QVWESGSEHYV	104	DDSDRPS	189	GGNNIGYDSVH	274
E6.	QVWESTSDHPT	105	YDNDRPS	190	GGNNIGATTV	275
F3.	QVWDSSSDHWV	106	YDSDRPS	191	GGNNIGSKSVH	276
H4.	QVWDSSSGHRGV	107	DDSDRPS	192	GGNNIVSKGVH	277
H5.	QVWDSATDHVV	108	SDRDRPS	193	GGNNLGSKIVH	278
B5.	QVWDSDRHHVV	109	DDYGRPS	194	AGNNIGGKSVQ	279
G6.	QVWDINDDYAV	110	QDTKRPS	195	SGDNLGHTNAC	280
C1.	QQYVSSPPMYT	111	GASSRAT	196	TASQSVSSTYLT	281
F1.	QQYVTYPLT	112	GASNLEG	197	RASQNIDYDLA	282
A3.	QQYDSVPLT	113	GASTLES	198	QASQVIDKYVN	283
B4.	QQYEDLPSF	114	EASNLET	199	QASQDIFHYLN	284
B6.	QQYGSFPYS	115	AASNRAT	200	RASQSFGSNYLA	285
F2.	QQYQNPPFT	116	GASNLER	201	QASQFIHIYLN	286
D3.	QQYKTFPHT	117	AASYLQT	202	RASQDVGIYVA	287
G2.	QQYHSYPYT	118	KVSTLES	203	RASQDINTWLA	288
A4.	QQYNNVLRT	119	EASNLET	204	QASQDISNWLN	289
G4.	QQYNKWPTF	120	GASTRAT	205	RVSQNVFSDLA	290
D5.	QQYYNWPPWT	121	AASTLHY	206	RASDNIGNYLN	291
A1.	QQRSNGVTF	122	EASTRAT	207	RASQSVSSFLA	292
H2.	QHYHTYPFT	123	QASSLKT	208	RATESISIWLA	293
E2.	QQYYLTPTFTVT	124	WASTRES	209	KSSQSVLYSSNNKNYL A	294
F4.	QQTNTFPLT	125	RATNLQS	210	RASQDISSWLA	295
C5.	QQYHTTPYT	126	WASTRES	211	KSSQSVLYSSNNRNYL A	296
E5.	QQSFSSPWT	127	AASNLQS	212	RASQTFTSHLN	297

F6.	QQSFTTLVT	128	SASTLQS	213	RASQSVNVYLN	298
G5.	CQQFNSYPLS	129	DASTLQT	214	RASQDISSSLA	299
A5.	GADHGSGSNLVYV	130	VGTGGIVGSRGD	215	TVSSGYRSYEVD	300
D6.	GADHGSGSDFVYV	131	VGTGGIVGSRGD	216	TLSSDYSSYNVD	301
E4.	AAWDDSLNGPV	132	TNNQRPS	217	SGSSTNIGSNAVN	302
F5.	AAWDDRLSGPV	133	TTDRRPS	218	SGGGSNIGSNFGY	303
G1.	ATWDDDLSNPKWV	134	TTNQRPS	219	SGSSSNIGSNSVD	304
E3.	MQALQTSWT	135	LGSNRAS	220	RSSQSLLHSNGYNFLD	305
A2.	MQGTHWPYT	136	QVSTRDS	221	CDDTVSTLPARHP	306
D1.	MQSRNLPKT	137	EASSRFS	222	KSSQSLVHRDGKTYLY	307
C4.	MVWYSAWV	138	RSDSDRHQGS	223	TLSSGFNVVSYNIY	308
E1.	HVLDSSTIVI	139	RDTNRPS	224	AGNNIGTYYVH	309
A6.	HQYNNWPLYT	140	GASTRAT	225	RASQSVSSNLA	310
H1.	NSRDSSGYLLL	141	GKNTRPS	226	QGDSLRTYYAS	311
B2.	LLSYSGAGV	142	DASNKHS	227	GSSTGAVTSGHYPY	312
C2.	LQDYSFPYT	143	DSSTLQS	228	RPSQDIGTDLG	313
G3.	QAWDSSHAV	144	QDTKRPS	229	SGDELKYKYTC	314
H3.	QSEDSRGPV	145	KDTERPS	230	SGSTFPKLYSF	315
D4.	VQATHFPVT	146	KISNRFS	231	RSSESVVHDDGNTYLS	316
C6.	CSYTTGSTLYL	147	DVNRRPS	232	TGTSDDVGRYDYVS	317

Table 3. Heavy chain variable domain (VH) amino acid sequences of exemplary anti-PDGFRβ antibodies.

Clone name	VH Amino Acid Sequence	SEQ ID NO.
	Sequences of VH Primary Selection on recombinant human PDGFRβ	
A4 XB1511	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMG GIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAI HGGDRSYWGQGTLVTVSS	318
B4	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYAISWVRQAPGQGLEWMG GIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAI HGGDRSYWGQGTLVTVSS	319
G2	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYAISWVRQAPGQGLEWMG GIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSKDTAVYYCAI HGGDRSYWGQGTLVTVSS	320
	(XB1511) Framework Shuffled and selected with 2 rounds on human and 2 rounds on mouse PDGFRβ targets	
XB2202	QVQLVQSGAEVKKPGSSVRVSCKASGGTFSRHAISWVRQAPGQGLEWIG GILPILKTPNYAQRFQGRVTINADESTSTVYMEMSSLRSEDTAVYYCAT HGGDRSYWGQGTLVTVSS	321
C4.	QMQLVQSGAEVKKPGSSVRVSCKASGGTFSRHAISWVRQAPGQGLEWIG GILPILKTPNYAQRFQGRVTINADESTSTVYMEMSGLRSEDTAVYYCAT HGGDRSYWGQGTLVTVSS	322
B12.	QMQLVQSGAEVKKPGSSVRVSCKASGGTFSRHAISWVRQAPGQGLEWIG GILPILKTPNYAQRFQGRVTINADESTSTVYMEMSSLRSENTAVYYCAT HGGDRSYWGQGTLVTVSS	323
D07.	QMQLVQSGAEVKKPGSSVRVSCKASGGTFSRHAISWVRQAPGQGLEWIG GILPILKTPNYAQRFQGRVTINADESTSTVYMEMSSLRSDDTAVYYCAT HGGDRSYWGQGTLVTVSS	324
C05.	QMQLVQSGAEVKKPGSSVRVSCKASGGTFSRHAISWVRQAPGQGLEWIG GVLPILKTPNYAQRFQGRVTINADESTSTVYMELSSLRSEDTAVYYCAT HGGDRSYWGQGTLVTVSS	325
E05.	QVQLVQSGPKVKKPGSSVRVSCKASGGTFSRHAISWVRQAPGQGLEWIG GILPILKTPNYAQRFQGRVTINADESTSTVYMEMSSLRSEDTAVYYCAT HGGDRSYWGQGTLVTVSS	326
E2.	QMQLVQSGAEVKKPGASVKISCKTSGYTFTDYYIQWVRQAPGQGLEWVG WINPNSGNTGYAQKFQGRVTMTRDTSISTAYMELSSLRSEDTAVYYCAT HGGDYSYWGQGTLVTVSS	327
A3.	QVQLVQSGAEVKKPGASVRVSCKASGYTFSDYYIQWVRQAPGQGLEWMG WINPNSGGTYFAQKFQGRVTMTRDTSISTAYMELSSLTSDDTAVYYCAT HGGDRGYWGQGTLVTVSS	328

C3.	QMQLVQSGAEVKKPGASVKVSCKASGYTFTDYYIQWVRQAPGQGLEWIG GILPILKTPNYAQRFQGRVTINADESTSTVYMEMSSLRSEDTAVYYCAT HGGDRSYWGQGTLVTVSS	329
F10.	QMQLVQSGAEVKKPGASVKVSCKASGYTFTDYYIQWVRQAPGQGLEWMG WINPDSGGTYFAQKFQGRVAMTRDTSINTAYMELSSLRSDDTAVYYCAT HGGDRSYWGQGTLVTVSS	330
C12.	QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYIQWVRQAPGEGLEWMG WMNPDSGGTIYAQKFQGRVTMTRDTSISTAYMELSRLRPDDTAVYYCAT HGGDRSYWGQGTLVTVSS	331
H2.	QMQLVQSGAEVKNPGASVKVSCKASGYPFSAYYIQWVRQAPGQGLEWMG WLNPNSGDTHSAQKFQGRVTMTRDTSISTAYMELSGLTSDDTAVYYCAT HGGDRSYWGQGTLVTVSS	332
F11.	QMQLVQSGAEVKNPGASVKVSCKASGYPFSAYYIQWVRQAPGQGLEWMG WLNPNSGDTHSAQKFQGRVTMTRDTSISTAYMELSGPTSDDTAVYYCAT HGGDRSYWGQGTLVTVSS	333
B1.	QMQLVQSGAEVRKPGASVKVSCKASGYSFSDYYIHWVRQAPGQGLEWIG WINPNNGNTTYAQKFQGRVTMIRDTSISTAYMELSELRSDDTAVYYCAT HGGDRSYWGQGTLVTVSS	334
E11.	QVQLVQSGAEVEKPGASVKVSCKASGYTFTDYYIHWVRQAPGQGLEWMR GIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAT HGGDRSYWGQGTLVTVSS	335
H1.	EVQLLESGAEVKQPGASVKVSCKTSGYTFTDYHLHWVRQAPGQGLEWMG WINPNSGGTNSAPKFQGRVTMTRDTSISTAYMELSGLTSDDTAVYYCAT HGGDRSYWGQGTLVTVSS	336
E6.	QMQLVQSGAEVKRPGASVKVPCKASGYTFTDYYLHWVRQAPGQGLKWMG WINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSEDTAVYYCAT HGGDRSYWGQGTLVTVSS	337
A1.	QVQLVQSGPEVKKPGTSVKVSCKASGFTFTSSAVQWVRQARGQRLEWIG WIVVGSGNTNYAQKFQERVTITRDMSTSTAYMELSSLRSEDTAVYYCAT HGGDRSYWGQGTLVTVSS	338
H7.	QVQLVQSGPEVKKPGTSVKVSCKASGFTFTSSAMQWVRQARGQRLEWIG WIVVGSGNTNYAQKFQERVTITRDMSTSTAYMELSSLRSEDTAVYYCAT HGGDRSYWGQGTLVTVSS	339
G04.	QVQLVQSGAEVKKPGASVKVSCKASGFTFTSYAISWVRQARGQRLEWIG WIVVGSGNTNYAQKFQERVTITRDMSTSTAYMELSSLRSEDTAVYYCAT HGGDRSYWGQGTLVTVSS	340
B2.	QVQLVQSGAEVKKPGASVKVSCKASGYSFTNYQVQWVRQAPGQGLEWLG VINTGVGSTNYAQKFQGRVTMTRDTATSTVYMELSSLRSEDTAVYYCAT HGGDRSYWGQGTLVTVSS	341
A7.	QVQLVQSGAEVKKPGASVKVSCKASGYSFTNYPVQWVRQAPGQGLEWLG VINTGVGSTNYAQKFQGRVTMTRDTATSIVYMELSSLRSEDTAVYYCAT HGGDRSYWGQGTLVTVSS	342
H3.	QVQLVQSGAEVKKPGASVKVSCRASGYTFTNSFMQWVRQVPGQRLEWVG LSNPSGDYTVYAPKFQGRVTMTTDTATSTFYMELFSLRSDDTAVYYCAT HGGDRSYWGQGTLVTVSS	343
B4.	QVQLVQSGAEVKKPGASVKVSCRASGYTFTNSFMQWVRQVPGQRLEWVG LSNPSGDYTVYAPKLQGRVTMTTDTATGTFYMELFSLRSDDTAVYYCAT HGGDRSYWGQGTLVTVSS	344
H05.	EVQLVQSGGGVVQPGGSLRLSCAASGFTFRSYGMHWVRQAPGKGLEWVA FILFDGNNKYYADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCAT HGGDRSYWGQGTLVTVSS	345
D06.	QVQLVQSGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVA VISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK HGGDRSYWGQGTLVTVSS	346
F3.	QVQLVQSGAEVKKPGASVKVSCKASGYTFISHGMSWVRQAPGQGLEWMG WISADNGNTNYAQKFQERVTITRDMSTSTAYMELSSLRSEDTAVYYCAT HGGDRSYWGQGTLVTVSS	347
A12.	QVQLVQSGAEVKKPGASVKVSCKASGYTFISHGMSWVRQAPGQGLEWMG WISADNGNTKYAQKFQDRVTLTTDTSTSTAYLELRSLRSDDTAVYYCAT HGGDRSYWGQGTLVTVSS	348
G3.	QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMG GFDPEDGETIYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCAT HGGDRSYWGQGTLVTVSS	349
F05.	QVQLVQSGAEVKRPGASVKVSCKASGYTLTELSMHWVRQAPGKGLEWMG GFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCAT HGGDRSYWGQGTLVTVSS	350

H12.	QVQLVQSGAEVKKPGASVKVSCKASGYTFTDNYVHWVRQAPGQGLEWMG GIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAT HGGDRSYWGQGTLVTVSS	351
G12.	QVQLVQSGAEVKKPGSSVKVSCKASGGAFNAYPISWVRQAPGQGLEWMG GIIPVSGTPNYAQKFQGRVTITADKSTYTAYMELSSLRSEDTAVYYCAT HGGDRSYWGQGTLVTVSS	352
C06.	QMQLVQSGAEVKKPGASVKVSCMASGYTFTGHYIHWVRQAPGQGLEWMG GIIPIFGTANYAQKFQGRVTITADESTSTAYTELSSLRSEDTAVYYCAT HGGDRSYWGQGTLVTVSS	353
C11.	QVQLVQSGAAVKKPGASVKVSRKASGYTFTNDYIHWVRQAPGQGLEWMG GIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAT HGGDRSYWGQGTLVTVSS	354
F08.	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSSYIHWVRQAPGQGLEWMG GIIPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAMYYCAT HGGDRSYWGQGTLVTVSS	355
E9.	QVQLVESGAEVRKPGESLQISCKASGYRFTNYWIGWVRQMPGKGLEWMG ITYPADSTTVYSPSFQGQVTISADKSISTVFLQWSSLRSEDTAVYYCAT HGGDRSYWGQGTLVTVSS	356
E11.	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYWMHWVRQAPGKGLVWVS RINNDGSSTSYADSVKGRFTISRDTAKNTLYLQMNSLRAEDTAVYYCAT HGGDRSYWGQGTLVTVSS	357
H11.	QVQLLESGAEVKNPGASVKVSCKASGYPFSAYYIQWVRQAPGQGLEWMG WLNPNSGDTHSAQKFQGRVTMTRDTSISTAYMELSGLTSDDTAVYYCAT HGGDRSYWGQGTLVTVSS	358
C08.	EVQLLESEGGLVQPGGSLRLSCTASGFSFNAFWMHWVRQAPGKGLEWVS RISIDGTTTTYADSVQGRFTISRDNARNTLYLQMNSLRAEDAAVYYCAT HGGDRSYWSQGTLVTVSS	359
	(XB1511) Framework Shuffled and selected with human PDGFRβ and off rate selection	
XB2708	QVQLVQSGGGVVQPGGSLRLSCAASGFTSRSYGMHWVRQAPGKGLEWVA FILFDGNNKYYADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCAT HGGDRSYWGQGTLVTVSS	360
D03.	QVQLVQSGGGLVQPGGSLRLSCVASGFTFGNDWMHWVRQAPGKGLVWVS RINADGTSTAYAESVKGRFTVSRDNAKNTLYLQMNGLRAEDTAVYYCAT HGGDRSYWGQGTLVTVSS	361
A10.	QVQLVQSGGGLVQPGRSLRLSCAASGFTFDDYAMNWVRQAPGKGLEWVS LIYSDGSTYYADSVKGRFTISRDNSKKTLYLQMNNLRVEDTAVYYCATH GGDRSYWGQGTLVTVSS	362
C09.	QVQLVQSGGALVQPGGSLRLSCAASGFTLSNNAMSWVRQAPGKRLEWVS AIDGSGGTTYYAGSVKGRFTISSDNSKNTVFLQMNSLRAEDTAVYYCAT HGGDRSYWGQGTLVTVSS	363
A06.	QVQLVQSGGGLVQPGGSLRLSCAASGFTFSGHWMHWVRQVPGKGLVWVS HISNDGSITRYADSVKGRFTVARDNAKNTMYLQMNSLRAEDTAVYYCAT HGGDRSYWGQGTLVTVSS	364
C05.	QVQLVQSGGGLVKPGGSLRLSCAASGFIFSSNWMHWVRQVPGKGLEWVS RIKTDGSSTSYADSVKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCAT HGGDRSYWGQGTLVTVSS	365
H01.	QVQLVQSGGGLVQPGGSLRLSCAASGFTLSSDWMHWVRQAPGKGLVWVS RISSDGSTTAYADSVRGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCAT HGGDRSYWGQGTLVTVSS	366
G04.	QVQLVQSGGGLVQPGGSLRLSCAASGFTLSSDWMHWVRQAPGKGLVWVS RISSDGSTTAYADSVRGRFTISRDNTKNTLYLQMNSLRAEDTAVYYCAT HGGDRSYWGQGTLVTVSS	367
G07.	QVQLVQSGGGLVQPGGSLRLSCAASGFTFSSDWMHWVRQAPGEGLVWVS RISSDGSSTAYADSVKGRFTISRDNAKNTVSLQMNSLRAEDTAVYYCAT HGGDRSYWGQGTLVTVSS	368

Table 4. Light chain variable domain (VL) amino acid sequences of exemplary anti-PDGFRβ antibodies.

Clone name	VL Amino Acid Sequence	SEQ ID NO.
	PR2 VL sequences from XB1511 pairing	
B10.	QSVLTQSPDLQSVTPREKLTITCRASQTIGSTLHWYQQKPGQSPRLVIKY AYQSVSGVPSRFSGSGSGTEFTLTINGLEAEDAATYYCHQSSSLPWTFGQ GTKLTVL	369
H10.	QSVLTQSPDFQSVSPKDKVTITCRASQSIGSSLHWYQQKPGQSPKLLIKY SSQSFSGVPSRFSGSASGTEFTLTITGLEAEDAATYYCHQSSSLPHTFGQ GTKVTVL	370
F10.	QSVLTQSPEFQSVTPKEKVTITCRASQSIGSGLHWYQQKPHQSPKLLIRY ASQSMSGVPSRFSGSGSGTDFTLTISRLEVEDAAMYYCHQSSSLPWTFGQ GTKVTVL	371
B12.	QSVLTQSPDFQSVTPKQNVTFTCRASQSIGIKLHWYQQKPDQSPKVLIKY ASQPFSGVPSRFSGRGSGTDFTLTINSLEAEDAATYYCHQTSSLPLTFGG GTKVTVL	372
B11.	QSVLTQSPGTLSLSPGERATLSCRASQSVSSNYLAWYQQKPGQAPRLLIY GASSRASGIPVRVSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPWTFG QGTKLTVL	373
E7.	QSVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIY GASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPQTFG QGTKLTVL	374
E8.	QSVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIY GASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPPYTF GQGTKLTVL	375
H8.	QSVLTQSPGTLSLSPGERATLSCRASQSVSSNYLAWYLQKPGQAPRLLIS GASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYAGSPFTFG PGTKLTVL	376
H12.	QSVLTQSPGTLSLSPGERATLSCRASQSVRSSYVAWYQQKPGQAPRLLIS GASRRATGIPDRFTGSGSGTDFTLTISRLEPEDFAVYHCQQFGSSPWTFG QGTKLTVL	377
F8.	QSVLTQPPSASGTPGQRVTISCSGGRSNIGGNAVNWYQQKPGQAPRLLIH VASRRVTGIPDRFSGSGSGTDFTLTISRLEPEDFAIYYCQQYGSSPLTFG GGTKLTVL	378
D11.	QSVLTQSPGTLSLSPGERATLSCRASQNITSNFFAWYQQKPGQAPRLLIY GASSRATGIPDRISGSGSGTDFTLTISRLEPEDFALYYCQQYGASPRTFG QGTQLTVL	379
G8.	QSVLTQSPGTLSLSPGDRATLSCRASQSLSGTYLAWYQQKPGQAPRLLIY DASNRAAGIPKRFSGSGSRTDFTLTISRVDPADSAVYYCQQYGSALLTFG GGTKVTVL	380
H9.	QSVLTQSPGTLSLSPGESATLSCRASEDIYNNYLAWYQHKRGQPPRLLIF RASTRATGIPTRFSGSGSGRDFVLTINRLEPEDFAVYYCQQYGNSWTFGQ GTKLTVL	381
H11.	QSVLTQSPDFQSVTPKEKVTITCRASQSIGSSLHWYQQKPDQSPKLLITF ASQSFSGVPSRFSGSGSGTDFTLTINSLEAEDAATYYCHQSRNLPFTFGP GTKLTVL	382
G12.	QSVLTQSPDFQSVTPKEEVTITCRASESIGTALHWYQQKPDQSPKLLIKY SSQSISGVPSRFVGRGSETEFTLTINSLEAENAATYYCHQSRSFPLTFGQ GTQLTVL	383
E11.	QSVLTQSPGTLSLSPGERATLSCRTSQILHSQYLAWYQQKRGQAPRLLIF RASTRATGIPERFSGSGSGRDFVLTISRLEPEDSAVYYCQQYETSWTFGQ GTKVTVL	384
F12.	QSVLTQDPVVSVALGQTVRITCQGDTLRTCYASWYQQRPRQAPILVIYGE NNRPSGIPARFSGSSSGSTASLTITGAQAEDEGDYYCHCRDGLNHLVFGG GTKVTVL	385
C8.	QSVLTQPPSVSAAPGQKVTISCSGSTSNIGKNFVSWYQHLPGTAPKLLIY DNYQRFSGIPDRFSGFKSGTSATLSITGLQTADEADYYCGTWDSSLSVVI FGGGTKLTVL	386
A8.	QAGLTQSPDFQSVTPKERVTITCRASRYIGSNLHWYQQKPDQPPKLLIKL ASQSFSGVPPRFSGGGSGTDFTLTINGLEAEDAATYYCHQTGSFPYTFGQ GTKLTVL	387
B8.	QAVLTQEPSLTVSPGGTVTLTCGSSTGAVTSGHSPFWFQQRPGQAPRTLI YDTSNKQSWTPARFSGSLLGGKAALTLSGAQPEDEAEYYCLLSYSGPRVV FGGGTKVTVL	388

F7.	QAVVTQSPDSLAVSLGERATISCKSSXSLLYRSNNKNYLAWYQQKPGQPP RLLISWASTRESGVPDRFSGSGSGTDFTLTVSRLRAEDAAVYYCQQSYRT PFSFGPGTKVTVL	389
B7.	SYVLTQPLSVSVALGQTARISCGGANIANKNVHWYQLQPGQAPVLVIYRD SNRPSGIPERFSGSNSGNTATLTITRAQARDEADYYCQVWDSSSVIIGGG TKVTVL	390
G9.	SYVLTQDPAVSVALGQTVRITCQGDSLRTYYASWYRQKPGQAPVLVFYGK DNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCKSRDSSAMRWVFG GGTKLTVL	391
A9.	NFMLTQEPSLTVSPGGTVTLTCGSSTGAVTSGHYPYWFQQKPGQVPRTFI YDTHNRHSWTPVRFSGSLFGGKAALTLSGAQPEDEAEYYCLLYFNPTRVF GGGTKLTV	392
A11.	NFMLTQPPSASASLGASVTLTCTLSSGYSNYKVDWYQQRPGKGPRFVMRV GTGGIVGSKGDGIPDRFSVLGSGLNRYLTIKNIQEEDESDYHCGADHGRV FGGGTKLTVL	393
E12.	QPVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYHLRPGQAPVLVIYFD SDRPSGIPERFSGSNSGNTATLTISRVEAGDEADYYCQVWHSGVIFGGGT KLTVL	394
H7.	QPVLTQSLDFQSVTPKEKVTITCRASQNIGNSLHWYQQKPNQSPKVLIKY ASQSFSGVPSRFSGSGFGTDFTLTINSLEPEDAATYYCHQSRSSHTFGQG TKLTVL	395
A10.	EIVLTQSPGNLSLSPGERATLSCTRCTGNIASHFVQWYQQRPGSSPTTVI FGNNQRPSGVSDRFSGSIDSSSNSASLTISRLKTEDEADYYCQSFDVYSH EVVFGGGTKLTVL	396
C11.	QTVVTQTPVSLSVTPGQPASISCKSSQSLLNSDDGKTYLYWYLQRPGQPP HLLIYEVSKRFSGVPDRFSGSGSGTDFTLRISRVEAEDVGVFYCMQSTHF PFTFGPGTKVTVL	397
D10.	NIQMTQSPVSLSASLGDTVSITCQASHDISNYLNWYQQKPGKAPKLLIYD ASHLEAGVPSRFRGSGSGTDFTLTINRLEPEDFAVYYCQQYDSPPWTFGQ GTKLTVL	398
D12.	DVVLTQSPGTMSLSPGERATLSCRASQSVSRTYLAWHQQKPGQAPRLLIY GASSRAAGIPDRFSGSGSGTDFTLSISRLEPEDFAVYYCQQHDTSQWTFG QGTKLTVL	399
C7.	DIVMTQTPLSLSVTPGQPASISCKSSQSLLHSDGKTHLFWYLQRPGQSPQ LLIYEVSGRFSGVSERFSGSGSGTDFTLKISRVEAEDVGVYYCMQGLHIP HTFGQGTKVEIK	400
D7.	DIVMTQSPLSLPVTLGQPASISCRSSHSLVHSDGNIYLNWYHQRPGQSPR RLIYSVSKRDSGVPDRFSGSGSRTDFTLKISRVEAEDVGVYFCMQSTHQW TFGQGTKVEIK	401
C9.	VIWMTQSPSTVSASVGDRVTITCRASQSISSWLAWYQQKPGKAPNLLIYE ASRLESGIPSRFSGSGSGTEFTLTXSSLQPDDFATYYCQQYDSYSRTFGQ GTKVAIK	402
C12.	DVVMTQSPSSLSASVGDRVTITCRTSQGIRNYLSWYQQKPAKAPKLLIHG ASGLQSGVPSRFSGSGSGTNFTLTISSLQPEDFATYYCQQSFSMRTFGQG TKVEIK	403
D8.	EIVMTQSPGTLTLSPGEGATLSCRASQSVTSNYLAWYQQRPGASSLQSGQ APRLLIYDASNRATGIPDRFSGSGFGTDFTLTISRLEPEDFAVYYCQQYV NSRTFGQGTKVEIK	404
D9.	EIVMTQSPVTLSVSPGERATLSCRASQSVSSKLAWYQQKPGQAPRLLIYG ASTRATGIPARFSGSGSGTEFTLTISSLQSEDFAIYYCQQYNDFFTFGPG TKVDIK	405
G7.	EIVLTQTPLSSPVTLGQPASISCRSSESPVHSDGNIYLSWLHQRPGQPPR LLLYKISNRMSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCMQATQFP SFGQGTKLEIK	406
G11.	EIVLTQSPGTLSLSPGEGATLSCRASQSVSSRNLAWYQQKPGQAPRLLIY GGSIRASGTSTRFSGSGSGTDFTLTINRLEPEDFAVYYCQQYGDSVFTFG PGIKVDIK	407
F9.	NIQMTQSPSSLSASVGDRVNITCRASDNIGNYLNWYQHKPGKAPTVLIYA ASTLHYGVPSRFSGRGSGTDFTVTISSLQPEDSATYYCQQSYSTPRTFGQ GTRVELK	408
E9.	AIQMTQSPSSLSASVGDRVTITCRASESISNYLNWYQQKPGKAPKLLLSA ASRLQSGVPSRFSGSGSGTDFTLTITSLQPEDLATYYCQESYSTLLYTFG QGTKLEIK	409
	VL sequences from XB2202 VL pairing	
B1.	SYELTQPPSVSVAPGKTASITCGGNNIGYDSVHWYQQKPGQAPVLVVFDD SDRPSGIPERFSGSNSGNTATLTISRVEAGDEADYYCQVWESGSEHYVFG	410

	TGTQLTVL	
E6.	LPVLTQPPSVSVAPGQTARISCGGNNIGATTVHWYQHRPGQAPVSVIFYD NDRPSGIPERFSGSNSGNTATLTISRVEAGDEADYYCQVWESTSDHPTFG GGTQLTVL	411
F3.	QSVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVIYYD SDRPSGIPERFSGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHWVFG GGTKLTVL	412
H4.	SYELTQSPSVSVPPGQTARITCGGNNIVSKGVHWYQQRPGQAPVLVVYDD SDRPSGIPERFAGFNSGNTATLTISRVEAGDEADYYCQVWDSSSGHRGVF GGGTKVTVL	413
H5.	SYELTQPPSVSMAPGKTARITCGGNNLGSKIVHWYQQKPGQAPVVVIYSD RDRPSGVPERFSGSNSGNSATLTISGVEAGDEADYYCQVWDSATDHVVFG GGTKLTVL	414
B5.	SYELTQPPSVSVAPGQTATITCAGNNIGGKSVQWYQQKPGQAPVVVVYDD YGRPSGIPERVSGSNSGNTATLTLTRVEAGDEADYYCQVWDSDRHHVVFG GGTKLTVL	415
G6.	QLVLTQPPSVSVSPGQTASITCSGDNLGHTNACWYQQNPGQSPVLVIYQD TKRPSGIPERFSGSNSGNPATLTIXRVXAGDEANYYCQVWDINDDYAVFG TGTXLTVL	416
C1.	QSVLTQSPGTLSLSPGERATLSCTASQSVSSTYLTWYQQKPGQAPRLLIY GASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYVSSPPMYT FGQL	417
F1.	DIQMTQSPSTLSASVGDRVTISCRASQNIDYDLAWYQXKPGKAPXLLIYG ASNLEGGVPSXFSGXGSGTEFTLTISSLQPDXSATYYCQQYVTYPLTFGQ GTRLEIK	418
A3.	AIQMTQSPSSLSASVGDRVTMTCQASQVIDKYVNWYRQRPGKAPELLIYG ASTLESGVPSRFSGSGSGTQFTFSITSVQPEDFATYICQQYDSVPLTFGP GTILDVKRTVA	419
B4.	DIQLTQSPSSLSASIGDRVTITCQASQDIFHYLNWFQQKPGKAPKLLIYE ASNLETGVPSRFSGSGSVTDFTFTISSLQPEDIATYYCQQYEDLPSFGGG TKVDIKRTVA	420
B6.	EIVLTQSPGTLSLSPGERATLSCRASQSFGSNYLAWYQHKPGQAPRLLIF AASNRATGIPDRFTGSASGTDFTLTINRVEPEDLAVYYCQQYGSFPYSFG QGTKLEIK	421
F2.	NIQMTQSPSSLSASVGDRVTITCQASQFIHIYLNWYQQKLGKAPKLLIYG ASNLERGVPSRFSGRGSETDFTFTIDSLQPEDIATYFCQQYQNPPFTFGG GIKVEINGTVA	422
D3.	AIRMTQSPSSLSASIGDRISVTCRASQDVGIYVAWFQQKPGKPPRSLIYA ASYLQTAVPPKFRGSGSGTDFTLTISDLQPDDFATYYCQQYKTFPHTFGQ GTKLDFKRTVA	423
G2.	VIWMTQSPSTLSASVGDRVTITCRASQDINTWLAWYQQKPGKAPKLLMFK VSTLESGDFSRFSGSGSGTEFTLTVSSLQPDDSAIYYCQQYHSYPYTFGQ GTRLEIK	424
A4.	DVVMTQSPSSLSASVGDRVTITCQASQDISNWLNWYQQKPGKAPKLLIYE ASNLETGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQYNNVLRTFGQ GTKVEIK	425
G4.	EIVMTXSPATLSVSPGERVTLSCRVSQNVFSDLAWYQRKTGQSPRLLIHG ASTRATGIPTRFSGSGSGTEFTLTISSLXSDDFAVYYCQQYNKWPTFGQG TKVEIK	426
D5.	AIQLTQSPSSLSASVGDRVNITCRASDNIGNYLNWYQHKPGKAPTVLIYA ASTLHYGVPSRFSGRGSGTDFTVTISSLRSDDFAVYYCQQYYNWPPWTFG QGTTVDIKRTVA	427
A1.	EIVLTQSPATLSLSPGERATLSCRASQSVSSFLAWYQQKPGQAPRLLIFE ASTRATGISARFSGSGSGTDFTLTISTLEPEDFAVYYCQQRSNGVTFGQG TRLEIK	428
H2.	DIQMTQSPSTLSASVGDTVTITCRATESISIWLAWYQQEPGKAPNLLVSQ ASSLKTGVPSRFSASGSGTEFTLTISSLHPDDFATYVCQHYHTYPFTFGP GTKVDMKRTVA	429
E2.	EIVLTQSPDSXAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPP RLLIYWASTRESGVPDRFSGSGSGTDFTLTISRLQAEDVAVYYCQQYYLT PTFTVTFGQGTKLEIK	430
F4.	DIQLTQSPSSVSASVGDRVTITCRASQDISSWLAWYQQKPGKAPKFLIYR ATNLQSGVPSRFSGSGSGTDFTLTISSLQPGDFATYYCQQTNTFPLTFGG GTKVEVKRTVA	431
C5.	DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNRNYLAWYQKKPGQPP KLLFYWASTRESGVSDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYHTT	432

	PYTFGQGTKLEIK	
E5.	VIWMTQSPSSLSASVGDRVSITCRASQTFTSHLNWYQQKPGQPPKLLIFA ASNLQSGVPSRFSGSGSGTDFTLTINGLQATDFATYYCQQSFSSPWTFGQ GTTVDVKGTVA	433
F6.	DIQMTQSPSSLSASVGDRVTITCRASQSVNVYLNWYQQKPGKAPKLLIYS ASTLQSGVPSRFTGSGSRTDFTLTINGLQPEDFATYYCQQSFTTLVTFGP GTRVDVTRTVA	434
G5.	DIQMTQSPSSLSASVGDRVTITCRASQDISSSLAWYQQKPGKAPKPLIYD ASTLQTGVPSRFSGRASGTDFTLTIDSLQPEDFATYCCQQFNSYPLSFGG GTKVELKRTVA	435
A5.	SYELTQPPSASASLGASVTLTCTVSSGYRSYEVDWFQQRPGKGPRFVMRV GTGGIVGSRGDGIPDRFSVWGSGLNRYLTIEDIQEEDESDYYCGADHGSG SNLVYVFGTGTKVTVL	436
D6.	QLVLTQPPSASASLGASVTLTCTLSSDYSSYNVDWYQQRPGMGPRFLMRV GTGGIVGSRGDGIPDRFSVKGSGLNRYLTIKNIQEEDESDYYCGADHGSG SDFVYVFGIGTKLTVL	437
E4.	QSVLTQPPSASGTPGQRVTISCSGSSTNIGSNAVNWYQQLPRTAPKLLIY TNNQRPSGVPDRFSGSKSGTSASLAISGLQSEDEANYYCAAWDDSLNGPV FGGGTQLTVL	438
F5.	QSVLTQPPSASGTPGQTVIISCSGGGSNIGSNFGYWYQQFPGTAPKLLIY TTDRRPSGVPDRFSGSKSGTTASLAISGLRSEDEADYYCAAWDDRLSGPV FGGGTQLTVL	439
G1.	QTVVTQPPSVSGTPGQRVTISCSGSSSNIGSNSVDWYQQFPGSAPKLLIY TTNQRPSGVPDRFSGSKSGTSASLAISGLRSEDEADYYCATWDDDLSNPK WVFGGGTKLTVL	440
E3.	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNFLDWYLQKPGQSPQ LLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGIYYCMQALQTS WTFGQGTKLEIK	441
A2.	DICRIRPLIRLTIGTITIYNYNGCCDDTVSTLPARHPWTAGLHLQSPRRL MYQVSTRDSGVPDRFSGSGSGTDFTLRISRVEAEDVGVYYCMQGTHWPYT FGQGTKLEIRRTVA	442
D1.	DIVMTQTPLSLSVTPGQPAAISCKSSQSLVHRDGKTYLYWYLQKPGHSPQ LLVYEASSRFSGVPDRISGSASGTQFTLNISRVEAEDVGLYYCMQSRNLP KTFGQGTKVEIK	443
C4.	SYELTQPTSLSASPGASASLTCTLSSGFNVVSYNIYWYQQKPGSPPQYLL RYRSDSDRHQGSGVPSRFSGSKDASANAGILVISALQSDDEADYYCMVWY SAWVFGGG	444
E1.	SYELTQPLSVSVALGQTATITCAGNNIGTYYVHWYQQRPGQAPVLVMYRD TNRPSGISDRFSGSNSGDTATLTICGVQVGDEADYYCHVLDSSTIVIFGG GTQLTVL	445
A6.	QSVLTQSPATLSVSPGERASLSCRASQSVSSNLAWYQQKPGQAPRLLIYG ASTRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCHQYNNWPLYTFG QGTKLTVL	446
H1.	QSVLTQDPAVPVALGQTVRITCQGDSLRTYYASWYQQKPGQAPLLVIYGK NTRPSGIPVRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGYLLLFG TGTKLTVL	447
B2.	QAVLTQEPSLTVSPGGTVTLTCGSSTGAVTSGHYPYWFQQKPGQAPRTLI YDASNKHSWTPARFSGSLLGGKAALTLSGAQPEDEAEYYCLLSYSGAGVF GTGTKVTVL	448
C2.	DIQMTQSPSSLSASVGDRVAIACRPSQDIGTDLGWYQQKPGKAPKLLIFD SSTLQSGVPSRFSGSLSGTDFILTITNLQPEDFATYYCLQDYSFPYTFGQ GTKLQIKRTVA	449
G3.	SYVLTQPPSVSVSPGQTASITCSGDELKYKYTCWYHQKPGQSPVLLIYQD TKRPSGIPERFSGSRSENTATLTISGTQAMDEADYYCQAWDSSHAVFGRG TQLTVL	450
H3.	H3SYVLTQPPSVSVFPGQTARITCSGSTFPKLYSFWYQQKTGQAPLLVIY KDTERPSGIPERFSGSTSGTTVTLIISGVQPEDDADYYCQSEDSRGPVFG GGTKVTVL	451
D4.	GVVMTQTPLSSLVTLGQPASISCRSSESVVHDDGNTYLSWLQQRPGQPPR LLIYKISNRFSGVPDRFSGSGAGTDFTLKISRVEPEDVGVYYCVQATHFP VTFGGGTRVEIK	452
C6.	QSALTQPASVSASPGQSVTISCTGTSDDVGRYDYVSWYQQHPGGAPKLIL YDVNRRPSGVSDRFSGSKSANKASLTISGLQADDEGDYYCCSYTTGSTLY LFGTGTQLTVL	453

In certain embodiments, the antibody, or antigen binding fragment thereof, comprises a heavy chain CDR3 sequence of SEQ ID NO:1 together with one or more CDR region amino acid sequences selected from the group consisting of SEQ ID NOs: 2-317. In exemplary embodiments, the antibody, or antigen binding fragment thereof, comprises HCDR3, HCDR2 and HCDR1 amino acid sequences selected from the group consisting of SEQ ID NO: 1, 2 and 3; 1, 2 and 34; 1, 3 and 35; 1, 4 and 35; 1, 5 and 36; 1, 6 and 36; 1, 3 and 36; 1, 7 and 36; 1, 8 and 36; 1, 9 and 36; 1, 10 and 38; 1, 2 and 38; 1, 11 and 39; 1, 12 and 40; 1, 13 and 41; 1, 13 and 42; 1, 13 and 33; 1, 14 and 43; 1, 14 and 44; 1, 15 and 45; 1, 16 and 45; 1, 17 and 46; 1, 18 and 47; 1, 19 and 47; 1, 20 and 48; 1, 2 and 49; 1, 21 and 50; 1, 2 and 51; 1, 2 and 52; 1, 2 and 53; 1, 22 and 54; 1, 23 and 55; 1, 24 and 56; 1, 25 and 46; 1, 26 and 57; 1, 27 and 58; 1, 28 and 59; 1, 29 and 60; 1, 30 and 61; 1, 31 and 62; and, 1, 32 and 62, respectively.

In other embodiments, the antibody, or antigen binding fragment thereof, further comprises the LCDR3, LCDR2 and LCDR1 amino acid sequences selected from the group consisting of SEQ ID NO: 63, 148 and 233; 64, 149 and 234; 65, 150 and 235; 66, 151 and 236; 67, 152 and 237; 68, 153 and 238; 69, 154 and 239; 70, 155 and 240; 71, 156 and 241; 72, 157 and 242; 73, 158 and 243; 741, 159 and 244; 75, 160 and 245; 76, 161 and 246; 77, 162 and 247; 78, 163 and 248; 79, 164 and 249; 80, 165 and 250; 81, 166 and 251; 82, 167 and 252; 83, 168 and 253; 84, 169 and 254; 85, 170 and 255; 86, 171 and 256; 87, 172 and 257; 88, 173 and 258; 89, 174 and 259; 90, 175 and 260; 91, 176 and 261; 92, 177 and 262; 93, 178 and 263; 94, 179 and 264; 95, 180 and 265; 96, 181 and 266; 97, 182 and 267; 98, 183 and 268; 99, 184 and 269; 100, 185 and 270; 101, 186 and 271; 102, 187 and 272; 103, 188 and 273; 104, 189 and 274; 105, 190 and 275; 106, 191 and 276; 107, 192 and 277; 108, 193 and 278; 109, 194 and 279; 110, 195 and 280; 111, 196 and 281; 112, 197 and 282; 113, 198 and 283; 114, 199 and 284; 115, 200 and 285; 116, 201 and 286; 117, 202 and 287; 118, 203 and 288; 119, 204 and 289; 120, 205 and 290; 121, 206 and 291; 122, 207 and 292; 123, 208 and 293; 124, 209 and 294; 125, 210 and 295; 126, 211 and 296; 127, 212 and 297; 128, 213 and 298; 129, 214 and 299; 130, 215 and 300; 131, 216 and 301; 132, 217 and 302; 133, 218 and 303; 134, 219 and 304; 135, 220 and 305; 136, 221 and 306; 137, 222 and 307; 138, 223 and 308; 139, 224 and 309; 140, 225 and 310; 141, 226 and 311; 142, 227 and 312; 143, 228 and 313; 144, 229 and 314; 145, 220 and 315; 146, 231 and 316; and, 147, 232 and 317, respectively.

In other embodiments, the antibody, or antigen binding fragment thereof, comprises the HCDR3 amino acid sequence set forth in SEQ ID NO: 1, and LCDR3, LCDR2 and LCDR1 amino acid sequences selected from the group consisting of SEQ ID NO: 63, 148 and 233; 64, 149 and 234; 65, 150 and 235; 66, 151 and 236; 67, 152 and 237; 68, 153 and 238; 69, 154 and 239; 70, 155 and 240; 71, 156 and 241; 72, 157 and 242; 73, 158 and 243; 741, 159 and 244; 75, 160 and 245; 76, 161 and 246; 77, 162 and 247; 78, 163 and 248; 79, 164 and 249; 80, 165 and 250; 81, 166 and 251; 82, 167 and 252; 83, 168 and 253; 84, 169 and 254; 85, 170 and 255; 86, 171 and 256; 87, 172 and 257; 88, 173 and 258; 89, 174 and 259; 90, 175 and 260; 91, 176 and 261; 92, 177 and 262; 93, 178 and 263; 94, 179 and 264; 95, 180 and 265; 96, 181 and 266; 97, 182 and 267; 98, 183 and 268; 99, 184 and 269; 100, 185 and 270; 101, 186 and 271; 102, 187 and 272; 103, 188 and 273; 104, 189 and 274; 105, 190 and 275; 106, 191 and 276; 107, 192 and 277; 108, 193 and 278; 109, 194 and 279; 110, 195 and 280; 111, 196 and 281; 112, 197 and 282; 113, 198 and 283; 114, 199 and 284; 115, 200 and 285; 116, 201 and 286; 117, 202 and 287; 118, 203 and 288; 119, 204 and 289; 120, 205 and 290; 121, 206 and 291; 122, 207 and 292; 123, 208 and 293; 124, 209 and 294; 125, 210 and 295; 126, 211 and 296; 127, 212 and 297; 128, 213 and 298; 129, 214 and 299; 130, 215 and 300; 131, 216 and 301; 132, 217 and 302; 133, 218 and 303; 134, 219 and 304; 135, 220 and 305; 136, 221 and 306; 137, 222 and 307; 138, 223 and 308; 139, 224 and 309; 140, 225 and 310; 141, 226 and 311; 142, 227 and 312; 143, 228 and 313; 144, 229 and 314; 145, 220 and 315; 146, 231 and 316; and, 147, 232 and 317, respectively.

In other embodiments, the antibody, or antigen binding fragment thereof, comprises HCDR3, HCDR2 and HCDR1 amino acid sequences selected from the group consisting of SEQ ID NO: 1, 2 and 3; 1, 2 and 34; 1, 3 and 35; 1, 4 and 35; 1, 5 and 36; 1, 6 and 36; 1, 3 and 36; 1, 7 and 36; 1, 8 and 36; 1, 9 and 36; 1, 10 and 38; 1, 2 and 38; 1, 11 and 39; 1, 12 and 40; 1, 13 and 41; 1, 13 and 42; 1, 13 and 33; 1, 14 and 43; 1, 14 and 44; 1, 15 and 45; 1, 16 and 45; 1, 17 and 46; 1, 18 and 47; 1, 19 and 47; 1, 20 and 48; 1, 2 and 49; 1, 21 and 50; 1, 2 and 51; 1, 2 and 52; 1, 2 and 53; 1, 22 and 54; 1, 23 and 55; 1, 24 and 56; 1, 25 and 46; 1, 26 and 57; 1, 27 and 58; 1, 28 and 59; 1, 29 and 60; 1, 30 and 61; 1, 31 and 62; and, 1, 32 and 62, respectively, and LCDR3, LCDR2 and LCDR1 amino acid sequences selected from the group consisting of SEQ ID NO: 63, 148 and 233; 64, 149 and 234; 65, 150 and 235; 66, 151 and 236; 67, 152 and 237; 68, 153 and 238; 69, 154 and 239; 70, 155 and 240; 71, 156 and 241; 72, 157 and 242; 73,

158 and 243; 741, 159 and 244; 75, 160 and 245; 76, 161 and 246; 77, 162 and 247; 78, 163 and 248; 79, 164 and 249; 80, 165 and 250; 81, 166 and 251; 82, 167 and 252; 83, 168 and 253; 84, 169 and 254; 85, 170 and 255; 86, 171 and 256; 87, 172 and 257; 88, 173 and 258; 89, 174 and 259; 90, 175 and 260; 91, 176 and 261; 92, 177 and 262; 93, 178 and 263; 94, 179 and 264; 95, 180 and 265; 96, 181 and 266; 97, 182 and 267; 98, 183 and 268; 99, 184 and 269; 100, 185 and 270; 101, 186 and 271; 102, 187 and 272; 103, 188 and 273; 104, 189 and 274; 105, 190 and 275; 106, 191 and 276; 107, 192 and 277; 108, 193 and 278; 109, 194 and 279; 110, 195 and 280; 111, 196 and 281; 112, 197 and 282; 113, 198 and 283; 114, 199 and 284; 115, 200 and 285; 116, 201 and 286; 117, 202 and 287; 118, 203 and 288; 119, 204 and 289; 120, 205 and 290; 121, 206 and 291; 122, 207 and 292; 123, 208 and 293; 124, 209 and 294; 125, 210 and 295; 126, 211 and 296; 127, 212 and 297; 128, 213 and 298; 129, 214 and 299; 130, 215 and 300; 131, 216 and 301; 132, 217 and 302; 133, 218 and 303; 134, 219 and 304; 135, 220 and 305; 136, 221 and 306; 137, 222 and 307; 138, 223 and 308; 139, 224 and 309; 140, 225 and 310; 141, 226 and 311; 142, 227 and 312; 143, 228 and 313; 144, 229 and 314; 145, 220 and 315; 146, 231 and 316; and, 147, 232 and 317, respectively.

In other embodiments, the antibody, or antigen binding fragment thereof, comprises at least one of the VH amino acid sequences set forth in SEQ ID NO: 318-368.

In other embodiments, the antibody, or antigen binding fragment thereof, comprises at least one of the VL amino acid sequences set forth in SEQ ID NO: 369-453.

In other embodiments, the antibody, or antigen binding fragment thereof, comprises the VH region amino acid sequence set forth in SEQ ID NO: 318, 321, or 360 paired with a VL region amino acid sequences selected from the group consisting of: SEQ ID NO: 369-453.

In certain embodiments, the antibody, or antigen binding fragment thereof, comprises one or more CDR amino acid sequence selected from the group consisting of SEQ ID NO: 1-317, wherein the one or more CDR region amino acid sequences comprises at least one or more conservative amino acid substitutions (e.g., 1, 2, 3, 4, or 5 conservative amino acid substitutions). Conservative amino acid substitutions include the substitution of an amino acid in one class by an amino acid of the same class, where a class is defined by common physicochemical amino acid side chain properties and

high substitution frequencies in homologous proteins found in nature, as determined, for example, by a standard Dayhoff frequency exchange matrix or BLOSUM matrix. Six general classes of amino acid side chains have been categorized and include: Class I (Cys); Class II (Ser, Thr, Pro, Ala, Gly); Class III (Asn, Asp, Gln, Glu); Class IV (His, Arg, Lys); Class V (Ile, Leu, Val, Met); and Class VI (Phe, Tyr, Trp). For example, substitution of an Asp for another class III residue such as Asn, Gln, or Glu, is a conservative substitution. Thus, a predicted nonessential amino acid residue in an anti-PDGFRβ antibody is preferably replaced with another amino acid residue from the same class. Methods of identifying amino acid conservative substitutions which do not eliminate antigen binding are well-known in the art (see, e.g., Brummell *et al.*, Biochem. 32:1180-1187 (1993); Kobayashi *et al.* Protein Eng. 12(10):879-884 (1999); and Burks *et al.* Proc. Natl. Acad. Sci. USA 94:412-417 (1997)).

In another embodiment, the present invention provides anti-PDGFRβ antibodies, or antigen binding fragment thereof, that comprise a VH and/or VL region amino acid sequence with about 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, identity to the VH region amino acid sequence set forth in SEQ ID NO: 318-368, and/or the VL region amino acid sequence set forth in SEQ ID NO: 369-453, respectively.

In another aspect, the present invention provides anti-PDGFRβ antibodies that bind to the same epitope and/or cross compete with an antibody, or antigen binding fragment thereof comprising the VH domain amino acid sequence set forth in SEQ ID NO: 318. Such antibodies can be identified using routine competition binding assays including, for example, surface plasmon resonance (SPR)-based competition assays.

In another aspect, the present invention provides a diverse library of unpaired VH domains wherein each member of the library binds specifically to human PDGFRβ and wherein diversity lies in the FR1-FR3 regions. In a preferred embodiment, each member of the library comprises an identical heavy chain CDR3 (e.g., the amino acid sequence set forth in SEQ ID NO: 1) amino acid sequence that binds specifically to human PDGFRβ, and wherein diversity lies in the FR1-FR3 regions.

In another aspect, the present invention provides a diverse library of stable VH/VL pairs wherein each member of the library binds to human PDGFRβ Preferably each member of the library comprises a VH domain comprising the CDR3 amino acid sequence set forth in SEQ ID NO: 1. The stable VH/VL pairs can be selected using any

methods known in the art including, without limitation those set forth in US provisional patent application 61/453,106, which is hereby incorporated by reference in its entirety.

Any type of VH or VL domain expression library can be employed in the methods of the invention. Suitable expression libraries include, without limitation, nucleic acid display, phage display, and cell surface display libraries (e.g., yeast, mammalian, and bacterial cells). In a preferred embodiment, the library is a nucleic acid display library generated according to the methods set forth in WO2010/011944, which is hereby incorporated by reference in its entirety. Methods for screening expression libraries are well known in the art. See, for example, Antibody Engineering: Methods and Protocols. Methods in Molecular Biology Volume 248, (B.K.C. Lo, Ed) Humana Press, 2004 (ISBN: 1-58829-092-1), which is hereby incorporated by reference in its entirety.

III. Modified Binding Polypeptides

In certain embodiments, binding polypeptides of the invention may comprise one or more modifications. Modified forms of binding polypeptides of the invention can be made using any techniques known in the art.

i) Reducing Immunogenicity

In certain embodiments, binding polypeptides (e.g., antibodies or antigen binding fragments thereof) of the invention are modified to reduce their immunogenicity using art-recognized techniques. For example, antibodies, or fragments thereof, can be chimericized, humanized, and/or deimmunized.

In one embodiment, an antibody, or antigen binding fragments thereof, of the invention may be chimeric. A chimeric antibody is an antibody in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies, or fragments thereof, are known in the art. See, e.g., Morrison, Science 229:1202 (1985); Oi *et al.*, BioTechniques 4:214 (1986); Gillies *et al.*, J. Immunol. Methods 125:191-202 (1989); U.S. Pat. Nos. 5,807,715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their entireties. Techniques developed for the production of "chimeric antibodies" (Morrison *et al.*, Proc. Natl. Acad. Sci. 81:851-855 (1984);

Neuberger *et al.*, Nature 312:604-608 (1984); Takeda *et al.*, Nature 314:452-454 (1985)) may be employed for the synthesis of said molecules. For example, a genetic sequence encoding a binding specificity of a mouse anti-PDGFRβ antibody molecule may be fused together with a sequence from a human antibody molecule of appropriate biological activity. As used herein, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region, e.g., humanized antibodies.

In another embodiment, an antibody, or antigen binding portion thereof, of the invention is humanized. Humanized antibodies have a binding specificity comprising one or more complementarity determining regions (CDRs) from a non-human antibody and framework regions from a human antibody molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen *et al.*, U.S. Pat. No. 5,585,089; Riechmann *et al.*, Nature 332:323 (1988), which are incorporated herein by reference in their entireties.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Pat. Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka *et al.*, Protein Engineering 7(6):805-814 (1994); Roguska. *et al.*, PNAS 91:969-973 (1994)), and chain shuffling (U.S. Pat. No. 5,565,332).

In some embodiments, de-immunization can be used to decrease the immunogenicity of PDGFRβ binding polypeptides (e.g., antibody, or antigen binding portion thereof). As used herein, the term "de-immunization" includes alteration of polypeptide (e.g., an antibody, or antigen binding portion thereof) to modify T cell epitopes (see, e.g., WO9852976A1, WO0034317A2). For example, VH and VL sequences from the starting PDGFRβ-specific antibody, or antigen binding portion thereof, of the invention may be analyzed and a human T cell epitope "map" may be generated from each V region showing the location of epitopes in relation to

complementarity-determining regions (CDRs) and other key residues within the sequence. Individual T cell epitopes from the T cell epitope map are analyzed in order to identify alternative amino acid substitutions with a low risk of altering activity of the final antibody. A range of alternative VH and VL sequences are designed comprising combinations of amino acid substitutions and these sequences are subsequently incorporated into a range of PDGFRβ-specific antibodies or fragments thereof for use in the diagnostic and treatment methods disclosed herein, which are then tested for function. Typically, between 12 and 24 variant antibodies are generated and tested. Complete heavy and light chain genes comprising modified V and human C regions are then cloned into expression vectors and the subsequent plasmids introduced into cell lines for the production of whole antibody. The antibodies are then compared in appropriate biochemical and biological assays, and the optimal variant is identified.

ii) Effector Functions and Fc Modifications

Binding polypeptides of the invention may comprise an antibody constant region (e.g. an IgG constant region e.g., a human IgG constant region, e.g., a human IgG1 or IgG4 constant region) which mediates one or more effector functions. For example, binding of the C1 component of complement to an antibody constant region may activate the complement system. Activation of complement is important in the opsonisation and lysis of cell pathogens. The activation of complement also stimulates the inflammatory response and may also be involved in autoimmune hypersensitivity. Further, antibodies bind to receptors on various cells via the Fc region, with a Fc receptor binding site on the antibody Fc region binding to a Fc receptor (FcR) on a cell. There are a number of Fc receptors which are specific for different classes of antibody, including IgG (gamma receptors), IgE (epsilon receptors), IgA (alpha receptors) and IgM (mu receptors). Binding of antibody to Fc receptors on cell surfaces triggers a number of important and diverse biological responses including engulfment and destruction of antibody-coated particles, clearance of immune complexes, lysis of antibody-coated target cells by killer cells (called antibody-dependent cell-mediated cytotoxicity, or ADCC), release of inflammatory mediators, placental transfer and control of immunoglobulin production. In preferred embodiments, the binding polypeptides (e.g., antibodies or antigen binding fragments thereof) of the invention bind to an Fc-gamma receptor. In alternative embodiments, binding polypeptides of the

invention may comprise a constant region which is devoid of one or more effector functions (e.g., ADCC activity) and/or is unable to bind Fcγ receptor.

Certain embodiments of the invention include anti-PDGFRβ antibodies in which at least one amino acid in one or more of the constant region domains has been deleted or otherwise altered so as to provide desired biochemical characteristics such as reduced or enhanced effector functions, the ability to non-covalently dimerize, increased ability to localize at the site of a tumor, reduced serum half-life, or increased serum half-life when compared with a whole, unaltered antibody of approximately the same immunogenicity. For example, certain antibodies, or fragments thereof, for use in the diagnostic and treatment methods described herein are domain deleted antibodies which comprise a polypeptide chain similar to an immunoglobulin heavy chain, but which lack at least a portion of one or more heavy chain domains. For instance, in certain antibodies, one entire domain of the constant region of the modified antibody will be deleted, for example, all or part of the CH2 domain will be deleted.

In certain other embodiments, binding polypeptides comprise constant regions derived from different antibody isotypes (e.g., constant regions from two or more of a human IgG1, IgG2, IgG3, or IgG4). In other embodiments, binding polypeptides comprises a chimeric hinge (i.e., a hinge comprising hinge portions derived from hinge domains of different antibody isotypes, e.g., an upper hinge domain from an IgG4 molecule and an IgG1 middle hinge domain). In one embodiment, binding polypeptides comprise an Fc region or portion thereof from a human IgG4 molecule and a Ser228Pro mutation (EU numbering) in the core hinge region of the molecule.

In certain embodiments, the Fc portion may be mutated to increase or decrease effector function using techniques known in the art. For example, the deletion or inactivation (through point mutations or other means) of a constant region domain may reduce Fc receptor binding of the circulating modified antibody thereby increasing tumor localization. In other cases it may be that constant region modifications consistent with the instant invention moderate complement binding and thus reduce the serum half life and nonspecific association of a conjugated cytotoxin. Yet other modifications of the constant region may be used to modify disulfide linkages or oligosaccharide moieties that allow for enhanced localization due to increased antigen specificity or flexibility. The resulting physiological profile, bioavailability and other biochemical effects of the modifications, such as tumor localization, biodistribution and serum half-life, may easily

be measured and quantified using well know immunological techniques without undue experimentation.

In certain embodiments, an Fc domain employed in an antibody of the invention is an Fc variant. As used herein, the term "Fc variant" refers to an Fc domain having at least one amino acid substitution relative to the wild-type Fc domain from which said Fc domain is derived. For example, wherein the Fc domain is derived from a human IgG1 antibody, the Fc variant of said human IgG1 Fc domain comprises at least one amino acid substitution relative to said Fc domain.

The amino acid substitution(s) of an Fc variant may be located at any position (i.e., any EU convention amino acid position) within the Fc domain. In one embodiment, the Fc variant comprises a substitution at an amino acid position located in a hinge domain or portion thereof. In another embodiment, the Fc variant comprises a substitution at an amino acid position located in a CH2 domain or portion thereof. In another embodiment, the Fc variant comprises a substitution at an amino acid position located in a CH3 domain or portion thereof. In another embodiment, the Fc variant comprises a substitution at an amino acid position located in a CH4 domain or portion thereof.

The binding polypeptides of the invention may employ any art-recognized Fc variant which is known to impart an improvement (e.g., reduction or enhancement) in effector function and/or FcR binding. Said Fc variants may include, for example, any one of the amino acid substitutions disclosed in International PCT Publications WO88/07089A1, WO96/14339A1, WO98/05787A1, WO98/23289A1, WO99/51642A1, WO99/58572A1, WO00/09560A2, WO00/32767A1, WO00/42072A2, WO02/44215A2, WO02/060919A2, WO03/074569A2, WO04/016750A2, WO04/029207A2, WO04/035752A2, WO04/063351A2, WO04/074455A2, WO04/099249A2, WO05/040217A2, WO05/070963A1, WO05/077981A2, WO05/092925A2, WO05/123780A2, WO06/019447A1, WO06/047350A2, and WO06/085967A2 or U.S. Pat. Nos. 5,648,260; 5,739,277; 5,834,250; 5,869,046; 6,096,871; 6,121,022; 6,194,551; 6,242,195; 6,277,375; 6,528,624; 6,538,124; 6,737,056; 6,821,505; 6,998,253; and 7,083,784, each of which is incorporated by reference herein. In one exemplary embodiment, a binding polypeptide of the invention may comprise an Fc variant comprising an amino acid substitution at EU position 268 (e.g., H268D or H268E). In another exemplary embodiment, a binding polypeptide of the invention may comprise an

amino acid substitution at EU position 239 (e.g., S239D or S239E) and/or EU position 332 (e.g., I332D or I332Q).

In certain embodiments, a binding polypeptide of the invention may comprise an Fc variant comprising an amino acid substitution which alters the antigen-independent effector functions of the antibody, in particular the circulating half-life of the binding polypeptide. Such binding polypeptides exhibit either increased or decreased binding to FcRn when compared to binding polypeptides lacking these substitutions, therefore, have an increased or decreased half-life in serum, respectively. Fc variants with improved affinity for FcRn are anticipated to have longer serum half-lives, and such molecules have useful applications in methods of treating mammals where long half-life of the administered antibody is desired, e.g., to treat a chronic disease or disorder. In contrast, Fc variants with decreased FcRn binding affinity are expected to have shorter half-lives, and such molecules are also useful, for example, for administration to a mammal where a shortened circulation time may be advantageous, e.g. for in vivo diagnostic imaging or in situations where the starting antibody has toxic side effects when present in the circulation for prolonged periods. Fc variants with decreased FcRn binding affinity are also less likely to cross the placenta and, thus, are also useful in the treatment of diseases or disorders in pregnant women. In addition, other applications in which reduced FcRn binding affinity may be desired include those applications in which localization the brain, kidney, and/or liver is desired. In one exemplary embodiment, the altered binding polypeptides (e.g., antibodies or antigen binding fragments thereof) of the invention exhibit reduced transport across the epithelium of kidney glomeruli from the vasculature. In another embodiment, the altered binding polypeptides (e.g., antibodies or antigen binding fragments thereof) of the invention exhibit reduced transport across the blood brain barrier (BBB) from the brain, into the vascular space. In one embodiment, an antibody with altered FcRn binding comprises an Fc domain having one or more amino acid substitutions within the "FcRn binding loop" of an Fc domain. The FcRn binding loop is comprised of amino acid residues 280-299 (according to EU numbering). Exemplary amino acid substitutions which altered FcRn binding activity are disclosed in International PCT Publication No. WO05/047327 which is incorporated by reference herein. In certain exemplary embodiments, the binding polypeptides (e.g., antibodies or antigen binding fragments thereof) of the invention comprise an Fc domain

having one or more of the following substitutions: V284E, H285E, N286D, K290E and S304D (EU numbering).

In other embodiments, binding polypeptides, for use in the diagnostic and treatment methods described herein have a constant region, e.g., an IgG1 or IgG4 heavy chain constant region, which is altered to reduce or eliminate glycosylation. For example, a binding polypeptides (e.g., antibodies or antigen binding fragments thereof) of the invention may also comprise an Fc variant comprising an amino acid substitution which alters the glycosylation of the antibody Fc. For example, said Fc variant may have reduced glycosylation (e.g., N- or O-linked glycosylation). In exemplary embodiments, the Fc variant comprises reduced glycosylation of the N-linked glycan normally found at amino acid position 297 (EU numbering). In another embodiment, the antibody has an amino acid substitution near or within a glycosylation motif, for example, an N-linked glycosylation motif that contains the amino acid sequence NXT or NXS. In a particular embodiment, the antibody comprises an Fc variant with an amino acid substitution at amino acid position 228 or 299 (EU numbering). In more particular embodiments, the antibody comprises an IgG1 or IgG4 constant region comprising an S228P and a T299A mutation (EU numbering).

Exemplary amino acid substitutions which confer reduce or altered glycosylation are disclosed in International PCT Publication No. WO05/018572, which is incorporated by reference herein. In preferred embodiments, the antibodies, or fragments thereof, of the invention are modified to eliminate glycosylation. Such antibodies, or fragments thereof, may be referred to as "agly" antibodies, or fragments thereof, (e.g. "agly" antibodies). While not being bound by theory, it is believed that "agly" antibodies, or fragments thereof, may have an improved safety and stability profile in vivo. Exemplary agly antibodies, or fragments thereof, comprise an aglycosylated Fc region of an IgG4 antibody which is devoid of Fc-effector function thereby eliminating the potential for Fc mediated toxicity to the normal vital organs that express PDGFRβ. In yet other embodiments, antibodies, or fragments thereof, of the invention comprise an altered glycan. For example, the antibody may have a reduced number of fucose residues on an N-glycan at Asn297 of the Fc region, i.e., is afucosylated. In another embodiment, the antibody may have an altered number of sialic acid residues on the N-glycan at Asn297 of the Fc region.

iii) Covalent Attachment

Binding polypeptides of the invention may be modified, e.g., by the covalent attachment of a molecule to the binding polypeptide such that covalent attachment does not prevent the binding polypeptide from specifically binding to its cognate epitope. For example, but not by way of limitation, the antibodies, or fragments thereof, of the invention may be modified by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, etc. Additionally, the derivative may contain one or more non-classical amino acids.

Binding polypeptide (e.g., antibodies, or fragments thereof) of the invention may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalent and non-covalent conjugations) to polypeptides or other compositions. For example, anti-PDGFRβ antibodies may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionuclides, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Pat. No. 5,314,995; and EP 396,387.

Binding polypeptides may be fused to heterologous polypeptides to increase the in vivo half life or for use in immunoassays using methods known in the art. For example, in one embodiment, PEG can be conjugated to the binding polypeptides of the invention to increase their half-life in vivo. Leong, S. R., *et al.*, Cytokine 16:106 (2001); Adv. in Drug Deliv. Rev. 54:531 (2002); or Weir *et al.*, Biochem. Soc. Transactions 30:512 (2002).

Moreover, binding polypeptides of the invention can be fused to marker sequences, such as a peptide to facilitate their purification or detection. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, Calif., 91311), among others, many of which are commercially available. As described in Gentz *et al.*, Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the "HA" tag, which corresponds to an

epitope derived from the influenza hemagglutinin protein (Wilson *et al.*, Cell 37:767 (1984)) and the "flag" tag.

Binding polypeptides of the invention may be used in non-conjugated form or may be conjugated to at least one of a variety of molecules, e.g., to improve the therapeutic properties of the molecule, to facilitate target detection, or for imaging or therapy of the patient. Binding polypeptides of the invention can be labeled or conjugated either before or after purification, when purification is performed. In particular, binding polypeptides of the invention may be conjugated to therapeutic agents, prodrugs, peptides, proteins, enzymes, viruses, lipids, biological response modifiers, pharmaceutical agents, or PEG.

The present invention further encompasses binding polypeptides of the invention conjugated to a diagnostic or therapeutic agent. The binding polypeptides can be used diagnostically to, for example, monitor the development or progression of a immune cell disorder (e.g., CLL) as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment and/or prevention regimen. Detection can be facilitated by coupling the binding polypeptides to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. See, for example, U.S. Pat. No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, .beta.-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include 125I, 131I, 111In or 99Tc.

Binding polypeptides for use in the diagnostic and treatment methods disclosed herein may be conjugated to cytotoxins (such as radioisotopes, cytotoxic drugs, or toxins) therapeutic agents, cytostatic agents, biological toxins, prodrugs, peptides, proteins, enzymes, viruses, lipids, biological response modifiers, pharmaceutical agents,

immunologically active ligands (e.g., lymphokines or other antibodies wherein the resulting molecule binds to both the neoplastic cell and an effector cell such as a T cell), or PEG.

In another embodiment, an anti-PDGFRβ antibody for use in the diagnostic and treatment methods disclosed herein can be conjugated to a molecule that decreases tumor cell growth. In other embodiments, the disclosed compositions may comprise antibodies, or fragments thereof, coupled to drugs or prodrugs. Still other embodiments of the present invention comprise the use of antibodies, or fragments thereof, conjugated to specific biotoxins or their cytotoxic fragments such as ricin, gelonin, Pseudomonas exotoxin or diphtheria toxin. The selection of which conjugated or unconjugated antibodyto use will depend on the type and stage of cancer, use of adjunct treatment (e.g., chemotherapy or external radiation) and patient condition. It will be appreciated that one skilled in the art could readily make such a selection in view of the teachings herein.

It will be appreciated that, in previous studies, anti-tumor antibodies labeled with isotopes have been used successfully to destroy tumor cells in animal models, and in some cases in humans. Exemplary radioisotopes include: 90Y, 125I, 131I, 123I, 111In, 105Rh, 153Sm, 67Cu, 67Ga, 166Ho, 177Lu, 186Re and 188Re. The radionuclides act by producing ionizing radiation which causes multiple strand breaks in nuclear DNA, leading to cell death. The isotopes used to produce therapeutic conjugates typically produce high energy alpha- or beta-particles which have a short path length. Such radionuclides kill cells to which they are in close proximity, for example neoplastic cells to which the conjugate has attached or has entered. They have little or no effect on non-localized cells. Radionuclides are essentially non-immunogenic.

IV. Expression of Binding Polypeptides

Following manipulation of the isolated genetic material to provide binding polypeptides of the invention as set forth above, the genes are typically inserted in an expression vector for introduction into host cells that may be used to produce the desired quantity of the claimed antibodies, or fragments thereof.

The term "vector" or "expression vector" is used herein for the purposes of the specification and claims, to mean vectors used in accordance with the present invention as a vehicle for introducing into and expressing a desired gene in a cell. As known to

those skilled in the art, such vectors may easily be selected from the group consisting of plasmids, phages, viruses and retroviruses. In general, vectors compatible with the instant invention will comprise a selection marker, appropriate restriction sites to facilitate cloning of the desired gene and the ability to enter and/or replicate in eukaryotic or prokaryotic cells.

Numerous expression vector systems may be employed for the purposes of this invention. For example, one class of vector utilizes DNA elements which are derived from animal viruses such as bovine papilloma virus, polyoma virus, adenovirus, vaccinia virus, baculovirus, retroviruses (RSV, MMTV or MOMLV) or SV40 virus. Others involve the use of polycistronic systems with internal ribosome binding sites. Additionally, cells which have integrated the DNA into their chromosomes may be selected by introducing one or more markers which allow selection of transfected host cells. The marker may provide for prototrophy to an auxotrophic host, biocide resistance (e.g., antibiotics) or resistance to heavy metals such as copper. The selectable marker gene can either be directly linked to the DNA sequences to be expressed, or introduced into the same cell by cotransformation. Additional elements may also be needed for optimal synthesis of mRNA. These elements may include signal sequences, splice signals, as well as transcriptional promoters, enhancers, and termination signals. In particularly preferred embodiments the cloned variable region genes are inserted into an expression vector along with the heavy and light chain constant region genes (preferably human) synthetic as discussed above.

In other preferred embodiments the binding polypeptides, or fragments thereof, of the invention may be expressed using polycistronic constructs. In such expression systems, multiple gene products of interest such as heavy and light chains of antibodies may be produced from a single polycistronic construct. These systems advantageously use an internal ribosome entry site (IRES) to provide relatively high levels of polypeptides of the invention in eukaryotic host cells. Compatible IRES sequences are disclosed in U.S. Pat. No. 6,193,980 which is incorporated herein. Those skilled in the art will appreciate that such expression systems may be used to effectively produce the full range of polypeptides disclosed in the instant application.

More generally, once a vector or DNA sequence encoding an antibody, or fragment thereof, has been prepared, the expression vector may be introduced into an appropriate host cell. That is, the host cells may be transformed. Introduction of the

plasmid into the host cell can be accomplished by various techniques well known to those of skill in the art. These include, but are not limited to, transfection (including electrophoresis and electroporation), protoplast fusion, calcium phosphate precipitation, cell fusion with enveloped DNA, microinjection, and infection with intact virus. See, Ridgway, A. A. G. "Mammalian Expression Vectors" Chapter 24.2, pp. 470-472 Vectors, Rodriguez and Denhardt, Eds. (Butterworths, Boston, Mass. 1988). Most preferably, plasmid introduction into the host is via electroporation. The transformed cells are grown under conditions appropriate to the production of the light chains and heavy chains, and assayed for heavy and/or light chain protein synthesis. Exemplary assay techniques include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), or flourescence-activated cell sorter analysis (FACS), immunohistochemistry and the like.

As used herein, the term "transformation" shall be used in a broad sense to refer to the introduction of DNA into a recipient host cell that changes the genotype and consequently results in a change in the recipient cell.

Along those same lines, "host cells" refers to cells that have been transformed with vectors constructed using recombinant DNA techniques and encoding at least one heterologous gene. In descriptions of processes for isolation of polypeptides from recombinant hosts, the terms "cell" and "cell culture" are used interchangeably to denote the source of antibody unless it is clearly specified otherwise. In other words, recovery of polypeptide from the "cells" may mean either from spun down whole cells, or from the cell culture containing both the medium and the suspended cells.

In one embodiment, the host cell line used for antibody expression is of mammalian origin; those skilled in the art can determine particular host cell lines which are best suited for the desired gene product to be expressed therein. Exemplary host cell lines include, but are not limited to, DG44 and DUXB11 (Chinese Hamster Ovary lines, DHFR minus), HELA (human cervical carcinoma), CVI (monkey kidney line), COS (a derivative of CVI with SV40 T antigen), R1610 (Chinese hamster fibroblast) BALBC/3T3 (mouse fibroblast), HAK (hamster kidney line), SP2/O (mouse myeloma), BFA-1c1BPT (bovine endothelial cells), RAJI (human lymphocyte), 293 (human kidney). In one embodiment, the cell line provides for altered glycosylation, e.g., afucosylation, of the antibodyexpressed therefrom (e.g., PER.C6.RTM. (Crucell) or FUT8-knock-out CHO cell lines (Potelligent.RTM. Cells) (Biowa, Princeton, N.J.)). In

one embodiment NS0 cells may be used. CHO cells are particularly preferred. Host cell lines are typically available from commercial services, the American Tissue Culture Collection or from published literature.

In vitro production allows scale-up to give large amounts of the desired polypeptides. Techniques for mammalian cell cultivation under tissue culture conditions are known in the art and include homogeneous suspension culture, e.g. in an airlift reactor or in a continuous stirrer reactor, or immobilized or entrapped cell culture, e.g. in hollow fibers, microcapsules, on agarose microbeads or ceramic cartridges. If necessary and/or desired, the solutions of polypeptides can be purified by the customary chromatography methods, for example gel filtration, ion-exchange chromatography, chromatography over DEAE-cellulose and/or (immuno-)affinity chromatography.

Genes encoding the binding polypeptides, or fragments thereof, of the invention can also be expressed non-mammalian cells such as bacteria or yeast or plant cells. In this regard it will be appreciated that various unicellular non-mammalian microorganisms such as bacteria can also be transformed; i.e. those capable of being grown in cultures or fermentation. Bacteria, which are susceptible to transformation, include members of the enterobacteriaceae, such as strains of Escherichia coli or Salmonella; Bacillaceae, such as Bacillus subtilis; Pneumococcus; Streptococcus, and Haemophilus influenzae. It will further be appreciated that, when expressed in bacteria, the polypeptides can become part of inclusion bodies. The polypeptides must be isolated, purified and then assembled into functional molecules.

In addition to prokaryotes, eukaryotic microbes may also be used. Saccharomyces cerevisiae, or common baker's yeast, is the most commonly used among eukaryotic microorganisms although a number of other strains are commonly available. For expression in Saccharomyces, the plasmid YRp7, for example, (Stinchcomb *et al.*, Nature, 282:39 (1979); Kingsman *et al.*, Gene, 7:141 (1979); Tschemper *et al.*, Gene, 10:157 (1980)) is commonly used. This plasmid already contains the TRP1 gene which provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example ATCC No. 44076 or PEP4-1 (Jones, Genetics, 85:12 (1977)). The presence of the trpl lesion as a characteristic of the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan.

V. Pharmaceutical Formulations and Methods of Administration of Binding Polypeptides.

In another aspect, the invention provides pharmaceutical compositions comprising an anti-PDGFRβ antibody, or fragment thereof.

Methods of preparing and administering antibodies, or fragments thereof, of the invention to a subject are well known to or are readily determined by those skilled in the art. The route of administration of the antibodies, or fragments thereof, of the invention may be oral, parenteral, by inhalation or topical. The term parenteral as used herein includes intravenous, intraarterial, intraperitoneal, intramuscular, subcutaneous, rectal or vaginal administration. The intravenous, intraarterial, subcutaneous and intramuscular forms of parenteral administration are generally preferred. While all these forms of administration are clearly contemplated as being within the scope of the invention, a form for administration would be a solution for injection, in particular for intravenous or intraarterial injection or drip. Usually, a suitable pharmaceutical composition for injection may comprise a buffer (e.g. acetate, phosphate or citrate buffer), a surfactant (e.g. polysorbate), optionally a stabilizer agent (e.g. human albumin), etc. However, in other methods compatible with the teachings herein, the polypeptides can be delivered directly to the site of the adverse cellular population thereby increasing the exposure of the diseased tissue to the therapeutic agent.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. In the subject invention, pharmaceutically acceptable carriers include, but are not limited to, 0.01-0.1M and preferably 0.05M phosphate buffer or 0.8% saline. Other common parenteral vehicles include sodium phosphate solutions, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers, such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present such as for example, antimicrobials, antioxidants, chelating agents, and inert gases and the like. More particularly, pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the

extemporaneous preparation of sterile injectable solutions or dispersions. In such cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and will preferably be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols, such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

In any case, sterile injectable solutions can be prepared by incorporating an active compound (e.g., an antibody by itself or in combination with other active agents) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated herein, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle, which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying, which yields a powder of an active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The preparations for injections are processed, filled into containers such as ampoules, bags, bottles, syringes or vials, and sealed under aseptic conditions according to methods known in the art. Further, the preparations may be packaged and sold in the form of a kit such as those described in co-pending U.S. Ser. No. 09/259,337 and U.S. Ser. No. 09/259,338 each of which is incorporated herein by reference. Such articles of manufacture will preferably have labels or package inserts indicating that the associated compositions are useful for treating a subject suffering from, or predisposed to autoimmune or neoplastic disorders.

Effective doses of the stabilized antibodies, or fragments thereof, of the present invention, for the treatment of the above described conditions vary depending upon many different factors, including means of administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic. Usually, the patient is a human, but non-human mammals including transgenic mammals can also be treated. Treatment dosages may be titrated using routine methods known to those of skill in the art to optimize safety and efficacy.

For passive immunization with an antibody of the invention, the dosage may range, e.g., from about 0.0001 to 100 mg/kg, and more usually 0.01 to 5 mg/kg (e.g., 0.02 mg/kg, 0.25 mg/kg, 0.5 mg/kg, 0.75 mg/kg, 1 mg/kg, 2 mg/kg, etc.), of the host body weight. For example dosages can be 1 mg/kg body weight or 10 mg/kg body weight or within the range of 1-10 mg/kg, preferably at least 1 mg/kg. Doses intermediate in the above ranges are also intended to be within the scope of the invention.

Subjects can be administered such doses daily, on alternative days, weekly or according to any other schedule determined by empirical analysis. An exemplary treatment entails administration in multiple dosages over a prolonged period, for example, of at least six months. Additional exemplary treatment regimes entail administration once per every two weeks or once a month or once every 3 to 6 months. Exemplary dosage schedules include 1-10 mg/kg or 15 mg/kg on consecutive days, 30 mg/kg on alternate days or 60 mg/kg weekly. In some methods, two or more monoclonal antibodies with different binding specificities are administered simultaneously, in which case the dosage of each antibody administered may fall within the ranges indicated.

Antibodies, or fragments thereof, of the invention can be administered on multiple occasions. Intervals between single dosages can be, e.g., daily, weekly, monthly or yearly. Intervals can also be irregular as indicated by measuring blood levels of polypeptide or target molecule in the patient. In some methods, dosage is adjusted to achieve a certain plasma antibody or toxin concentration, e.g., 1-1000 ug/ml or 25-300 ug/ml. Alternatively, antibodies, or fragments thereof, can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the antibody in the patient. In general, humanized antibodies show the longest half-life, followed by chimeric antibodies and

nonhuman antibodies. In one embodiment, the antibodies, or fragments thereof, of the invention can be administered in unconjugated form. In another embodiment, the antibodies of the invention can be administered multiple times in conjugated form. In still another embodiment, the antibodies, or fragments thereof, of the invention can be administered in unconjugated form, then in conjugated form, or vise versa.

The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, compositions containing the present antibodies or a cocktail thereof are administered to a patient not already in the disease state to enhance the patient's resistance. Such an amount is defined to be a "prophylactic effective dose." In this use, the precise amounts again depend upon the patient's state of health and general immunity, but generally range from 0.1 to 25 mg per dose, especially 0.5 to 2.5 mg per dose. A relatively low dosage is administered at relatively infrequent intervals over a long period of time. Some patients continue to receive treatment for the rest of their lives.

In therapeutic applications, a relatively high dosage (e.g., from about 1 to 400 mg/kg of antibody per dose, with dosages of from 5 to 25 mg being more commonly used for radioimmunoconjugates and higher doses for cytotoxin-drug conjugated molecules) at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and preferably until the patient shows partial or complete amelioration of symptoms of disease. Thereafter, the patent can be administered a prophylactic regime.

In one embodiment, a subject can be treated with a nucleic acid molecule encoding a polypeptide of the invention (e.g., in a vector). Doses for nucleic acids encoding polypeptides range from about 10 ng to 1 g, 100 ng to 100 mg, 1 ug to 10 mg, or 30-300 ug DNA per patient. Doses for infectious viral vectors vary from 10-100, or more, virions per dose.

Therapeutic agents can be administered by parenteral, topical, intravenous, oral, subcutaneous, intraarterial, intracranial, intraperitoneal, intranasal or intramuscular means for prophylactic and/or therapeutic treatment. Intramuscular injection or intravenous infusion are preferred for administration of a antibody of the invention. In some methods, therapeutic antibodies, or fragments thereof, are injected directly into the cranium. In some methods, antibodies, or fragments thereof, are administered as a sustained release composition or device, such as a Medipad^{TM} device.

Agents of the invention can optionally be administered in combination with other agents that are effective in treating the disorder or condition in need of treatment (e.g., prophylactic or therapeutic). Preferred additional agents are those which are art recognized and are standardly administered for a particular disorder.

Effective single treatment dosages (i.e., therapeutically effective amounts) of 90Y-labeled antibodies of the invention range from between about 5 and about 75 mCi, more preferably between about 10 and about 40 mCi. Effective single treatment non-marrow ablative dosages of 131I-labeled antibodies range from between about 5 and about 70 mCi, more preferably between about 5 and about 40 mCi. Effective single treatment ablative dosages (i.e., may require autologous bone marrow transplantation) of 131I-labeled antibodies range from between about 30 and about 600 mCi, more preferably between about 50 and less than about 500 mCi. In conjunction with a chimeric modified antibody, owing to the longer circulating half life vis-a-vis murine antibodies, an effective single treatment non-marrow ablative dosages of iodine-131 labeled chimeric antibodies range from between about 5 and about 40 mCi, more preferably less than about 30 mCi. Imaging criteria for, e.g., the 111In label, are typically less than about 5 mCi.

While a great deal of clinical experience has been gained with 131I and .90Y, other radiolabels are known in the art and have been used for similar purposes. Still other radioisotopes are used for imaging. For example, additional radioisotopes which are compatible with the scope of the instant invention include, but are not limited to, 123I, 125I, 32P, 57Co, 64Cu, 67Cu, 77Br, 81Rb, 81Kr, 87Sr, 113In, 127Cs, 129Cs, 132I, 197Hg, 203Pb, 206Bi, 177Lu, 186Re, 212Pb, 212Bi, 47Sc, 105Rh, 109Pd, 153Sm, 188Re, 199Au, 225Ac, 211A 213Bi. In this respect alpha, gamma and beta emitters are all compatible with in the instant invention. Further, in view of the instant disclosure it is submitted that one skilled in the art could readily determine which radionuclides are compatible with a selected course of treatment without undue experimentation. To this end, additional radionuclides which have already been used in clinical diagnosis include 125I, 123I, 99Tc, 43K, 52Fe, 67Ga, 68Ga, as well as 111In. Antibodies have also been labeled with a variety of radionuclides for potential use in targeted immunotherapy (Peirersz *et al.* Immunol. Cell Biol. 65: 111-125 (1987)). These radionuclides include 188Re and 186Re as well as 199Au and 67Cu to a lesser extent. U.S. Pat. No. 5,460,785

provides additional data regarding such radioisotopes and is incorporated herein by reference.

As previously discussed, the antibodies, or fragments thereof, of the invention, can be administered in a pharmaceutically effective amount for the in vivo treatment of mammalian disorders. In this regard, it will be appreciated that the disclosed antibodies, or fragments thereof, will be formulated so as to facilitate administration and promote stability of the active agent. Preferably, pharmaceutical compositions in accordance with the present invention comprise a pharmaceutically acceptable, non-toxic, sterile carrier such as physiological saline, non-toxic buffers, preservatives and the like. For the purposes of the instant application, a pharmaceutically effective amount of a antibody of the invention, conjugated or unconjugated to a therapeutic agent, shall be held to mean an amount sufficient to achieve effective binding to a target and to achieve a benefit, e.g., to ameliorate symptoms of a disease or disorder or to detect a substance or a cell. In the case of tumor cells, the polypeptide will be preferably be capable of interacting with selected immunoreactive antigens on neoplastic or immunoreactive cells and provide for an increase in the death of those cells. Of course, the pharmaceutical compositions of the present invention may be administered in single or multiple doses to provide for a pharmaceutically effective amount of the polypeptide.

In keeping with the scope of the present disclosure, the antibodies of the invention may be administered to a human or other animal in accordance with the aforementioned methods of treatment in an amount sufficient to produce a therapeutic or prophylactic effect. The polypeptides of the invention can be administered to such human or other animal in a conventional dosage form prepared by combining the antibody of the invention with a conventional pharmaceutically acceptable carrier or diluent according to known techniques. It will be recognized by one of skill in the art that the form and character of the pharmaceutically acceptable carrier or diluent is dictated by the amount of active ingredient with which it is to be combined, the route of administration and other well-known variables. Those skilled in the art will further appreciate that a cocktail comprising one or more species of polypeptides according to the present invention may prove to be particularly effective.

VI. Methods of Treating PDGFRβ-Associated Disease or Disorders

The binding polypeptides, or fragments thereof, of the invention are useful for antagonizing PDGFRβ activity. Accordingly, in another aspect, the invention provides methods for treating PDGFRβ-associated diseases or disorders by administering to a subject in need of thereof a pharmaceutical composition comprising one or more anti-PDGFRβ antibody, or antigen binding fragment thereof of the invention.

PDGFRβ-associated diseases or disorders amenable to treatment include, without limitation: Age related macular degeneration (AMD); restenosis, including coronary restenosis after angioplasty, atherectomy, or other invasive methods of plaque removal, and renal or peripheral artery restenosis after the same procedures; vascular proliferative phenomena and fibrosis associated with other forms of acute injury such as: pulmonary fibrosis associated with adult respiratory distress syndrome, renal fibrosis associated with nephritis, coronary stenosis associated with Kawasake's disease, and vascular narrowings associated with other arteritides such as Takayasha's disease; fibrotic processes, such as scleroderma, myofibrosis; and cancer (e.g., tumor cell proliferation and neovascularization)

One skilled in the art would be able, by routine experimentation, to determine what an effective, non-toxic amount of antibody (or additional therapeutic agent) would be for the purpose of treating a PDGFRβ-associated disease or disorder. For example, a therapeutically active amount of a polypeptide may vary according to factors such as the disease stage (e.g., stage I versus stage IV), age, sex, medical complications (e.g., immunosuppressed conditions or diseases) and weight of the subject, and the ability of the antibody to elicit a desired response in the subject. The dosage regimen may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily, or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation. Generally, however, an effective dosage is expected to be in the range of about 0.05 to 100 milligrams per kilogram body weight per day and more preferably from about 0.5 to 10, milligrams per kilogram body weight per day.

VII. Examples

The present invention is further illustrated by the following examples which should not be construed as further limiting. The contents of Sequence Listing, figures and all references, patents and published patent applications cited throughout this application are expressly incorporated herein by reference.

Example 1. Isolation of VH domains that Bind Specifically to Human PDGFRβ

VH domains that bind specifically to human PDGFRβ were selected using DNA display as set forth in WO2010/011944, which is hereby incorporated by reference in its entirety. Specifically, a naïve, human VH domain DNA display library derived from ten bone marrow donors was subject to six rounds of selection against human PDGFRβ. The selected binders were cloned and sequenced. From this screen, VH domain clones A4, B4 and G2 were selected, the amino acid sequences of which are set forth in Table 3.

Example 2. HCDR3 Shuffling

A. VH Library Construction

To screen for VH domains with improved binding characteristics, the HCDR3 sequence of clone A4 (designated XB1511) was shuffled into a naïve human VH library, which was further selected for binding to human and mouse PDGFRβ. Specifically, the DNA sequence coding for the HCDR3 of clone A4 (SEQ ID NO: 1) was synthesized and assembled into a library comprising framework regions 1-3 of naïve human VH domains amplified from bone marrow B cells and PBMCs using framework specific oligonucleotides. Human VH framework regions 1-3 were amplified using 5' VH family-specific and 3' generic FR3 reverse primers to create separate libraries of VH family framework regions. The VH family framework libraries and the XB1511 HCDR3 were shuffled by further PCR amplification using 5' T7TMV and 3' XB1511 FR3CDR3FR4 oligos. This also added a T7TMV promoter sequence at the 5' end for *in vitro* transcription/translation. A C-terminal Cμ3 sequence and a FLAG tag (for purification after translation) were also added by PCR using FR4 Cu3 Reverse and Y109 primers, respectively, together with the 5' T7TMV primer. The nucleic acid sequences of the oligonucleotides used for preparation of the HCDR3-shuffled VH library are set

forth in Table 5. A schematic representation of the VH library construction is set forth in Figure 1.

Table 5. Oligonucleotides for constructing HCDR3 shuffled VH libraries

Oligo	Sequence	SEQ ID NO.
FR3 Reverse	CGCACAGTAATACACGGC	454
VH1a	CAATTACTATTTACAATTACAATGCAGGTKCAGCTGGTGCAGTCTG	455
VH1b	CAATTACTATTTACAATTACAATGCAGGTCCAGCTTGTGCAGTCTG	456
VH1c	CAATTACTATTTACAATTACAATGSAGGTCCAGCTGGTACAGTCTG	457
VH1d	CAATTACTATTTACAATTACAATGCARATGCAGCTGGTGCAGTCTG	458
VH2	CAATTACTATTTACAATTACAATGCAGRTCACCTTGAAGGAGTCTG	459
VH3a	CAATTACTATTTACAATTACAATGGARGTGCAGCTGGTGGAGTCTG	460
VH3b	CAATTACTATTTACAATTACAATGCAGGTGCAGCTGGTGGAGTCTG	461
VH3c	CAATTACTATTTACAATTACAATGGAGGTGCAGCTGTTGGAGTCTG	462
VH4a	CAATTACTATTTACAATTACAATGCAGSTGCAGCTGCAGGAG	463
VH4b	CAATTACTATTTACAATTACAATGCAGGTGCAGCTACAGCAGTGG	464
VH5	CAATTACTATTTACAATTACAATGGARGTGCAGCTGGTGCAGTCTG	465
VH6	CAATTACTATTTACAATTACAATGCAGGTACAGCTGCAGCAGTCAG	466
VH7	CAATTACTATTTACAATTACAATGCAGGTGCAGCTGGTGCAATCTG	467
T7TMVUTR	TAATACGACTCACTATAGGGACAATTACTATTTACAATTACA	468
XB1511 FR3CDR3FR4 Reverse	TGAGGAGACGGTGACCAGGGTTCCCTGGCCCCAGTAGCTCCTGTCG CCCCCATGTKTCGCACAGTAATACACGGC	469
FR4 Cu3 Reverse	GGAGACGAGGGGGAAAAGGGTTGAGGAGACGGTGACCAG	470
Y109	TTTTTTTTTTTTTTTTTTTTAAATAGCGGATGCTAAGGACGACTTG TCGTCGTCGTCCTTGTAGTCGGAGACGAGGGGGAAAAGGGT	471

B. Library Screening

The HCDR3 shuffled VH domain library was then transcribed into an mRNA library and subjected to selection with dsDNA display technology as set forth in WO2010/011944. The selection was carried out with human and mouse PDGFRβ at alternate round for 4 rounds. Kinetic controlled on- and off-rate selection was applied at successive rounds to increase the stringency of selection, and thus select for VH domains with high affinity for PDGFRβ. Specifically, selection was performed as follows: Round 1 (R1) with 10 nM of immobilized human PDGFRβ; R2 with immobilized 100 nM mouse PDGFRβ; R3 with 10 nM soluble human PDGFRβ and competed with 200 nM immobilized human PDGFRβ for 24 hours and 120 hours; and R4 with 10 nM mouse PDGFRβ. The R4 binding pool was subcloned for DNA sequencing. Analysis of the sequences of the R4 binding pool showed that the HCDR3 of XB1511 was present in a variety of different framework contexts. No wild type

parental sequence was obtained from the set of sequences analyzed. The amino acid sequences of the selected VH domains are set forth in Table 3, herein.

C. Binding Specificity of Selected HCDR3 Shuffled VH domains

The R4 binding pool selected above was assessed for binding to both human and mouse PDGFRβ using a ^{35}S Met-labelled i*n vitro* translated library. Specifically, binding of the pool to epoxy beads, 100 nM of human IgG, human PDGFRβ and mouse PDGFRβ were assessed. As shown in Figure 2, the parental XB1511 VH domain showed specific binding to human PDGFRβ, and undetectable binding to mouse PDGFRβ. The framework shuffled pre-selected library showed weak binding to human PDGFRβ. However, in contrast, the R4 framework shuffled library showed significant binding to both human and mouse PDGFRβ.

Example 3. Identification of stable VL/VH pairs

A. Construction of VL DNA libraries

Human VL libraries (Vkappa and Vlamda) were constructed from B cells of young healthy donors (Allcells) by RT-PCR. To ensure the diversity of the library, 300 million bone marrow mononuclear cells and 100 million peripheral blood mononuclear cells were obtained from ten donors and used for naive VH and VL library construction. A schematic of the library generation method is set forth in Figure 3.

Oligonucleotide primers for cDNA synthesis and subsequent PCR amplification of the Vkappa and Vlamda sequences were designed as set forth in Table 4. Specifically, multiple sense primers were designed from the Vκ and Vλ FR1 regions of each family with an upstream UTR sequence. The anti-sense primers for κ and λ gene amplification were designed from the constant regions nested to Cκ1 (Cκ2) or Jλ with the same Cκ2 downstream (JλCκ2). The Vκ and Vλ libraries carry the same C-terminal sequence for PCR amplification during the selection cycles.

mRNA was prepared from individual donors using a FastTrack mRNA preparation kit (Invitrogen) following the protocol provided by the kit. First strand cDNA was synthesized from the isolated mRNA using primers specific for the light chain kappa and lambda constant regions (Cκ1 and Cλ1).

PCR amplification of the Vkappa and Vlamda sequences was performed with Cκ2 and Vκ family specific or JλCκ2 mix and Vλ family specific primers using cDNA

as a template. The PCR was performed for individual Vκ and Vλ families and individual donors for 18-20 cycles. After gel purification, Vκ and Vλ libraries from each different source were pooled to generate the final Vκ and Vλ libraries.

Table 6. Oligonucleotides for constructing human Vλ and Vκ DNA display libraries

Oligo	Sequence	SEQ ID NO.
Ck1	CAACTGCTCATCAGATGGCGG	472
Cl1	CAGTGTGGCCTTGTTGGCTTG	473
Ck2	AGATGGTGCAGCCACAGTTCG	474
Jl1-3Ck2	AGATGGTGCAGCCACAGTTCGTAGACGGTSASCTTGGTCCC	475
Jl7Ck2	AGATGGTGCAGCCACAGTTCGGAGACGGTCAGCTGGGTGCC	476
T7TMVUTR	TAATACGACTCACTATAGGGACAATTACTATTTACAATTACA	477
Vλ oligos		
UTRVk1a	CAATTACTATTTACAATTACAATGRACATCCAGATGACCCAG	478
UTRVk1b	CAATTACTATTTACAATTACAATGGMCATCCAGTTGACCCAG	479
UTRVk1c	CAATTACTATTTACAATTACAATGGCCATCCRGATGACCCAG	480
UTRVk1d	CAATTACTATTTACAATTACAATGGTCATCTGGATGACCCAG	481
UTRVk2a	CAATTACTATTTACAATTACAATGGATATTGTGATGACCCAG	482
UTRVk2b	CAATTACTATTTACAATTACAATGGATRTTGTGATGACTCAG	483
UTRVk3a	CAATTACTATTTACAATTACAATGGAAATTGTGTTGACRCAG	484
UTRVk3b	CAATTACTATTTACAATTACAATGGAAATAGTGATGACGCAG	485
UTRVk3c	CAATTACTATTTACAATTACAATGGAAATTGTAATGACACAG	486
UTRVk4a	CAATTACTATTTACAATTACAATGGACATCGTGATGACCCAG	487
UTRVk5a	CAATTACTATTTACAATTACAATGGAAACGACACTCACGCAG	488
UTRVk6a	CAATTACTATTTACAATTACAATGGAAATTGTGCTGACTCAG	489
UTRVk6b	CAATTACTATTTACAATTACAATGGATGTTGTGATGACACAG	490
Vλ oligos		
UTRVL1a	CAATTACTATTTACAATTACAATGCAGTCTGTGCTGACKCAG	491
UTRVL1b	CAATTACTATTTACAATTACAATGCAGTCTGTGYTGACGCAG	492
UTRVL2	CAATTACTATTTACAATTACAATGCAGTCTGCCCTGACTCAG	493
UTRVL3a	CAATTACTATTTACAATTACAATGTCCTATGWGCTGACTCAG	494
UTRVL3b	CAATTACTATTTACAATTACAATGTCCTATGAGCTGACACAG	495
UTRVL3c	CAATTACTATTTACAATTACAATGTCTTCTGAGCTGACTCAG	496
UTRVL3d	CAATTACTATTTACAATTACAATGTCCTATGAGCTGATGCAG	497
UTRVL4	CAATTACTATTTACAATTACAATGCAGCYTGTGCTGACTCAA	498
UTRVL5	CAATTACTATTTACAATTACAATGCAGSCTGTGCTGACTCAG	499
UTRVL6	CAATTACTATTTACAATTACAATGAATTTTATGCTGACTCAG	500

UTRVL7	CAATTACTATTTACAATTACAATGCAGRCTGTGGTGACTCAG	501
UTRVL8	CAATTACTATTTACAATTACAATGCAGACTGTGGTGACCCAG	502
UTRVL4/9	CAATTACTATTTACAATTACAATGCWGCCTGTGCTGACTCAG	503
UTRVL10	CAATTACTATTTACAATTACAATGCAGGCAGGGCTGACTCAG	504
	R = A/G, Y = C/T, K = G/T, M = A/C, S = G/C, W = A/T	

B. Generation of VL fusion libraries by dsDNA Display

Vκ and Vλ DNA libraries generated using the methods set forth in this Example were transcribed into mRNA libraries using the T7 Megascript kit (Invitrogen, Cat# AM1334). The mRNA was purified with RNeasy MinElute Cleanup Kit (Qiagen, Cat# 74204) following protocol provided by the kit. A total of 600 pmol of RNA (300 pmol of Vκ and Vλ libraries) was ligated and assembled with dsDNA display linkers and components as described in WO2010/011944. The assembled VL library was subjected to *in vitro* translation to create a fusion library in which each VL domain (phenotype) is stably fused to its coding sequence (genotype). ^{35}S Met was incorporated in the translation process to radiolabel the fusions. The library was then purified with oligo dT cellulose, converted into a dsDNA display library using the standard molecular biology techniques of reverse transcription, RNaseH digestion, 2nd strand DNA synthesis, followed by flag tag purification.

C. Identification of VL Pairs for XB1511, and XB2202 VH domains

XB1511 VH domain was translated as free protein (with incorporation of ^{35}S Met in the translation reaction) and affinity purified through a c-terminal flag tag. The XB1511 VH domain and a purified VL domain fusion library (prepared as above) were then mixed at an equal molar ratio and incubated at 25C overnight to allow for *in vitro* association of VH and VL fusion domains through their hydrophobic patch. The mixture was then contacted with PDGFRβ target pre-immobilized on Epoxy450 beads or in solution and captured by protein A beads, Complexes that bound to the immobilized PDGFRβ target were washed and eluted with 0.1N KOH. PCR was performed with VL specific primer sets to recover the VLs that bound to the PDGFRβ target, both as VH-VL pairs and as unpaired VL domains. The VL pairing was performed for 3 rounds, with low stringency (100 nM PDGFRβ) for the first 2 rounds and higher stringency (10 nM PDGFRβ) for the third round. The XB2202 VH domain

was also paired with the VL library similarly for two rounds. For each round of XB2202/VL pairing and selection, the stringency was increased by kinetic controlled on and off rate strategy to identify VL domains that paired stably with XB2202 VH domain and enhance the VH binding.

VL domain pools identified above were then cloned into Blunt Zero TOPO vector (Invitrogen) and VL-encoding DNA sequences were amplified from the resultant bacterial colonies by PCR using M13 forward and reverse primers. The individual amplified VL-encoding DNA sequences were then sequenced. The sequence data obtained from VL pools showed that a diverse repertoire of VLs was enriched through the process. Multiple families and frameworks were present in the pool. Several VLs were present as replicates or families. Distinct VL families could be identified and several VLs were present more than once. Exemplary VL sequences identified using the methods of the invention that pair with the PDGFRβ-binding VH domains XB1511 and XB2202 are set forth in Table 4 herein.

D. Evaluation of Identified VH and VL Pairs

To evaluate the characteristics of the identified VH-VL pairs, 10-12 scFVs from each pool were constructed and produced by either *in vitro* translation or by *E.coli* expression, followed by affinity purification.

A PDGFRβ binding ELISA assay was performed to assess the binding of the scFv to immobilized PDGFRβ and to determine the EC50. Specifically, 2 ug/mL of human PDGFRβ and human Fc or IgG in PBS was immobilized on Maxisorp plates at $4^{O}C$ overnight. The plate was then washed and blocked with superblock. *In vitro* translated crude scFv lysate was diluted 1:3 in 1X PBST. 100 ul of the diluted scFv lysate was loaded into each well of Maxisorp plates and incubated for 1 hour at room temperature. scFv that bound to immobilized PDGFRβ was detected by anti-flag antibody-HRP at 1:5000 dilution and a TMB substrate. The plate was read on a Molecular Device plate reader with end point assay at OD 450 nm. As shown in Figures 4, 5 and 6, in the ELISA binding assay, greater than 50% of the scFvs generated for XB1511 and XB2202 showed specific binding to PDGFRβ. In contrast, the unpaired VLs alone did not show binding to PDGFRβ (see Figure 7).

The affinity of several scFvs was determined by solution based equilibrium binding assay. Specifically, 120 pmol of scFv RNA was translated into free protein with

^{35}S Met incorporated. The translated reaction mixture was 3-fold diluted in binding buffer containing 1XPBS with 0.025% triton, 1mg/mL BSA and 0.1 mg/mL sssDNA. Human PDGFRβ was diluted in the same binding buffer to final concentrations from 100 nM to 0 nM. The diluted scFv mixture was incubated with hPDGFRβ in final volume of 100 ul on Kingfisher plates (Thermofisher Scientific, 97002084). Following incubation, 25 ul of protein A magnetic beads (Invitrogen) were used to capture the PDGFRβ from solution. The captured PDGFRβ was washed and eluted in kingfisher Reader (Thermofisher Scientific). The amount of scFv (labeled with ^{35}S Met) bound to the magnetic bead-immobilzed hPDGFRβ was counted using a scintillation counter and the Kd was calculated with Graph Pad Prism 5. For the XB1511-derived scFv tested, 2 scFv showed an 8-10 fold higher Kd, 1 showed 2.5 fold higher Kd, and 4 showed a similar Kd when compared to XB1511 VH alone (Figure 8). Only 1 scFv showed a lower K_D than XB1511 VH alone. As shown in Figure 9, both of the XB2202-derived scFv tested showed approximately an 8-10 fold better Kd when compared to XB2202 VH alone.

Example 4. Binding Affinity of Anti-PDGFRß VH domains to Human and Mouse PDGFRß

The R4 framework shuffled human and mouse PDGFRβ enriched VH domain pool selected in Example 2 was cloned into *E.coli* expression vectors, produced and purified. The binding kinetics of the VH domains to human and mouse PDFGR was determined using surface plasmon resonance on a Biacore T100. Briefly, human and mouse PDGFR-hIgG1-Fc chimeric fusion protein were separately immobilized using a Series CM5 sensorchip (CM5) coupled to anti-hIgG1 Fc monoclonal antibody. For each cycle, the PDGFR fusion protein was first captured, followed by the injection of VH for 115 seconds at a flow rate of 100uL/min (association). Immediately following the association phase is a dissociation phase of 600 seconds. The surface was regenerated at each cycle with a single injection of 3M MgCl2 (10uL/min, 60 seconds). Multiple concentrations of VH domain were injected (0.55nM – 40nM) and the resulting sensorgram were analyzed with T100 Evaluation software. The binding kinetics was determined using 1:1 binding curve fitting. The binding kinetics of VH domain clones XB2202 and XB2708 to human and mouse PDGFRβ are shown in Figures 10, 11 and 12, respectively. These results show that XB2202 and XB2708 have a 50 -150 fold

affinity improvement compared to parental XB1511. Specifically, XB2202 and XB2708 have Kds of 249pM and 93pM, respectively and off rates (Koff) of 1.86 $x10^{-3}$ and $9.267x10^{-4}$, respectively. Both XB2202 and XB2708 bound to human and mouse PDGFRβ. It is of particular note that, although they shared the same HCDR3, XB2202 was derived from a VH1 family germline sequence and XB2708 was derived from VH3 family germline sequence.

Example 5. Inhibition of PDGFBB Binding to PDGFRβ

The ability of the XB2202 VH domain, disclosed herein, to antagonize the binding of PDGFBB ligand to the human PDFGRb was assessed using surface plasmon resonance on a Biacore T100. Briefly, human PDGFR-hIgG1-Fc chimeric fusion protein was immobilized using a Series CM5 sensorchip coupled with anti-hIgG1 Fc monoclonal antibody. 10 nM of human PDGFBB was injected to pre-captured human PDGFRβ obtain the 100% binding response unit to PDGFRβ in the absence VH. For each successive cycle, the PDGFR fusion protein was first captured then VH domain was then injected for 120 seconds. After washing away unbound VH domain, 10 nM of PDGFBB was then injected for 120 seconds. The surface was regenerated at each cycle with a single injection of 3M MgCl2 (10uL/min, 60 seconds). Multiple concentrations of VH were injected (0.46nM – 60nM), the resulting sensorgram were analyzed with T100 Evaluation software, and the PDGFBB binding inhibition was calculated. As shown in Figure 13, XB2202 inhibits PDGFBB binding to human PDFGRb with an IC50 of less than 5nM.

Example 6. Inhibition of Pericyte Cell Migration

The ability of the XB2708 VH domain, disclosed herein, to antagonize PDGF-BB induced pericyte migration *in vitro* was determined. Primary human retinal pericytes were obtained from Cell Systems Corporation (Kirkland, WA) and cultured according to the manufacturer's suggestions using CSC full growth medium. Approximately 125 cells (2-5 passages) were seeded in each well of 384-well BIND© biosensor plates coated with human plasma fibronectin (5μg/ml in PBS) and blocked with BSA (1% in PBS) in serum-free medium containing 0.1% BSA. Cells were allowed to adhere and then serum-starve overnight. Following serum starvation, cells were incubated for 1 hour with various concentrations of VHs against human PDGFRβ receptor in a tissue culture incubator. Migration was stimulated at the end of antibody

pre-incubation by PDGF-BB addition to a final concentration of 5 ng/ml in serum-free medium. Well images were acquired using a BIND© Scanner every 18 minutes for 20 hours in a tissue culture incubator at 37°C with 5% CO_2 and >75% humidity. Collected data were analyzed using a Matlab-based centroid identification and tracking algorithm to calculate the speed of cells between the hours 10 and 16. The results set forth in Figure 14 show that XB2708 can antagonize PDGF-BB-induced pericyte migration with an IC50 of 0.54nM.

Example 7. Conversion of VH-VL Pairs to Heterotetrameric IgG and Demonstration of Biological Activity

XB1511 VH and D8 VL were expressed together in a heterotetrameric IgG in 293T cells. Cell culture supernatant was collected after 48 hours and 96 hours and the expressed IgG was purified with protein A agarose beads. The IgG was produced at 8 mg/L without any optimization. To evaluate the biological activity of the XB1511/D8 IgG, HFF-1 human foreskin fibroblasts were seeded in 384-well BIND biosensors and allowed to attach overnight in serum-free media. The fibroblast cells were then stimulated with 5 ng/mL or 10 ng/mL of PDGFBB ligand and allowed to migrate for 18 hours in the presence or absence of 100nM XB1511/D8 IgG. BIND Scanner images were captured every 15 minutes and software analysis tools used to measure the track lengths of individual cell migration responses. Track length is represented by a "heat map" from blue (no migration) to red (maximal migration). As shown in Figure 15, the XB1511/D8 IgG was able to completely block the PDGFBB-induced migration of human fibroblasts.

Example 8. scFv Thermostability

The thermostability of the XB2202 VH and XB2202/A4 scFv were determined. Specifically, 1 mg/mL of XB2202 and XB2202-A4 were incubated at 4°C, 37°C, 60°C and 70°C for 12 hours and a PDGFRβ binding ELISA was performed to test the binding activity of the protein after incubation. As shown in Figure 16, the XB2202 VH domain lost significant PDGFRβ binding activity after incubation at 60°C and completely lost binding activity after incubation at 70°C. The Tm of XB2202 was measured to be approximately 62°C. In contrast, the XB2202/A4 scFv was completely active after 12

hour incubation at 70°C, indicating that the Tm of the XB2202 scFv was greater than 70°C.

Example 9. Expression, Purification and Concentration of IgG1 Antibodies

XB1511/D8 and XB2202/A4 VH/VL pairs were separately expressed as full-length heterotetrameric IgG1 antibodies in 293T cells and purified. The amino acid sequences of the heavy and light chains of XB1511/D8 and XB2202/A4 IgG1 antibodies are set forth in Table 7, herein.

Cell culture supernatants were obtained by filtration and expressed antibodies purified using a two-step purification scheme. Specifically, Protein A affinity purification was performed, with antibody bound elution at pH3.5. The pH of the Protein A eluate was adjusted to pH 7 using 1M Tris, and purified further by ion exchange chromatography using a HiTrap Q XL column (GE Healthcare). The purified antibody was stored in PBS at pH7.

Table 7. Amino acid sequences of XB1511/D8 and XB2202/A4 VH/VL pairs formatted as full-length heterotetrameric IgG1 antibodies.

Antibody chain	Amino Acid Sequence (Signal sequences underlined)	SEQ ID NO.
XB1511 IgG1	GWSLILLFLVAVATRVLSQVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAIS WVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRS EDTAVYYCAIHGGDRSYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS RDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	505
D8 Ckappa	DFQVQIISFLLISASVIMSRGEIVMTQSPGTLTLSPGEGATLSCRASQSVTSN YLAWYQQRPGQAPRLLIYDASNRATGIPDRFSGSGFGTDFTLTISRLEPEDFA VYYCQQYVNSRTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTKVEIKRTVA APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT EQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	506
XB2202 IgG1	GWSLILLFLVAVATRVLSQVQLVQSGAEVKKPGSSVRVSCKASGGTFSRHAIS WVRQAPGQGLEWIGGILPILKTPNYAQRFQGRVTINADESTSTVYMEMSSLRS EDTAVYYCATHGGDRSYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS RDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	507
A4	DFQVQIISFLLISASVIMSRGDVVMTQSPSSLSASVGDRVTITCQASQDISNW LNWYQQKPGKAPKLLIYEASNLETGVPSRFSGSGSGTDFTFTISSLQPEDIAT	508

Ckappa	YYCQQYNNVLRTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTKVEIKRTVA APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT EQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	

The antibody expression level and the antibody concentration after each purification step was determined by measuring the A_{280} of the antibody solution. The purity and quality of the purified antibodies was determined by size-exclusion high-performance liquid chromatography (SEC-HPLC). The results of these experiments are set forth in Table 8, herein. These data show that when XB1511/D8 and XB2202/A4 VH/VL pairs are formatted as full-length heterotetrameric IgG1 antibodies, the resultant antibodies are highly manufacturable in that they are expressed at high levels, are easily purified to a high purity, and exhibit little aggregation.

Table 8. Analysis of expression and purification of XB1511/D8 and XB2202/A4 IgG1 antibodies.

Antibody	Culture volume	Antibody Expression level	Amount of antibody after protein A purification	Amount of antibody after ion-exchange purification	Antibody % recovery after 2 step purification	Antibody % purity	% antibody aggregates
XB1511/D8 IgG1	2.0L	24mg/L	46mg	44mg	97.8%	95.8%	2.4%
XB2202/A4 IgG1	1.4L	47mg/L	62mg	54mg	87%	96.9%	2.5%

The purified XB1511/D8 and XB2202/A4 IgG1 antibodies were further analysed for their ability to be concentrated. Specifically, solutions of each antibody were concentrated to 50mg/ml using centricon ultra filtration spin columns with 10kDa and 30kDa cut-off limits. The integrity of the concentrated solution was analyzed by SEC-HPLC. From this analysis it was determined that the 50mg/ml solution of XB1511/D8 IgG1 had a purity of about 96% and contained about 2.4% of antibody aggregates, whilst the 50mg/ml solution of XB2202/A4 IgG1 had a purity of about 97.8% and contained about 2.2% of antibody aggregates. These data demonstrate that XB1511/D8 and XB2202/A4 IgG1 antibodies are highly stable in a concentrated solution.

Example 10. Thermostability of XB1511/D8 and XB2202/A4 IgG1 Antibodies

The thermostability of XB1511/D8 and XB2202/A4 IgG1 antibodies were determined using a fluorescence based assay. Specifically, 5mg/ml of purified

XB1511/D8 IgG1, XB2202/A4 IgG1, or human IgG1 control were mixed with Sypro orange dye (Sigma) and the temperature of the mixture increased in 1 degree increments from 25°C to 95°C. The Sypro orange dye incorporates into the IgG when the temperature increases and the IgG unfolds. The fluorescent signal produced by the association of Sypro orange dye with the IgGs was monitored using a BioRad CFX96 instrument. In this assay, the negative regression of the Sypro orange signal was used to identify the peak melting (i.e. T_m) point for each protein.

From this analysis it was determined that XB1511/D8 and XB2202/A4 have melting temperatures (T_m) of 67°C to 70°C, respectively. This compared well to the human IgG1 control antibody which exhibited a T_m of 72°C. This data demonstrate that the VH and VH/VL pairs of the invention are capable of being formatted into highly thermostable full-length IgG molecules.

Example 11. Binding Affinities of XB2202 VH, scFv and IgG1 antibodies to Human

The binding kinetics of XB2202 VH domain, XB2202/A4 scFv and XB2202/A4 IgG1 to human PDFGR were determined using surface plasmon resonance on a Biacore T100. Briefly, recombinant human PDGFR-hIgG1-Fc chimeric fusion protein (R&D, #385-PR-100/CF) was immobilized on a Series CM5 sensorchip coupled with anti-hIgG1 Fc monoclonal antibody(for the VH and ScFv assays) or anti-6His antibody (for the IgG assay). XB2202 VH domain, XB2202/A4 scFv and XB2202/A4 IgG1 were flown over the surface at 50 or 100ul/min for 3 min at different concentrations (75, 50, 25, 10, 5, and 1 nM) and allowed to dissociate for 10 min. The data was analyzed using the Biacore T100 analysis software using a 1:1 model. Mass transport was checked and avoided to allow accurate measurements. All data was double referenced according to Biacore standard protocol.

The binding kinetics of XB2202 VH domain, XB2202/A4 scFv and XB2202/A4 IgG1 antibodies to human PDGFRβ are shown in Table 9, herein. These data show that XB2202 VH domain, XB2202/A4 scFv and XB2202/A4 IgG1each have a high binding affinity for PDGFRβ. It is of particular note that XB2202/A4 scFv and XB2202/A4 IgG1 exhibit an improved off-rate ($1.54x10^{-3}$ s^{-1} and $1.56x10^{-3}$ s^{-1}, respectively) compared to that of the unpaired XB2202 VH domain alone ($2.95x10^{-3}$ s^{-1}).

Table 9. Binding Kinetics of XB2202 VH domain, XB2202/A4 scFv and XB2202/A4 IgG1 to human PDGFRβ

Antibody	On-rate ($M^{-1}s^{-1}$)	Off-rate (s^{-1})	Kd (M)
XB2202 VH	$1.30x10^{7}$	$2.95x10^{-3}$	$2.27x10^{-10}$
XB2202/A4 ScFv	$7.06x10^{5}$	$1.54x10^{-3}$	$2.18x10^{-9}$
XB2202/A4 IgG1	$9.80x10^{5}$	$1.56x10^{-3}$	$1.59x10^{-9}$

Example 12. Functional Analysis of Anti-PDGFRβ Antibodies Using *in vivo* Mouse Models

The ability of the anti-PDGFRβ antibodies disclosed herein to inhibit PDGF-induced vascularization *in vivo* is evaluated using the developing retina vasculature model, the corneal neovascularization model, and/or the choroidal neovascularization model described in Nobuo et al. Am. J. Path, (2006) 168(6), 2036-2052 (which is incorporated by reference herein in its entirety). In these assays antibodies are administered to mice as VH domains, scFv, and/or full length IgG.

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 step or element or integer or group of steps or elements or integers but not the exclusion of any other step or element or integer or group of elements or integers.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present disclosure as it existed before the priority date of each of the appended claims.

2012347972 26 Apr 2018

CLAIMS:

1. An isolated binding polypeptide comprising a variable heavy (VH) domain that specifically binds to PDGFRβ, wherein the VH domain comprises CDR3, CDR2, and CDR1 amino acid sequences, wherein the CDR3 comprises the amino acid sequence set forth in SEQ ID NO: 1, the CDR2 comprises the amino acid sequence set forth in SEQ ID NO: 3, and the CDR1 comprises the amino acid sequence set forth in SEQ ID NO: 35.

2. An isolated binding polypeptide comprising a variable heavy (VH) domain that specifically binds to PDGFRβ, wherein the VH domain comprises CDR3, CDR2, and CDR1 amino acid sequences, wherein the CDR3 comprises the amino acid sequence set forth in SEQ ID NO: 1, the CDR2 comprises the amino acid sequence set forth in SEQ ID NO: 25, and the CDR1 comprises the amino acid sequence set forth in SEQ ID NO: 46.

3. The binding polypeptide of claim 1 or 2, wherein the VH domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 321-324, 345, and 360.

4. The binding polypeptide of any one of the preceding claims, further, comprising a variable light (VL) domain comprising a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 63-147, a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 148-232, and a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 233-317.

5. The binding polypeptide of claim 4, wherein the VL domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 369-453.

6. The binding polypeptide of any one of the preceding claims, which inhibits the activity of PDGFRβ.

7. The binding polypeptide of claim 6, wherein the activity of PDGFRβ is inhibited by antagonizing PDGF binding to PDGFRβ.

8. The binding polypeptide of claim 6, wherein the activity of PDGFRβ is inhibited by antagonizing PDGFRβ dimerization.

9. The binding polypeptide of any one of the preceding claims, which binds to PDGFRβ with a Kd of less than 250pM.

10. The binding polypeptide of any one of the preceding claims, which binds to PDGFRβ with a Kd of less than 100pM.

11. The binding polypeptide of any one of the preceding claims, which binds to PDGFRβ with an off-rate of less than 10^{-3} s^{-1}.

12. The binding polypeptide of any one of the preceding claims, which binds specifically to mouse and human PDGFRβ.

13. The binding polypeptide of any one of the preceding claims, which antagonizes PDGF binding to the PDGFRβ with an IC50 of less than 5nM.

14. The binding polypeptide of any one of the preceding claims, which inhibits ligand induced tyrosine phosphorylation of PDGFRβ with an IC50 of less than 4nM.

15. The binding polypeptide of any one of the preceding claims, which inhibits retinal pericyte migration with an IC50 of less than 6nM.

16. The binding polypeptide of any one of the preceding claims, comprising a VH domain with a melting temperature (Tm) of at least 68°C.

17. The binding polypeptide of any one of the preceding claims, which is an antibody.

18. The binding polypeptide of any one of the preceding claims, which is an scFv.

19. A recombinant expression vector comprising an isolated nucleic acid encoding the binding polypeptide of any one of the preceding claims.

20. A host cell comprising the recombinant expression vector of claim 19.

21. A method of producing a binding polypeptide that binds specifically to human PDGFRβ, comprising culturing the host cell of claim 20 under conditions such that a binding polypeptide that binds specifically to human PDGFRβ is produced by the host cell.

22. A pharmaceutical composition comprising the binding polypeptide of any one of claims 1 to 18 and one or more pharmaceutically acceptable carrier.

23. A method for treating a PDGFRβ-associated disease or disorder, the method comprising administering to a subject in need of thereof the pharmaceutical composition claim 22.

24. The method of claim 23, wherein the disease or disorder is age-related macular degeneration (AMD) or cancer.

25. Use of a binding polypeptide of any one of claims 1 to 18 in the manufacture of a medicament for treating a PDGFRβ-associated disease or disorder.

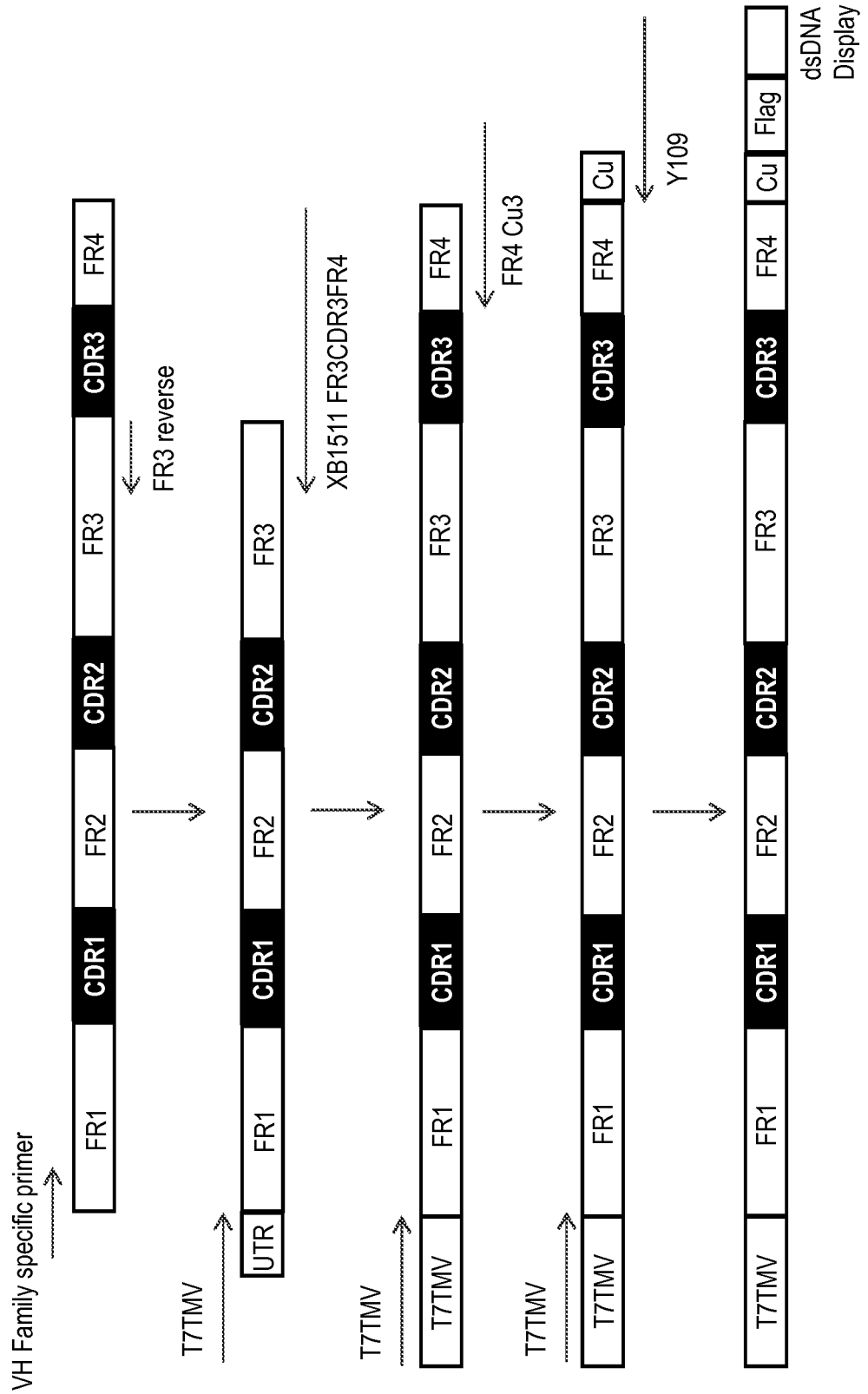

VH Family specific primer
FR1
CDR1
FR2
CDR2
FR3
CDR3
FR4
FR3 reverse
T7TMV
UTR
XB1511 FR3CDR3FR4
T7TMV
FR4 Cu3
T7TMV
Y109
Cu
dsDNA Display
Flag

Fig. 1

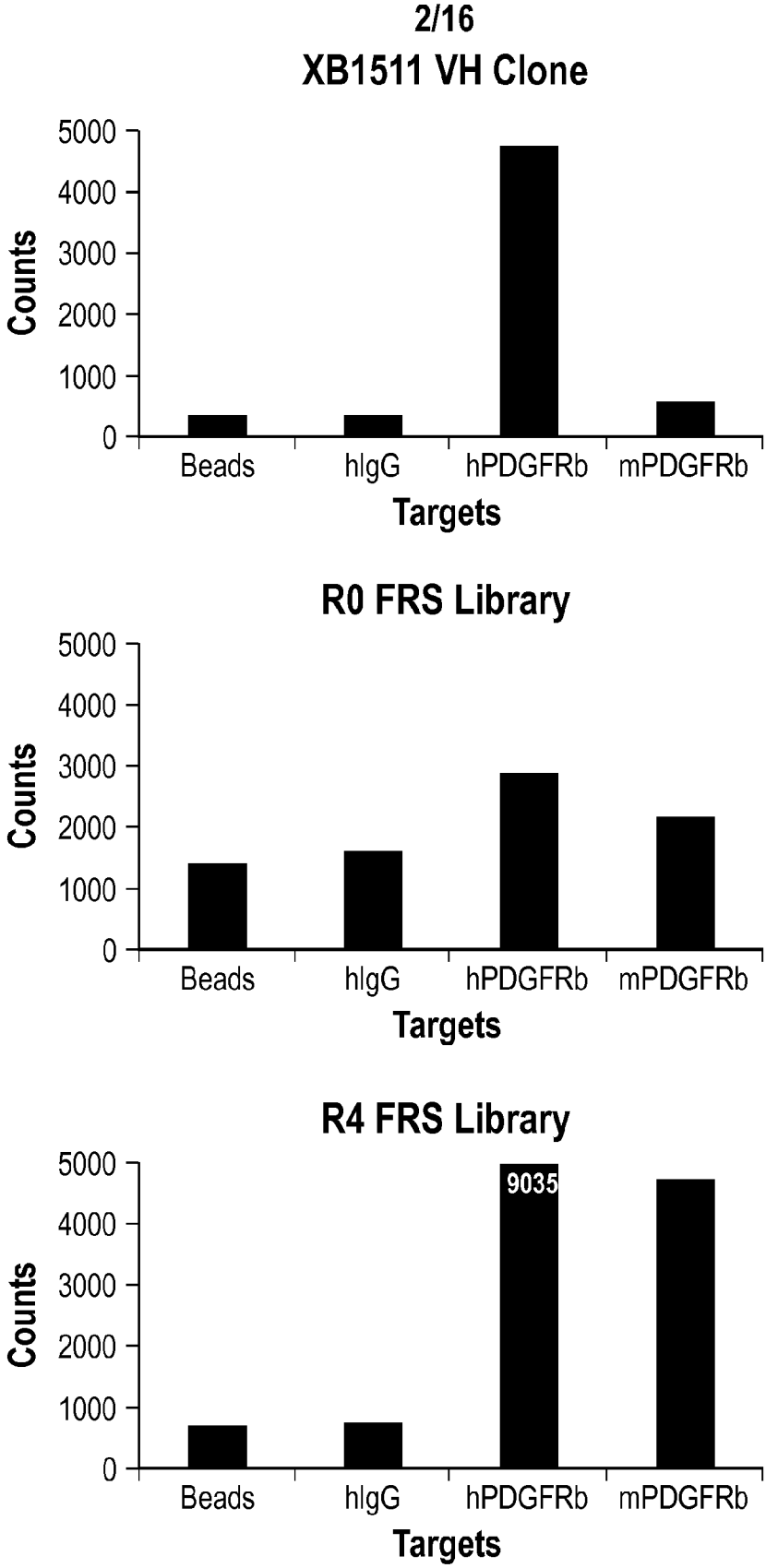

XB1511 VH Clone
Counts
5000
4000
3000
2000
1000
0
Beads
hIgG
hPDGFRb
mPDGFRb
Targets
R0 FRS Library
Counts
5000
4000
3000
2000
1000
0
Beads
hIgG
hPDGFRb
mPDGFRb
Targets
R4 FRS Library
Counts
5000
4000
3000
2000
1000
0
9035
Beads
hIgG
hPDGFRb
mPDGFRb
Targets

Fig. 2

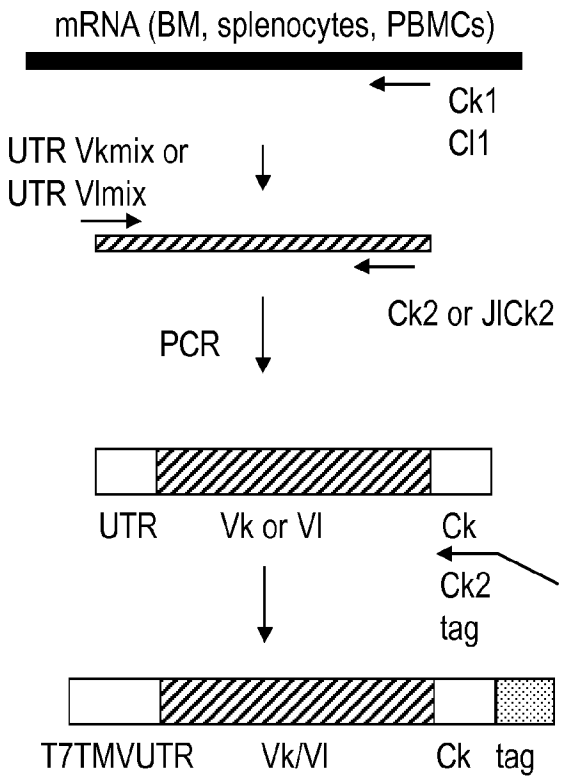

mRNA (BM, splenocytes, PBMCs)
Ck1
Cl1
UTR Vkmix or
UTR Vlmix
Ck2 or JlCk2
PCR
UTR
Vk or Vl
Ck
Ck2
tag
T7TMVUTR
Vk/Vl
Ck
tag

Fig. 3

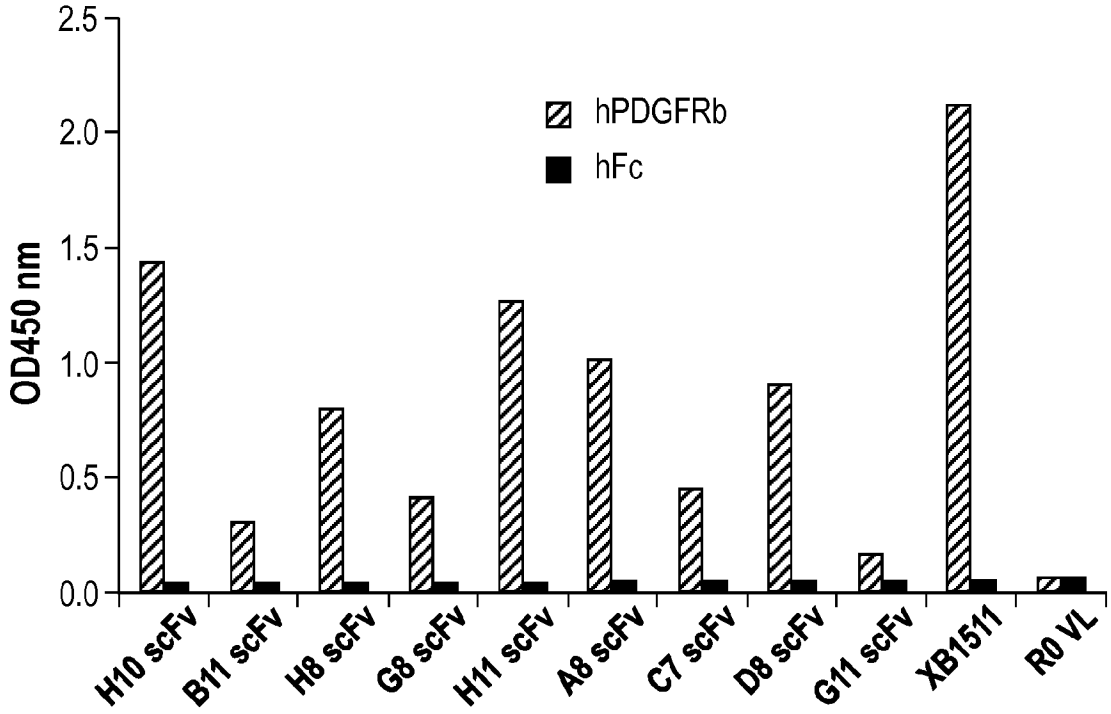

2.5
2.0
1.5
1.0
0.5
0.0
OD450 nm
hPDGFRb
hFc
H10 scFv
B11 scFv
H8 scFv
G8 scFv
H11 scFv
A8 scFv
C7 scFv
D8 scFv
G11 scFv
XB1511
R0 VL

Fig. 4

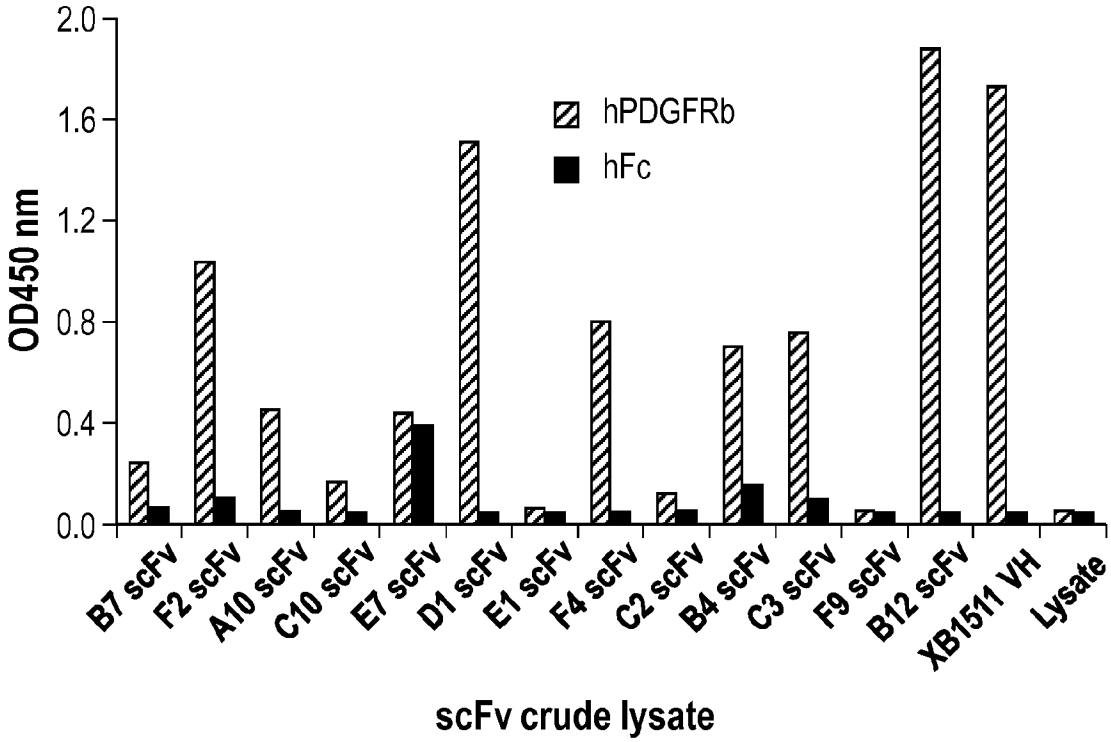

2.0
1.6
1.2
0.8
0.4
0.0
OD450 nm
hPDGFRb
hFc
B7 scFv
F2 scFv
A10 scFv
C10 scFv
E7 scFv
D1 scFv
E1 scFv
F4 scFv
C2 scFv
B4 scFv
C3 scFv
F9 scFv
B12 scFv
XB1511 VH
Lysate
scFv crude lysate

Fig. 5

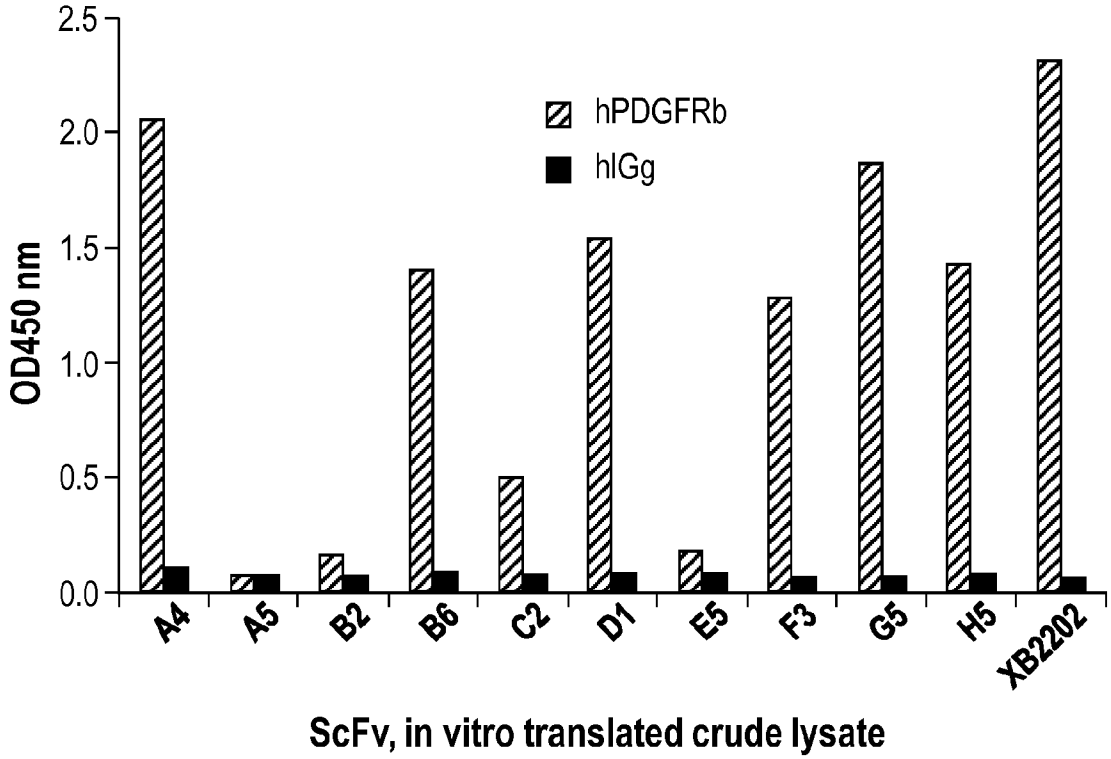

2.5
2.0
1.5
1.0
0.5
0.0
OD450 nm
hPDGFRb
hIGg
A4
A5
B2
B6
C2
D1
E5
F3
G5
H5
XB2202
ScFv, in vitro translated crude lysate

Fig. 6

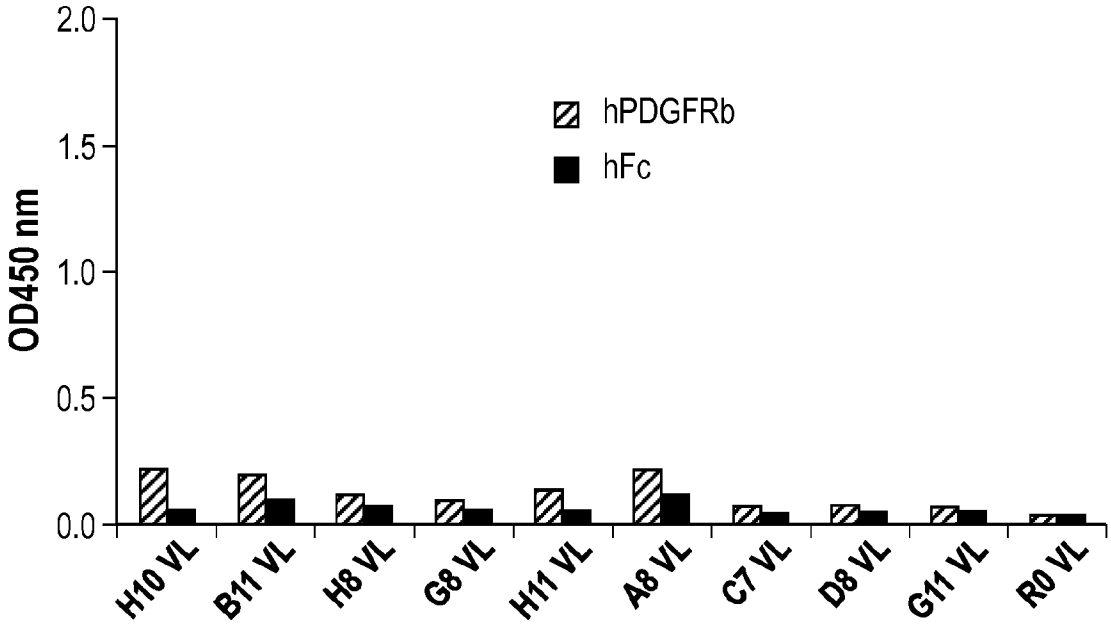
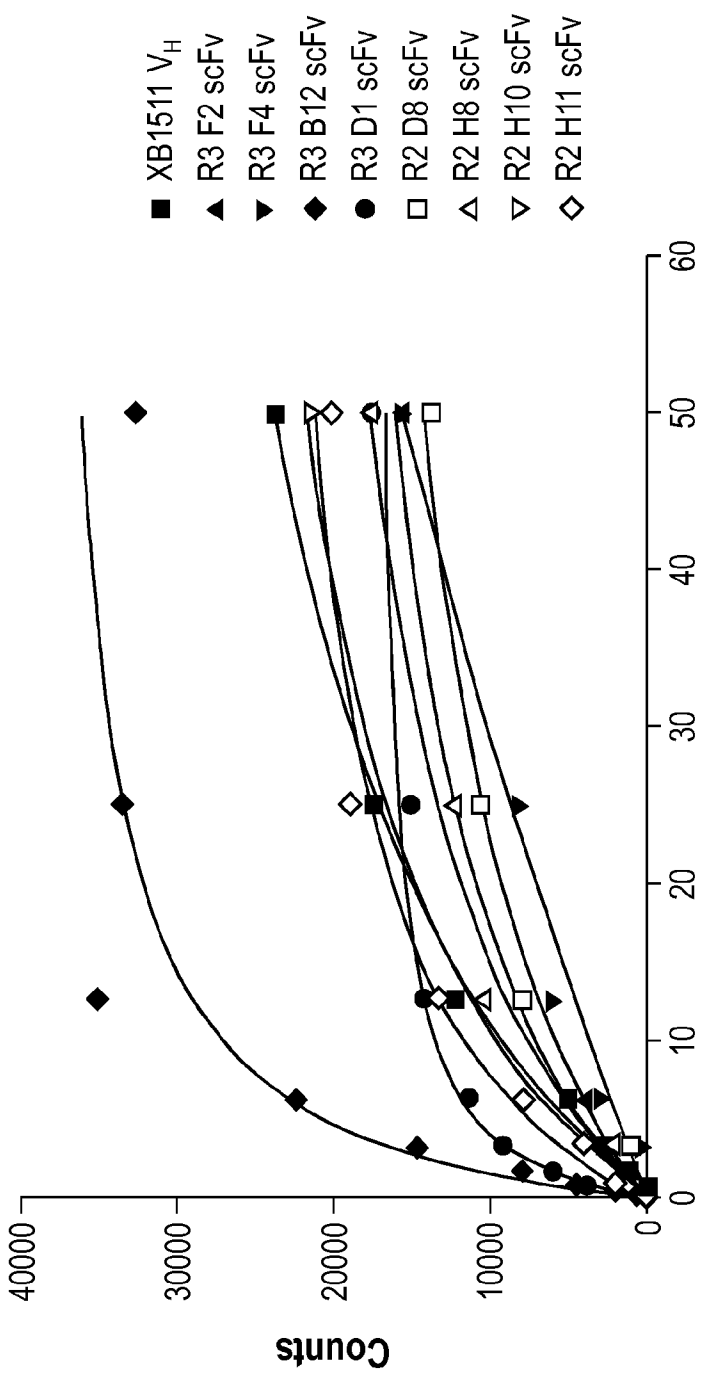

2.0
1.5
1.0
0.5
0.0
OD450 nm
hPDGFRb
hFc
H10 VL
B11 VL
H8 VL
G8 VL
H11 VL
A8 VL
C7 VL
D8 VL
G11 VL
R0 VL

Fig. 7



	XB1511 V_H	R3 F2 scFv	R3 F4 scFv	R3 B12 scFv	R3 D1 scFv	R2 D8 scFv	R2 H8 scFv	R2 H10 scFv	R2 H11 scFv
K_D (nM)	31.88	23.12	223.5	4.579	3.155	28.32	24.45	23.2	12.86

Fig. 8

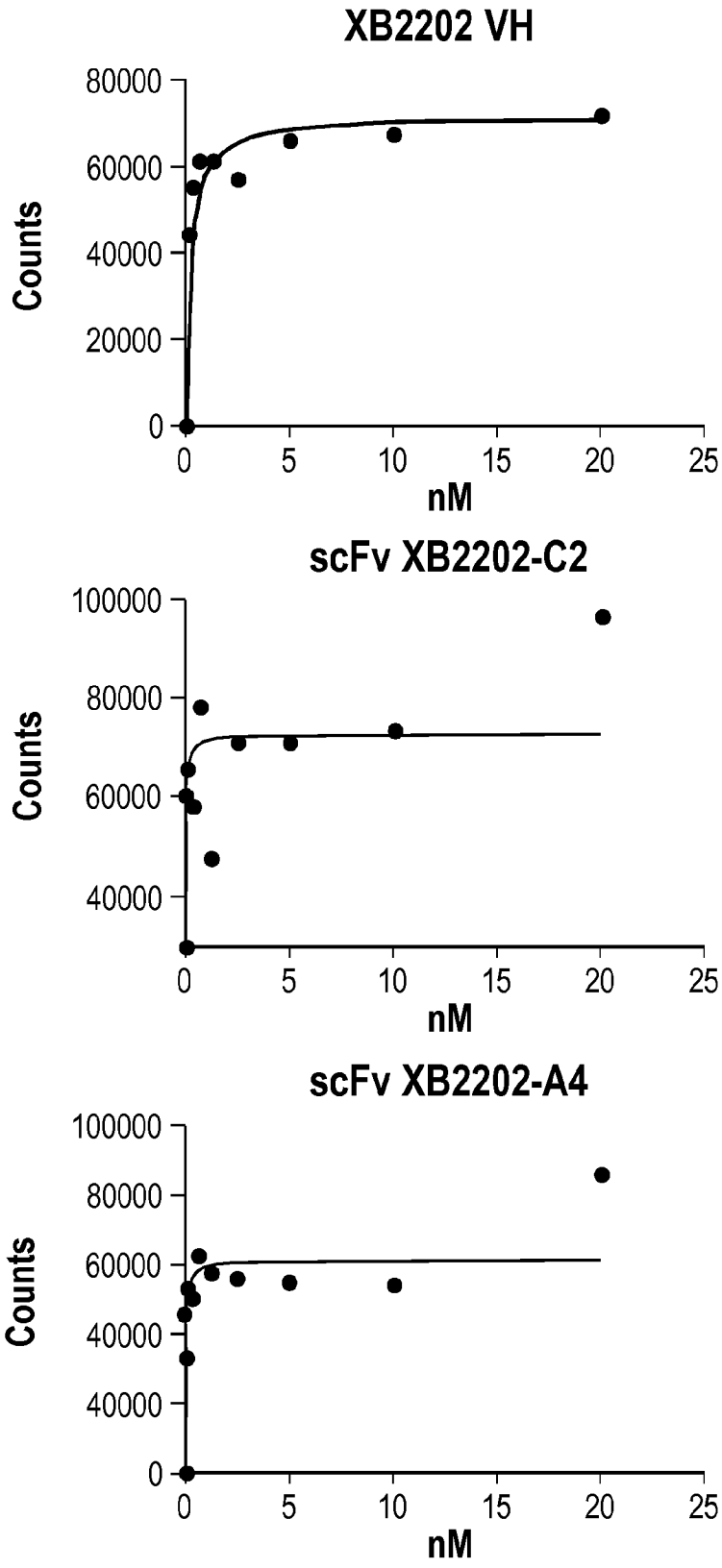

XB2202 VH
Counts
80000
60000
40000
20000
0
0
5
10
15
20
25
nM
scFv XB2202-C2
Counts
100000
80000
60000
40000
0
5
10
15
20
25
nM
scFv XB2202-A4
Counts
100000
80000
60000
40000
40000
0
5
10
15
20
25
nM

Fig. 9

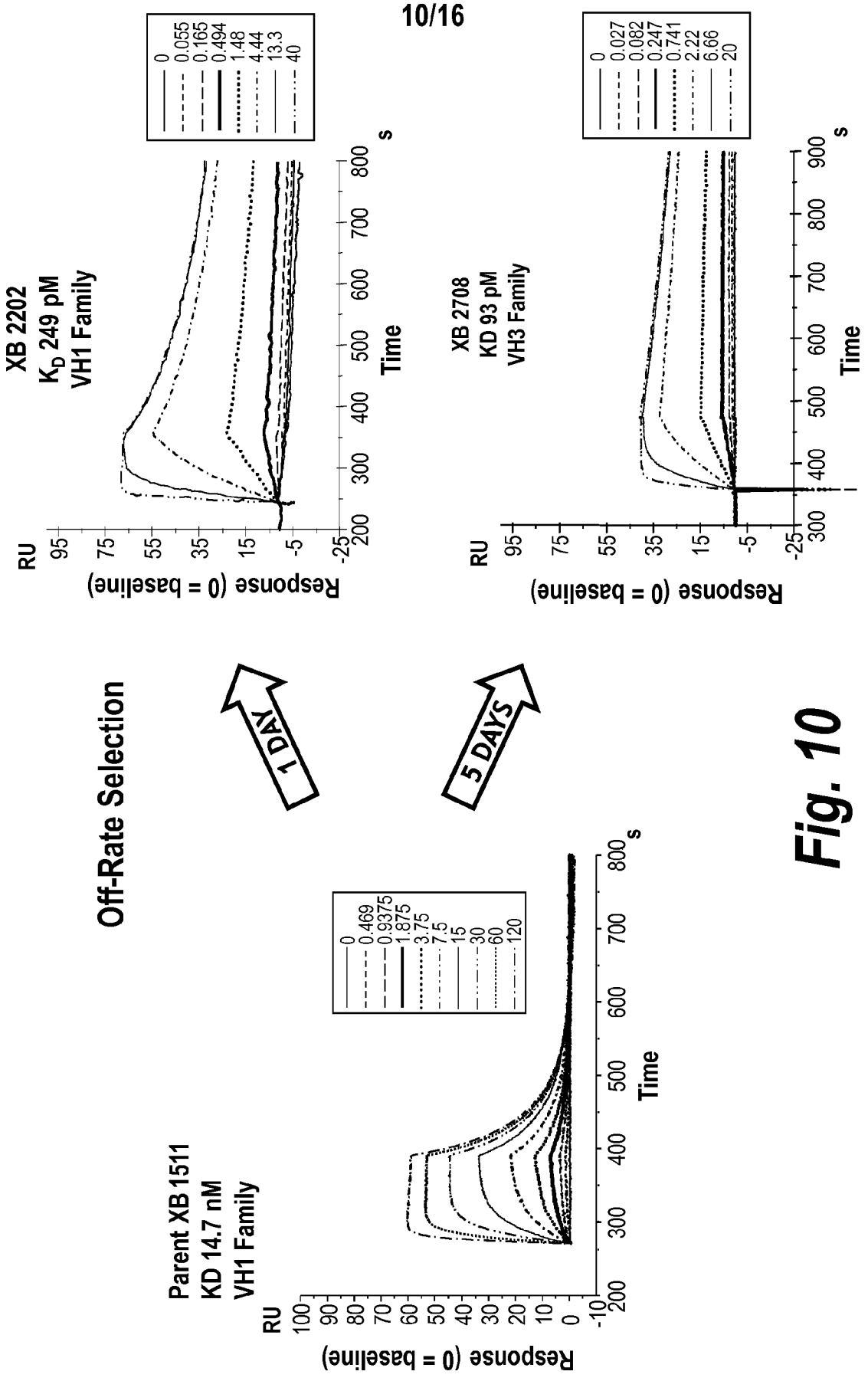

XB 2202
K_D 249 pM
VH1 Family
RU
Response (0 = baseline)
Time
s
0
0.055
0.165
0.494
1.48
4.44
13.3
40
XB 2708
KD 93 pM
VH3 Family
0
0.027
0.082
0.247
0.741
2.22
6.66
20
Off-Rate Selection
1 DAY
5 DAYS
Parent XB 1511
KD 14.7 nM
VH1 Family
0
0.469
0.9375
1.875
3.75
7.5
15
30
60
120

Fig. 10

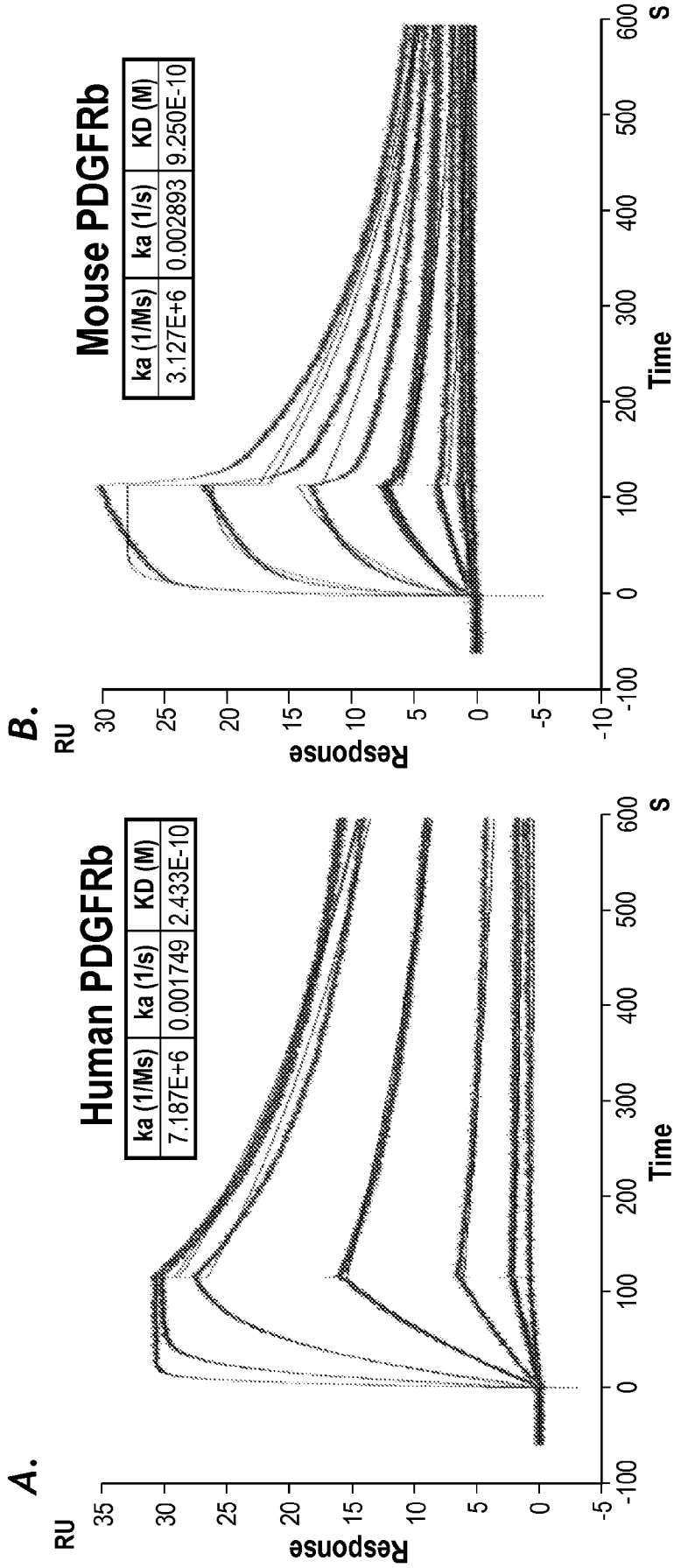

A.
Human PDGFRb
ka (1/Ms) ka (1/s) KD (M)
7.187E+6 0.001749 2.433E-10
RU
Response
Time
s
B.
Mouse PDGFRb
ka (1/Ms) ka (1/s) KD (M)
3.127E+6 0.002893 9.250E-10
RU
Response
Time
s

Fig. 11

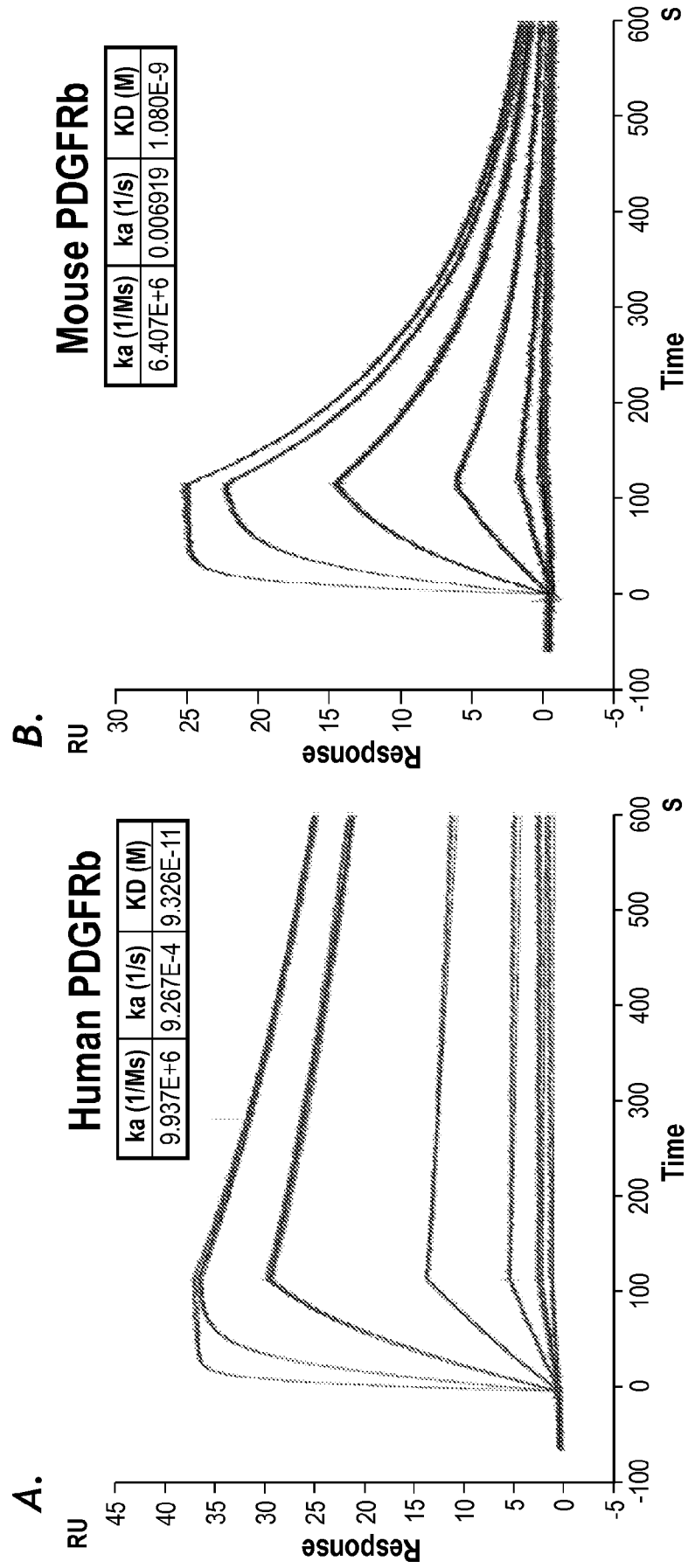

A.
Human PDGFRb
ka (1/Ms) | ka (1/s) | KD (M)
9.937E+6 | 9.267E-4 | 9.326E-11
RU
Response
Time
s
B.
Mouse PDGFRb
ka (1/Ms) | ka (1/s) | KD (M)
6.407E+6 | 0.006919 | 1.080E-9
RU
Response
Time
s

Fig. 12

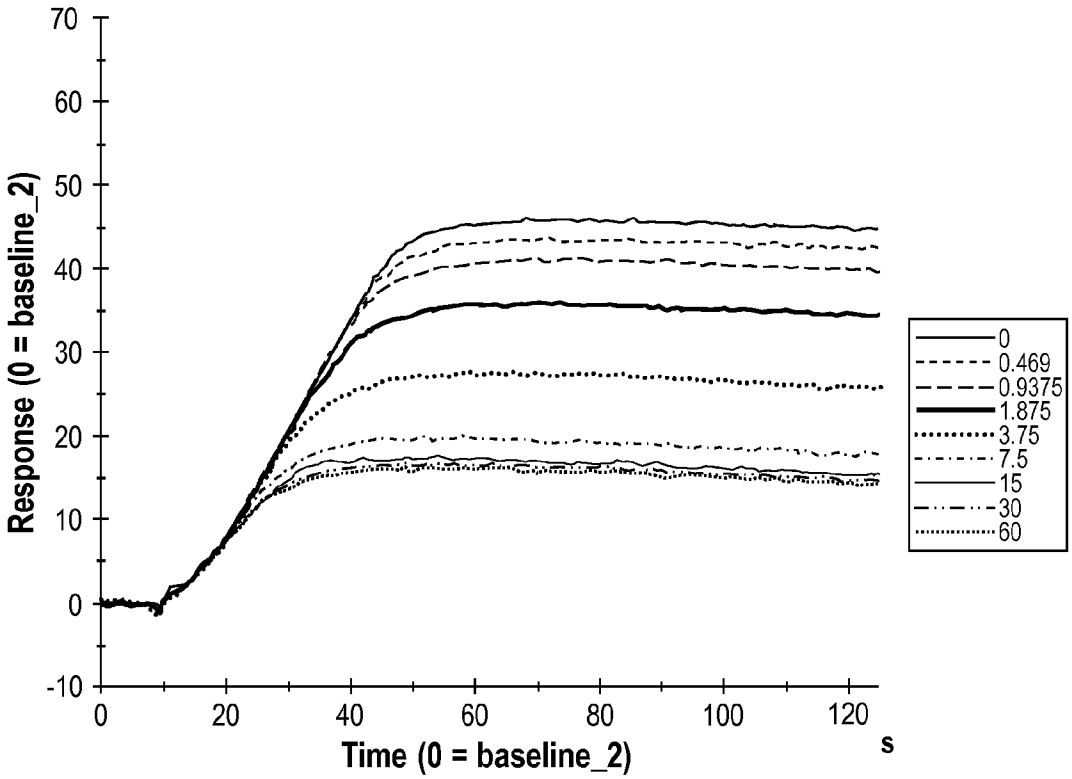

70
60
50
40
30
20
10
0
-10
Response (0 = baseline_2)
0
20
40
60
80
100
120
s
Time (0 = baseline_2)
0
0.469
0.9375
1.875
3.75
7.5
15
30
60

Fig. 13

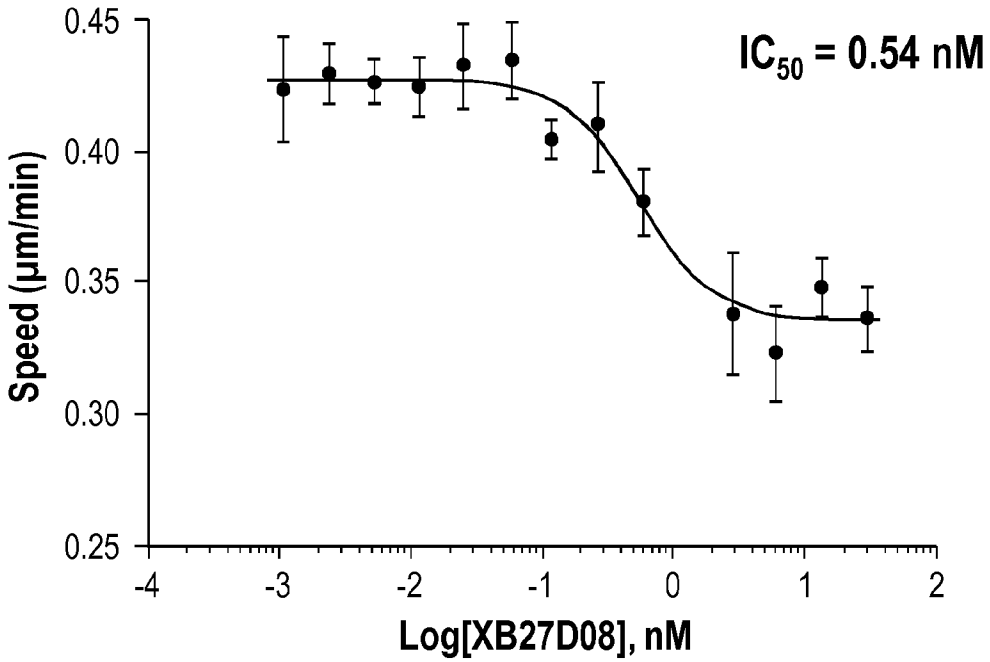

0.45
0.40
0.35
0.30
0.25
Speed (µm/min)
IC50 = 0.54 nM
-4
-3
-2
-1
0
1
2
Log[XB27D08], nM

Fig. 14

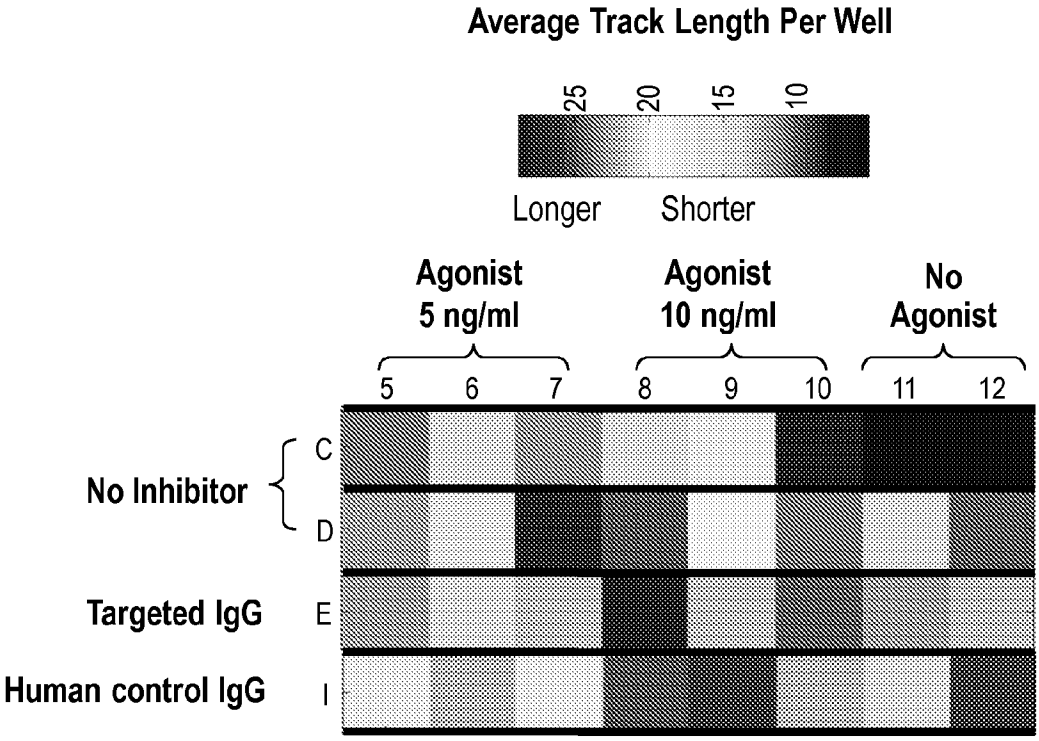
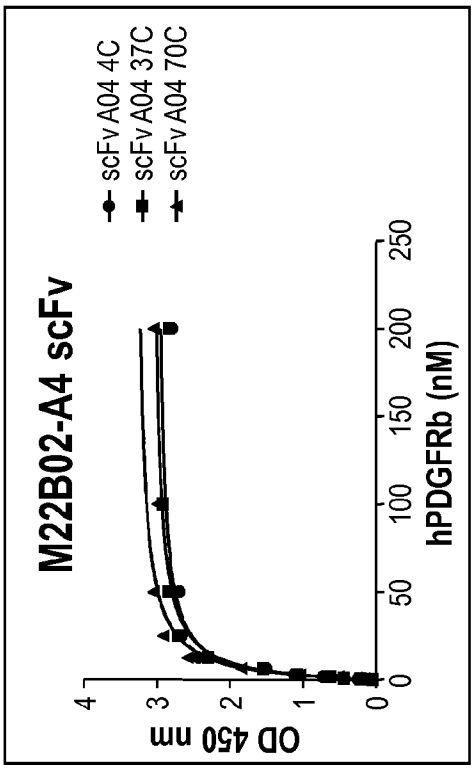
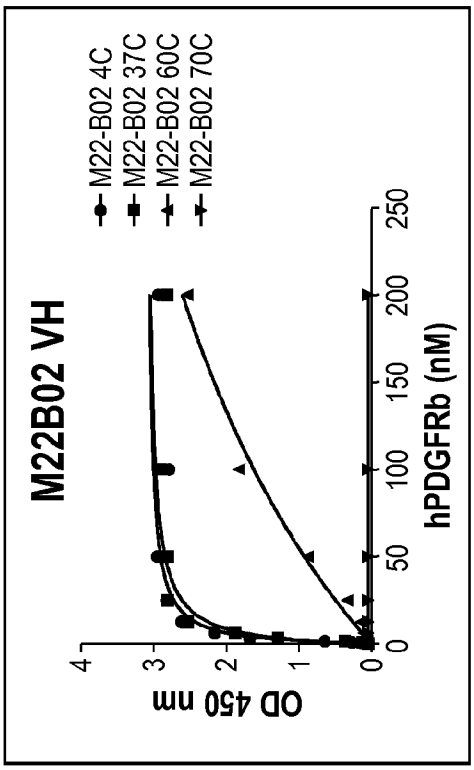

Average Track Length Per Well
25
20
15
10
Longer
Shorter
Agonist
5 ng/ml
Agonist
10 ng/ml
No
Agonist
5
6
7
8
9
10
11
12
No Inhibitor
C
D
Targeted IgG
E
Human control IgG
I

Fig. 15



Tm >70C



Tm ~ 62C

Fig. 16