



DIRECCIÓN DE NUEVAS CREACIONES
PRESENTACIÓN DE OPOSICIÓN

1. Oposición a solicitud de:

- ☒ Patente de invención
☐ Registro de Diseño Industrial
☐ Patente de Modelo de Utilidad
☐ Esquema de Trazado de Circuitos Integrados

Número de expediente: 12-056-244

Solicitante: BOEHRINGER INGELHEIM INTERNATIONAL GMBH.

Apoderado: CARLOS REINALDO OLARTE.

Título de la Nueva Creación: MOLECULAS DE UNION BIESPECIFICAS PARA LA TERAPIA ANTI-ANGIOGENESIS.

Gaceta: 652 de 17 de septiembre de 2012, No. de publicación 1388.

2. Datos del Opositor

Persona Natural

☐ Persona Jurídica

☒

TECNOQUIMICAS S.A.

Nombre o denominación / Razón social completa

Documento de identificación: C.C. ☐ C.E. ☐ NIT ☒ Otro ☐ Cuál

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Autorizo expresamente a la Superintendencia de Industria y Comercio para que todas las comunicaciones sean enviadas a través de la dirección de correo electrónico

3. Datos del representante o apoderado:

REYES VILLAMIZAR JOSE LUIS

79'152.473

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Apellidos y Nombre

Documento de identificación

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En caso de haber presentado poder general para asuntos que se adelanten ante la Delegatura de Propiedad Industrial, sírvase indicar el número de su radicación

2

Justificación: VER ANEXO

SI



NO

X

☒

X

JOSÉ LUIS REYES VILLAMIZAR

Firma

44655

T.P.

Señor:

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

E.

S.

D.

REF: EXPEDIENTE 12-056-244

TÍTULO SOLICITADO: "MOLÉCULAS DE UNIÓN BIESPECÍFICAS PARA LA TERAPIA ANTI-ANGIOGÉNESIS"

PUBLICACIÓN: Gaceta 652 de 17 de septiembre de 2012, N° de publicación 1388.

PRIORIDAD: EP 09172137.3 de 02/10/2009
EP 10175316.8 de 03/09/2010

SOLICITANTE: BOEHRINGER INGELHEIM INTERNATIONAL GMBH

APODERADO: CARLOS REINALDO OLARTE GARCIA

TRÁMITE: PRESENTACION DE OPOSICIÓN

JOSÉ LUIS REYES VILLAMIZAR, mayor de edad, identificado como aparece al pie de mi firma, abogado en ejercicio, titular de la tarjeta profesional número 44655 del Consejo Superior de la Judicatura, obrando en calidad de apoderado de la sociedad **TECNOQUÍMICAS S.A.**, en ejercicio de lo dispuesto por el artículo 42 de la Decisión 486 de 2000, muy respetuosamente me permito formular la siguiente

I. PETICIÓN

Sírvase negar el beneficio patentario a la solicitud contenida en el expediente **12-056-244**, por no cumplir con lo dispuesto en la Decisión 486 de 2000, con base en los fundamentos que se expondrán a continuación.

II. LEGÍTIMO INTERÉS

Asiste a **TECNOQUÍMICAS S.A.**, legítimo interés para presentar esta oposición, pues la eventual concesión de la solicitud restringiría injustificadamente el empleo de ciertas sustancias, derivados y procesos que a nuestro juicio carecen de nivel inventivo, en detrimento de sus legítimos intereses comerciales. De igual manera mis clientes están interesados, por su misma condición de industrias farmacéuticas, en evitar la concesión de privilegios de patente que incumplan los requisitos materiales de patentabilidad establecidos en las normas vigentes, y que supongan un riesgo para la libre competencia.

III. FUNDAMENTOS DE LA OPOSICIÓN

La solicitud colombiana en trámite 12-056-244 que se refiere a una molécula de unión biespecífica que comprende un componente de unión a Dll4 (siglas en inglés de *Delta Like Ligand 4*) y un componente de unión a VEGF (Factor de Crecimiento Vascular Endotelial), no reúne los requisitos necesarios para otorgársele el privilegio de patente de invención, como se demuestra a través de los siguientes argumentos:

- **Concisión y Soporte en la descripción**

Se anota que el capítulo reivindicatorio presentado ante la SIC no cumple con el requisito de concisión y soporte en la descripción ya que como se demuestra a continuación, la reivindicación 1 no define el objeto reclamado adecuadamente por sus características estructurales sino por su función de unirse a Dll4 y VEGF de la siguiente forma:

1-. Una molécula de unión biespecífica que comprende un componente de unión a Dll4 y un componente de unión a VEGF.

Como se observa, la molécula comprende un componente de unión a Dll4, el cual no se encuentra caracterizado por su secuencia de aminoácidos o por el certificado de depósito de material biológico que daría sustento a cualquier técnico medio para llevar a cabo el objeto reclamado. De acuerdo a lo anterior, cualquier molécula biespecífica capaz de unirse a dichos receptores sería cubierta por el

ámbito de las reivindicaciones, donde dichas moléculas no poseerían sustento en la descripción.

Ahora bien, en modalidades particulares del objeto reclamado, se puede encontrar cierta definición de la estructura de la molécula biespecífica en términos de una fórmula que igualmente reclama una cantidad no determinada de posibles moléculas señalando únicamente dos CDRs mediante una fórmula, pero sin determinar las posiciones de las mismas CDRs dentro de la molécula, lo cual también influye en la actividad, inmunogenicidad y demás propiedades de la molécula, veamos por ejemplo la reivindicación 5:

5- Una molécula de unión biespecífica de una cualquiera de las Reivindicaciones 2 a 4, en la que dicho componente de unión a Dll4 y dicho componente de unión a VEGF comprende al menos un dominio variable sencillo de inmunoglobulina de unión a VEGF y al menos un dominio variable sencillo de inmunoglobulina de unión a Dll4, respectivamente, en el que dicho dominio variable sencillo de inmunoglobulina tiene cuatro regiones flanqueantes y tres regiones determinantes de la complementariedad CDR1, CDR2 y CDR3, respectivamente, en las que

a) una CDR de dicho al menos un dominio variable sencillo de inmunoglobulina de unión a Dll4 tiene una secuencia de aminoácidos seleccionada de

i Arg Ala Pro Asp Thr Arg Leu Xaa Pro Tyr Xaa Tyr Asp

Xaa se muestra la SEC ID N° 1, en la que

Xaa en la posición 8 es Arg, Ala o Glu;

Xaa en la posición 11 es Leu o Glu; y

Xaa en la posición 14 es Tyr o His; y

ii Asp Arg Tyr Ile Trp Ala Arg Gln Gly Glu Tyr Trp Gly Ala

Tyr Xaa Asp Tyr como se muestra en la SEC ID N°: 2, en la que

Xaa es Gln, Ala o Tyr; y en la que

b) una CDR de dicho dominio al menos un dominio variable sencillo de inmunoglobulina de unión a VEGF tiene la secuencia de aminoácido Ser Arg Ala Tyr Gly Ser Ser Arg Leu Arg Leu Ala Asp Thr Tyr Xaa Tyr, mostrada en la SEC ID N°: 3, en la que Xaa es Asp o Glu;

en la que dicho dominio variable sencillo de inmunoglobulina de unión a VEGF es capaz de bloquear la interacción de VEGF165 recombinante humano con el VEGF-2 recombinante humano con una velocidad de inhibición $\geq 60\%$.

En dicha reivindicación además se observa que la molécula esta definida por la velocidad de inhibición, lo cual obedece a resultados a alcanzar de cualquier molécula de unión probada o no demostrando nuevamente la falta de concisión y sustento de la solicitud.

Adicionalmente, la falta de sustento es definitiva en reivindicaciones como:

22-. *Una molécula de unión biespecífica de una cualquiera de las Reivindicaciones 1 a 21, que comprende una o más moléculas enlazadoras y/o restos de ampliación de la semivida.*

30-. *Una molécula de ácido nucleico que codifica una molécula de unión biespecífica de una cualquiera de las Reivindicaciones 1 a 29 o un vector que lo contiene.*

31-. *Una célula hospedadora que contiene una molécula de ácido nucleico de la Reivindicación 30.*

32-. *Una composición farmacéutica que contiene al menos una molécula de unión a VEGF de una cualquiera de las Reivindicaciones 1 a 29, como el ingrediente activo.*

Estas reivindicaciones no cumplen con los requisitos mínimos para ser objeto de patente puesto que no mencionan las características técnicas para definir el objeto o categoría dado en cada una, por ejemplo, no cualquier una o más moléculas enlazadoras ha sido demostrada y evaluada por lo que la definición funcional únicamente aporta confusión; la molécula de ácido nucleico no se define por su secuencia de bases nitrogenadas, por lo que es especulativa; no cualquier célula hospedadora puede ser portadora del ácido nucleico no definido, pero que permita obtener la molécula biespecífica reclamada y por último, la composición farmacéutica no presenta sino un compuesto como ingrediente activo, lo cual no define una composición.

Por otro lado, y no menos importante, es el hecho que la reivindicación 32 menciona como característica excluyente la composición que contiene "al menos una molécula de unión a VEGF" lo que de otra manera quiere decir que se incluyen composiciones que comprenden como agente activo, anticuerpos Bevacizumab, Ranibizumab, entre otros.

Así, el objeto reclamado no cumple con los mínimos requisitos de claridad, concisión o sustento para ser merecedores del privilegio de patente.

Por otro lado, adicionalmente, la materia objeto de la solicitud a pesar de las deficiencias tampoco cumple con los requisitos de Novedad y Nivel Inventivo como veremos a continuación:

Objeciones a la Novedad.

Para demostrar que la materia es idéntica a la ya divulgada en el estado de la técnica se puede observar el documento:

- WO 2009/134776 "Dual variable domain immunoglobulins and uses thereof" de Ghayur Tariq, publicado el 5 de noviembre de 2009.

Dicho documento ha sido establecido como relevante para todas las reivindicaciones menos para las reivindicaciones 2 y 3 de acuerdo con el examen internacional ya que dichas reivindicaciones son las únicas que gozan de la prioridad EP 09172137.3 de 02/10/2009; a las demás reivindicaciones (1 y 4 a 32) se les reconoce únicamente la prioridad EP 10175316.8 de 03/09/2010 por lo que la fecha de búsqueda de los documentos del estado de la técnica incluyen todos los publicados antes del 03/09/2012.

Teniendo en cuenta lo anterior, el documento WO 2009/134776 destruye por completo la novedad de la solicitud ya que este describe moléculas biespecíficas que comprenden componentes de unión a DII4 así como componentes de unión a VEGF. El abstract menciona como eje central del documento proteínas de unión multiespecíficas, métodos para realizarlas y su uso específico en diagnóstico, prevención y/o tratamiento de enfermedades inflamatorias crónicas y agudas, cáncer y otras enfermedades; las moléculas que se describen en dicho documento tienen una región Fc y son usadas para el tratamiento de cáncer (ver página 26, línea 18 a página 38, línea 9 y página 143, líneas 9 a 30).

En el mismo documento se menciona que los anticuerpos biespecíficos ha sido producidos mediante tecnología quadroma basada en la fusión somática de dos líneas celulares que expresan anticuerpos monoclonales murinos con la deseada

especificidad. Esto demuestra una vez más que la tecnología de anticuerpos biespecíficos como el de la solicitud en estudio ya es conocida en el estado de la técnica y que únicamente se pone en práctica el cambio de las secuencias afines que se obtienen de combinar dos anticuerpos que se unen a diferentes receptores o antígenos.

En el siguiente aparte de la anterioridad mencionada (página 7) se describe específicamente la selección determinada de anticuerpos biespecíficos, nombrando particularmente la combinación VEGF y DLL4 (línea 6), veamos:

WO 2009/134776	PCT/US2009/041945
specific embodiment the binding protein is capable of binding pairs of targets selected from the group consisting of CD-20 and CD-19; CD-20 and CD-80; CD-20 and CD-22; CD-20 and CD-40; CD-3 and HER-2; CD-3 and CD-19; EGFR and HER-2; EGFR and CD-3; EGFR and IGF1,2; EGFR and IGF1R; EGFR and RON; EGFR and HGF; EGFR and c-MET; HER-2 and IGF1,2; 5 HER-2 and IGF1R; RON and HGF; VEGF and EGFR; VEGF and HER-2; VEGF and CD-20; VEGF and IGF1,2; VEGF and DLL4; VEGF and HGF; VEGF and RON; VEGF and NRP1; CD-20 and CD3; DLL-4 and PLGF; VEGF and PLGF; ErbB3 and EGFR; ErbB3 and HGF; HER-2 and ErbB3; c-Met and ErbB3; PLGF and HER-2; HER-2 and HER-2.	

De esta forma queda claro que cualquier anticuerpo o molécula de unión a los compuestos DLL4 y VEGF ya han sido divulgados en el estado de la técnica y que cualquier definición particular de secuencias diferentes que se unen a los mismos obedecen únicamente a una caracterización secuencial de dichos anticuerpos.

En todo el documento citado como anterioridad, existen ejemplos de moléculas biespecíficas de unión que residen en Dominios Variables Duales (DVD) y específicamente en la página 26 se mencionan los dominios característicos de la solicitud en estudio determinando diferentes cadenas de secuencias pesadas y ligeras dependiendo sus sitios de unión:

20 In an embodiment, the binding protein capable of binding VEGF (seq. 1) and DLL-4 (seq. 2) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 407 and SEQ ID NO. 409; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 408 and SEQ ID NO. 410. In an embodiment, the binding protein capable of binding VEGF (seq. 1) and DLL-4 (seq. 2) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 407 and a DVD light chain amino acid sequence of SEQ ID NO: 408. In another embodiment, the binding protein capable of binding VEGF (seq. 1) and DLL-4 (seq. 2) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 409 and a DVD light chain amino acid sequence of SEQ ID NO: 410.

De esta forma queda demostrado que la materia objeto de la solicitud no cumple con el requisito de novedad ya que todas sus características estaban anticipadas por el documento WO 2009/134776.

- Objeciones al Nivel inventivo

Adicionalmente al documento del estado de la técnica mencionado arriba, la descripción de la materia también era evidente según las enseñanzas de otras anterioridades que hacen que la solicitud no cumpla con los requisitos de nivel inventivo y que señalamos a continuación:

- La solicitud internacional WO2008/060705 de Yan Minhong "*Anti-DLL4 antibodies and methods using same*" publicado el 22 de mayo de 2008.
- La solicitud internacional WO2008/019144 de Wiegand Stanley, "*Therapeutic methods for treating vascular eye disorders with DLL4 antagonists*" publicado el 14 de febrero de 2008.

Para las reivindicaciones 2 y 3 de la solicitud en estudio (y en general para la materia descrita que reivindica la prioridad EP 09172137.3 de 02/10/2009), el documento WO 2008/060705 se establece también como el estado de la técnica más cercano ya que describe un método para tratar cáncer que involucra la coadministración de anticuerpos ant-DLL4 y anti-VEGF (ver el siguiente aparte del documento WO2008/060705 página 14).

In one aspect, the invention provides an article of manufacture comprising a container; and a composition contained within the container, wherein the composition comprises one or more anti-DLL4 antibodies of the invention. In one embodiment, the composition further comprises an anti-angiogenesis agent. In one embodiment the anti-angiogenesis agent is an anti-VEGF antibody, e.g., bevacizumab. In one embodiment, the composition comprises a nucleic acid of the invention. In one embodiment, a composition comprising an antibody further comprises a carrier, which in some embodiments is pharmaceutically acceptable. In one embodiment, a second composition is contained within the container, wherein the second composition comprises an anti-angiogenesis agent. In one embodiment, the anti-angiogenesis agent is an anti-VEGF antibody, e.g. bevacizumab. In one embodiment, an article of manufacture of the invention further comprises instructions for administering the composition(s) (for e.g., the antibody) to a subject (such as instructions for any of the methods described herein).

Este documento a pesar de no hacer mención de los anticuerpos duales, ya presentaba la solución del mismo problema técnico de la solicitud en trámite, al incluir en el tratamiento los dos anticuerpos Anti-DLL4 y Anti-VEGF y se había formulado la solución de incluirlos en las mismas patologías de uso en la solicitud en estudio, incluso, esta anterioridad describe anticuerpos multivalentes como parte de la invención determinados por los dominios duales presentados por la solicitud en estudio y los demás documentos mencionados en este memorial (ver página 62):

Multivalent Antibodies

A multivalent antibody may be internalized (and/or catabolized) faster than a bivalent antibody by a cell expressing an antigen to which the antibodies bind. The antibodies of the present invention can be multivalent antibodies (which are other than of the IgM class) with three or more antigen binding sites (e.g. tetravalent antibodies), which can be readily produced by recombinant expression of nucleic acid encoding the polypeptide chains of the antibody. The multivalent antibody can comprise a dimerization domain and three or more antigen binding sites. The preferred dimerization domain comprises (or consists of) an Fc region or a hinge region. In this scenario, the antibody will comprise an Fc region and three or more antigen binding sites amino-terminal to the Fc region. The preferred multivalent antibody herein comprises (or consists of) three to about eight, but preferably four, antigen binding sites. The multivalent antibody comprises at least one polypeptide chain (and preferably two polypeptide chains), wherein the polypeptide chain(s) comprise two or more variable domains. For instance, the polypeptide chain(s) may comprise VD1-(X1)_n-VD2-(X2)_n-Fc, wherein VD1 is a first variable domain, VD2 is a second variable domain, Fc is one polypeptide chain of an Fc region, X1 and X2 represent an amino acid or polypeptide, and n is 0 or 1. For instance, the polypeptide chain(s) may comprise: VH-CH1-flexible linker-VH-CH1-Fc region chain; or VH-CH1-VH-CH1-Fc region chain. The multivalent antibody herein preferably further comprises at least two (and preferably four) light chain variable domain polypeptides. The multivalent antibody herein may, for instance, comprise from about two to about eight

Por su parte, el documento WO2008/19144 (ver abstract), revela métodos para la realización de anticuerpos duales; por esta razón, cualquier técnico medio enfrentado al problema de obtener moléculas biespecíficas de unión para los tratamientos de cáncer se habría enfocado bajo las enseñanzas de cualquiera de los documentos citados a la realización de anticuerpos biespecíficos que se unen a DLL4 y VEGF con la misma probabilidad de éxito de la solicitud en trámite ya que dicha terapia enfocada a estos dos ligandos ya era conocida.

Para terminar y teniendo en cuenta que en la solicitud en cuestión no se observa ninguna ventaja sorprendente de secuencias específicas reclamadas sobre los anticuerpos del estado de la técnica, se evidencia que la misma únicamente se trata de una selección arbitraria de los anticuerpos biespecíficos anti-DLL4 y VEGF ya divulgados por el estado de la técnica.

De esta manera, lo reclamado y sus descripciones no son materia patentable ya que no cumplen con lo dispuesto en cuanto a Novedad y Nivel Inventivo por la Decisión 486 de la Comunidad Andina de Naciones.

IV PRUEBAS

Ruego que se tenga como tal, sin perjuicio de las que puedan presentarse al vencimiento de la prórroga solicitada, y de aquellas que el Despacho considere oficiosamente, las siguientes:

- La solicitud internacional WO 2009/134776 *"Dual variable domain immunoglobulins and uses thereof"* de Ghayur Tariq, publicado el 5 de noviembre de 2009. Portada, páginas 7, 23, 26, 38, 143 reivindicaciones
- La solicitud internacional WO2008/060705 de Yan Minhong *"Anti-DLL4 antibodies and methods using same"* publicado el 22 de mayo de 2008. Portada, páginas 1, 4, 62, 117 y reivindicaciones
- La solicitud internacional WO2008/19144 de Wiegand Stanley, *"Therapeutic methods for treating vascular eye disorders with DLL4 antagonists"* publicado el 14 de febrero de 2008. Portada, página 1.

REYES & REYES
A B O G A D O S

Señor Superintendente,



JOSÉ LUIS REYES VILLAMIZAR

C.C. 79.152.473 de Usaquén

T.P.A. 44.655 de C.S.J.

AA 3286373 NOTARIA TERCERA ENCARGADO



ESCRITURA NUMERO CUATRO MIL QUINIENTOS -
TREINTA Y NUEVE (No. 4.539) -----
En la ciudad de Cali, Capital del Depar-
tamento del Valle del Cauca, República de
Colombia, a los VEINTITRES (23) días -
del mes de OCTUBRE del año DOS MIL UNO --

(2.001), ante mi, JORGE ENRIQUE CAICEDO ZAMORANO, Notario
T E R C E R O (3o.) del Círculo de Cali, - - - - -

compareció JUAN MANUEL BARBERI OSPINA mayor de edad, vecino de Cali,
identificado con la cédula de ciudadanía número 14.949.561 de Cali, y dijo: ----.

PRIMERO.- Que obra en su carácter de Representante Legal de en nombre y
representación de TECNOQUIMICAS S.A. sociedad comercial organizada y existente
conforme a las leyes colombianas, con domicilio y sede principal en Cali constituida
por escritura pública número 2670 de la Notaría Segunda de Cali de fecha 12 de
junio de 1957, reformada en distintas oportunidades, según consta en el certificado
expedido por la Cámara de Comercio de Cali -----

SEGUNDO: Que en tal carácter, por este público instrumento, concede Poder Especial
pero amplio y suficiente a JOSE LUIS REYES VILLAMIZAR, mayor de edad, vecino de
Bogotá, identificado con C.C. # 79.152.473 de Isaquén, abogado en ejercicio, portador
de la T.P. 44655 del C.S. de la J., domiciliado en Bogotá, D.C., República de Colombia
con dirección carrera 7 No. 88.23 oficina 205, de la misma ciudad, como su representante
con poder especial, amplio y suficiente, para obtener la protección de nuestra propiedad
industrial y contra la competencia desleal, a cuyo efecto lo autorizo, para que nos
represente ante cualesquiera entidad o persona, ejerzan o no jurisdicción, especialmente
ante las del orden administrativo, contencioso-administrativo y judicial, en todo lo
concerniente a los siguientes asuntos: a) Obtención, formulación de observaciones y
oposición a patentes de invención, modelos y dibujos industriales y el registro de
licencias de uso de esos derechos; b) Las acciones de oposición, cancelación, nulidad,
medidas cautelares, concesión de licencias y en general los procesos civiles, penales,
administrativos o contencioso-administrativos relacionados con estos derechos u otros de
diferente naturaleza; acciones y procesos que promovamos o se nos promuevan; y c) para

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hago constar que esta fotocopia coincide
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13 AGO 2007
HILLY VALENTIN
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4539
0523

LUZ MERY CORREA
ELABORACION DE ESCRITURAS
Notaría 3ª de Cali

que en todos los asuntos arriba determinados, , tanto en los terminos señalados por los
artículos 42 y complementarios de la Decisión 486 de la Comunidad Andina como del
artículo 70 del C.P.C pueda sustituir este mandato, transigir, conciliar, admitir los hechos
del proceso, desistir, cancelar, recibir, renunciar, y ratificar los actos de agentes oficiosos
y ceder a titulo gratuito y/o oneroso, licenciar, inscribir contratos de cesión y de licencia,
inscripción de prendas, levantamiento de prendas, inscribir derechos de retención y en
general todos los asuntos que se deban tramitar ante la Superintendencia de Industria y
Comercio.-----

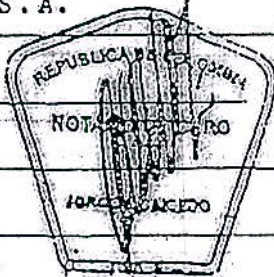
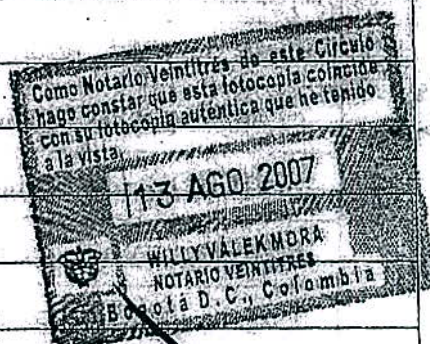
Leído el presente instrumento al otorgante, lo aprobó y en constancia firma conmigo el
Notario de todo lo cual doy fe.-----

Se agregan comprobantes. Derechos \$30.000.00 Decreto 1681 de
Septiembre 16 de 1996. Reformado mediante Resolución No. 5839
de Diciembre 27 del año 2.000 y Decreto 13267 de Julio 06 del
año 2.001. Se corrió en la hoja número AA 3286877. ENMENDA-
DO "OCTUBRE" VALE.

Juan m. Barberi

JUAN MANUEL BARBERI OSPINA

En rep. de TECNOQUIMICAS S.A.



El Notario Tercero de Cali

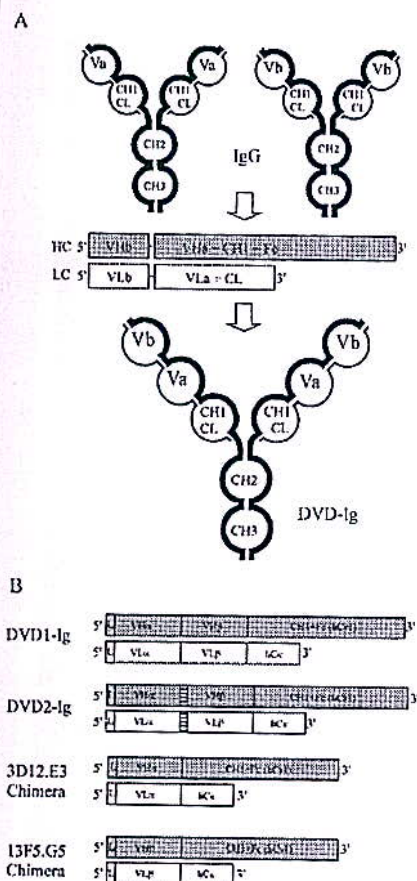
Dr. JORGE ENRIQUE CAICEDO ZAMORANO

- (51) International Patent Classification:
G01N 33/53 (2006.01)
- (21) International Application Number:
PCT/US2009/041945
- (22) International Filing Date:
28 April 2009 (28.04.2009)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
61/125,834 29 April 2008 (29.04.2008) US
61/134,283 8 July 2008 (08.07.2008) US
61/197,191 23 October 2008 (23.10.2008) US
61/199,009 12 November 2008 (12.11.2008) US
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(54) Title: DUAL VARIABLE DOMAIN IMMUNOGLOBULINS AND USES THEREOF

Figure 1



(57) Abstract: The present invention relates to engineered multivalent and multispecific binding proteins, methods of making, and specifically to their uses in the prevention, diagnosis, and/or treatment of disease.

specific embodiment the binding protein is capable of binding pairs of targets selected from the group consisting of CD-20 and CD-19; CD-20 and CD-80; CD-20 and CD-22; CD-20 and CD-40; CD-3 and HER-2; CD-3 and CD-19; EGFR and HER-2; EGFR and CD-3; EGFR and IGF1,2; EGFR and IGF1R; EGFR and RON; EGFR and HGF; EGFR and c-MET; HER-2 and IGF1,2;
 5 HER-2 and IGF1R; RON and HGF; VEGF and EGFR; VEGF and HER-2; VEGF and CD-20; VEGF and IGF1,2; VEGF and DLL4; VEGF and HGF; VEGF and RON; VEGF and NRP1; CD-20 and CD3; DLL-4 and PLGF; VEGF and PLGF; ErbB3 and EGFR; ErbB3 and HGF; HER-2 and ErbB3; c-Met and ErbB3; PLGF and HER-2; HER-2 and HER-2.

In an embodiment, the binding protein capable of binding CD-20 and CD-19 comprises a
 10 DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 99 and SEQ ID NO. 101; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 100 and SEQ ID NO. 102. In an embodiment, the binding protein capable of binding CD-20 and CD-19 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 99 and a DVD light chain amino acid sequence of SEQ ID NO: 100. In another embodiment, the
 15 binding protein capable of binding CD-20 and CD-19 has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 101 and a DVD light chain amino acid sequence of SEQ ID NO: 102.

In an embodiment, the binding protein capable of binding CD-20 and CD-3 (seq. 1) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID
 20 NO. 103 and SEQ ID NO. 105; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 104 and SEQ ID NO. 106. In an embodiment, the binding protein capable of binding CD-20 and CD-3 (seq. 1) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 103 and a DVD light chain amino acid sequence of SEQ ID NO: 104. In another embodiment, the binding protein capable of binding CD-20 and CD-3 (seq. 1) has a
 25 reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 105 and a DVD light chain amino acid sequence of SEQ ID NO: 106.

In an embodiment, the binding protein capable of binding CD-20 and CD-80 comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 107
 and SEQ ID NO. 109; and a DVD light chain amino acid sequence selected from the group
 30 consisting of SEQ ID NO. 108 and SEQ ID NO. 110. In an embodiment, the binding protein capable of binding CD-20 and CD-80 comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 107 and a DVD light chain amino acid sequence of SEQ ID NO: 108. In another embodiment, the binding protein capable of binding CD-20 and CD-80 has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 109 and a DVD light
 35 chain amino acid sequence of SEQ ID NO: 110.

In a third embodiment, the binding protein capable of binding VEGF (seq. 1) and PLGF (seq. 1) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 351 and SEQ ID NO. 353; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 352 and SEQ ID NO. 354. In an embodiment, the binding protein capable of binding VEGF (seq. 1) and PLGF (seq. 1) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 351 and a DVD light chain amino acid sequence of SEQ ID NO: 352. In another embodiment, the binding protein capable of binding VEGF (seq. 1) and PLGF (seq. 1) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 353 and a DVD light chain amino acid sequence of SEQ ID NO: 354.

In a fourth embodiment, the binding protein capable of binding VEGF (seq. 1) and PLGF (seq. 1) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 355 and SEQ ID NO. 357; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 356 and SEQ ID NO. 358. In an embodiment, the binding protein capable of binding VEGF (seq. 1) and PLGF (seq. 1) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 355 and a DVD light chain amino acid sequence of SEQ ID NO: 356. In another embodiment, the binding protein capable of binding VEGF (seq. 1) and PLGF (seq. 1) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 357 and a DVD light chain amino acid sequence of SEQ ID NO: 358.

In an embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 1) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 359 and SEQ ID NO. 361; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 360 and SEQ ID NO. 362. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 1) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 359 and a DVD light chain amino acid sequence of SEQ ID NO: 360. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 1) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 361 and a DVD light chain amino acid sequence of SEQ ID NO: 362.

In a second embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 1) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 363 and SEQ ID NO. 365; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 364 and SEQ ID NO. 366. In an embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 1) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 363 and a DVD light chain amino acid sequence of SEQ ID NO: 364. In another embodiment, the binding protein capable of binding EGFR (seq. 2) and ErbB3 (seq. 1) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 365 and a DVD light chain amino acid sequence of SEQ ID NO: 366.

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In an embodiment, the binding protein capable of binding EGFR (seq. 1) and ErbB3 (seq. 2) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 399 and SEQ ID NO. 401; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 400 and SEQ ID NO. 402. In an embodiment, the binding protein capable of binding EGFR (seq. 1) and ErbB3 (seq. 2) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 399 and a DVD light chain amino acid sequence of SEQ ID NO: 400. In another embodiment, the binding protein capable of binding EGFR (seq. 1) and ErbB3 (seq. 2) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 401 and a DVD light chain amino acid sequence of SEQ ID NO: 402.

10 In an embodiment, the binding protein capable of binding HGF (seq. 1) and ErbB3 (seq. 2) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 403 and SEQ ID NO. 405; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 404 and SEQ ID NO. 406. In an embodiment, the binding protein capable of binding HGF (seq. 1) and ErbB3 (seq. 2) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 403 and a DVD light chain amino acid sequence of SEQ ID NO: 404. In another embodiment, the binding protein capable of binding HGF (seq. 1) and ErbB3 (seq. 2) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 405 and a DVD light chain amino acid sequence of SEQ ID NO: 406.

20 In an embodiment, the binding protein capable of binding VEGF (seq. 1) and DLL-4 (seq. 2) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 407 and SEQ ID NO. 409; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 408 and SEQ ID NO. 410. In an embodiment, the binding protein capable of binding VEGF (seq. 1) and DLL-4 (seq. 2) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 407 and a DVD light chain amino acid sequence of SEQ ID NO: 408. In another embodiment, the binding protein capable of binding VEGF (seq. 1) and DLL-4 (seq. 2) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 409 and a DVD light chain amino acid sequence of SEQ ID NO: 410.

30 In a second embodiment, the binding protein capable of binding VEGF (seq. 1) and DLL-4 (seq. 2) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 411 and SEQ ID NO. 413; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 412 and SEQ ID NO. 414. In an embodiment, the binding protein capable of binding VEGF (seq. 1) and DLL-4 (seq. 2) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 411 and a DVD light chain amino acid sequence of SEQ ID NO: 412. In another embodiment, the binding protein capable of binding VEGF (seq. 1) and DLL-4 (seq. 2) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 413 and a DVD light chain amino acid sequence of SEQ ID NO: 414.

In a fourth embodiment, the binding protein capable of binding VEGF (seq. 3) and DLL-4 (seq. 4) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 591 and SEQ ID NO. 593; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 592 and SEQ ID NO. 594. In an embodiment, the binding protein capable of binding VEGF (seq. 3) and DLL-4 (seq. 4) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 591 and a DVD light chain amino acid sequence of SEQ ID NO. 592. In another embodiment, the binding protein capable of binding VEGF (seq. 3) and DLL-4 (seq. 4) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 593 and a DVD light chain amino acid sequence of SEQ ID NO. 594.

In an embodiment, the binding protein capable of binding HER-2 (seq. 1) and ErbB3 (seq. 1) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 595 and SEQ ID NO. 597; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 596 and SEQ ID NO. 598. In an embodiment, the binding protein capable of binding HER-2 (seq. 1) and ErbB3 (seq. 1) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 595 and a DVD light chain amino acid sequence of SEQ ID NO. 596. In another embodiment, the binding protein capable of binding HER-2 (seq. 1) and ErbB3 (seq. 1) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 597 and a DVD light chain amino acid sequence of SEQ ID NO. 598.

In a second embodiment, the binding protein capable of binding HER-2 (seq. 1) and ErbB3 (seq. 1) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 599 and SEQ ID NO. 601; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 600 and SEQ ID NO. 602. In an embodiment, the binding protein capable of binding HER-2 (seq. 1) and ErbB3 (seq. 1) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 599 and a DVD light chain amino acid sequence of SEQ ID NO. 600. In another embodiment, the binding protein capable of binding HER-2 (seq. 1) and ErbB3 (seq. 1) has a reverse orientation and comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 601 and a DVD light chain amino acid sequence of SEQ ID NO. 602.

In a third embodiment, the binding protein capable of binding HER-2 (seq. 1) and ErbB3 (seq. 1) comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 603 and SEQ ID NO. 605; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 604 and SEQ ID NO. 606. In an embodiment, the binding protein capable of binding HER-2 (seq. 1) and ErbB3 (seq. 1) comprises a DVD heavy chain amino acid sequence of SEQ ID NO. 603 and a DVD light chain amino acid sequence of SEQ ID NO. 604. In another embodiment, the binding protein capable of binding HER-2 (seq. 1)

MAG and RAGE that binds A-b and S100 A. Furthermore, neurite outgrowth inhibitors e.g., nogo and nogo receptor, also play a role in preventing nerve regeneration in immunological diseases like multiple sclerosis. Inhibition of nogo-nogo receptor interaction has been shown to enhance recovery in animal models of multiple sclerosis. Therefore, DVD-Ig molecules that can

5 block the function of one immune mediator eg a cytokine like IL-12 and a neurite outgrowth inhibitor molecule eg nogo or RGM may offer faster and greater efficacy than blocking either an immune or an neurite outgrowth inhibitor molecule alone.

8. Oncological disorders

Monoclonal antibody therapy has emerged as an important therapeutic modality for

10 cancer (von Mehren M, et al 2003 Monoclonal antibody therapy for cancer. Annu Rev Med.;54:343-69). Antibodies may exert antitumor effects by inducing apoptosis, redirected cytotoxicity, interfering with ligand-receptor interactions, or preventing the expression of proteins that are critical to the neoplastic phenotype. In addition, antibodies can target components of the tumor microenvironment, perturbing vital structures such as the formation of tumor-associated

15 vasculature. Antibodies can also target receptors whose ligands are growth factors, such as the epidermal growth factor receptor. The antibody thus inhibits natural ligands that stimulate cell growth from binding to targeted tumor cells. Alternatively, antibodies may induce an anti-idiotypic network, complement-mediated cytotoxicity, or antibody-dependent cellular cytotoxicity (ADCC). The use of dual-specific antibody that targets two separate tumor mediators will likely

20 give additional benefit compared to a mono-specific therapy. DVD Igs capable of binding the following pairs of targets to treat oncological disease are also contemplated: IGF1 and IGF2; IGF1/2 and HER-2; VEGFR and EGFR; CD20 and CD3; CD138 and CD20; CD38 and CD20; CD38 and CD138; CD40 and CD20; CD138 and CD40; CD38 and CD40; CD-20 and CD-19; CD-20 and EGFR; CD-20 and CD-80; CD-20 and CD-22; CD-3 and HER-2; CD-3 and CD-19;

25 EGFR and HER-2; EGFR and CD-3; EGFR and IGF1,2; EGFR and IGF1R; EGFR and RON; EGFR and HGF; EGFR and c-MET; HER-2 and IGF1,2; HER-2 and IGF1R; RON and HGF; VEGF and EGFR; VEGF and HER-2; VEGF and CD-20; VEGF and IGF1,2; VEGF and DLL4; VEGF and HGF; VEGF and RON; VEGF and NRP1; CD20 and CD3; VEGF and PLGF; DLL4 and PLGF; ErbB3 and EGFR; HGF and ErbB3; HER-2 and ErbB3; c-Met and ErbB3; HER-2 and

30 PLGF; HER-2 and HER-2.

In another embodiment, a DVD of the invention is capable of binding VEGF and phosphatidylserine; VEGF and ErbB3; VEGF and PLGF; VEGF and ROBO4; VEGF and BSG2; VEGF and CDCP1; VEGF and ANPEP; VEGF and c-MET; HER-2 and ERB3; HER-2 and

35 BSG2; HER-2 and CDCP1; HER-2 and ANPEP; EGFR and CD64; EGFR and BSG2; EGFR and CDCP1; EGFR and ANPEP; IGF1R and PDGFR; IGF1R and VEGF; IGF1R and CD20; CD20 and CD74; CD20 and CD30; CD20 and DR4; CD20 and VEGFR2; CD20 and CD52; CD20 and

We claim:

1. A binding protein comprising a polypeptide chain, wherein said polypeptide chain comprises VD1-(X1)_n-VD2-C-(X2)_n, wherein;

5 VD1 is a first heavy chain variable domain obtained from a first parent antibody or antigen binding portion thereof;

VD2 is a second heavy chain variable domain obtained from a second parent antibody or antigen binding portion thereof;

C is a heavy chain constant domain;

10 (X1)_n is a linker with the proviso that it is not CH1, wherein said (X1)_n is either present or absent; and

(X2)_n is an Fc region, wherein said (X2)_n is either present or absent,

wherein the binding protein is capable of binding a pair of antigens selected from the group consisting of CD-20 and CD-19; CD-20 and CD-80; CD-20 and CD-22; CD-20 and CD-40; CD-3 and HER-2; CD-3 and CD-19; EGFR and HER-2; EGFR and CD-3; EGFR and IGF1,2; EGFR and IGF1R; EGFR and RON; EGFR and HGF; EGFR and c-MET; HER-2 and IGF1,2; HER-2 and IGF1R; RON and HGF; VEGF and EGFR; VEGF and HER-2; VEGF and CD-20; VEGF and IGF1,2; VEGF and DLL4; VEGF and HGF; VEGF and RON; VEGF and NRP1; CD-20 and CD3; DLL-4 and PLGF; VEGF and PLGF, ErbB3 and EGFR, HER-2 and ErbB3, ErbB3 and HGF; HER-2 and PLGF, and HER-2 and HER-2.

20 2. The binding protein of claim 1, wherein VD1 and VD2 comprise an amino acid sequence selected from the group consisting of SEQ ID NOs: 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, and 93.

3. A binding protein comprising a polypeptide chain, wherein said polypeptide chain comprises VD1-(X1)_n-VD2-C-(X2)_n, wherein;

25 VD1 is a light heavy chain variable domain obtained from a first parent antibody or antigen binding portion thereof;

VD2 is a light heavy chain variable domain obtained from a second parent antibody or antigen binding portion thereof;

C is a light chain constant domain;

30 (X1)_n is a linker with the proviso that it is not CH1, wherein said (X1)_n is either present or absent; and

(X2)_n is an Fc region, wherein said (X2)_n is either present or absent,

wherein the binding protein is capable of binding a pair of antigens selected from the group consisting of CD-20 and CD-19; CD-20 and CD-80; CD-20 and CD-22; CD-20 and CD-40; CD-3 and HER-2; CD-3 and CD-19; EGFR and HER-2; EGFR and CD-3; EGFR and IGF1,2; EGFR and IGF1R; EGFR and RON; EGFR and HGF; EGFR and c-MET; HER-2 and IGF1,2; HER-2 and IGF1R; RON and HGF; VEGF and EGFR; VEGF and HER-2; VEGF and CD-20; VEGF and IGF1,2; VEGF and DLL4; VEGF and HGF; VEGF and RON; VEGF and NRP1; CD-20 and CD3; DLL-4 and PLGF; VEGF and PLGF, ErbB3 and EGFR, HER-2 and ErbB3, ErbB3 and HGF; HER-2 and PLGF, and HER-2 and HER-2.

4. The binding protein of claim 3, wherein the VD1 and VD2 light chain variable domains comprise an amino acid sequence selected from the group consisting of SEQ ID NOs: 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, and 94.

5. The binding protein of claim 1 or 3, wherein (X2)_n is absent.

6. A binding protein comprising first and second polypeptide chains, wherein ,
15 said first polypeptide chain comprises a first VD1-(X1)_n-VD2-C-(X2)_n, wherein

VD1 is a first heavy chain variable domain obtained from a first parent antibody or antigen binding portion thereof;

VD2 is a second heavy chain variable domain obtained from a second parent antibody or antigen binding portion thereof;

20 C is a heavy chain constant domain;

(X1)_n is a linker with the proviso that it is not CH1, wherein said (X1)_n is either present or absent; and

(X2)_n is an Fc region, wherein said (X2)_n is either present or absent; and

25 wherein said second polypeptide chain comprises a second VD1-(X1)_n-VD2-C-(X2)_n, wherein

VD1 is a first light chain variable domain obtained from a first parent antibody or antigen binding portion thereof;

VD2 is a second light chain variable domain obtained from a second parent antibody or antigen binding portion thereof;

30 C is a light chain constant domain;

(X1)_n is a linker with the proviso that it is not CH1, wherein said (X1)_n is either present or absent; and

- (X2)_n does not comprise an Fc region, wherein said (X2)_n is either present or absent, wherein the binding protein is capable of binding a pair of antigens selected from the group consisting of CD-20 and CD-19; CD-20 and CD-80; CD-20 and CD-22; CD-20 and CD-40; CD-3 and HER-2; CD-3 and CD-19; EGFR and HER-2; EGFR and CD-3; EGFR and IGF1,2; EGFR and IGF1R; EGFR and RON; EGFR and HGF; EGFR and c-MET; HER-2 and IGF1,2; HER-2 and IGF1R; RON and HGF; VEGF and EGFR; VEGF and HER-2; VEGF and CD-20; VEGF and IGF1,2; VEGF and DLL4; VEGF and HGF; VEGF and RON; VEGF and NRP1; CD-20 and CD3; DLL-4 and PLGF; VEGF and PLGF, ErbB3 and EGFR, HER-2 and ErbB3, ErbB3 and HGF; HER-2 and PLGF, and HER-2 and HER-2.
7. The binding protein of claim 6, wherein the VD1 and VD2 heavy chain variable domains comprise an amino acid sequence selected from the group consisting of SEQ ID NOs: 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, and 93 and wherein the VD1 and VD2 light chain variable domains comprise an amino acid sequence selected from the group consisting of SEQ ID NOs: 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, and 94.
8. The binding protein of claim 1, 3, or 6, wherein (X2)_n is an amino acid sequence selected from the group consisting of SEQ ID NOs 1-26.
9. The binding protein of claim 6, wherein the binding protein comprises two first polypeptide chains and two second polypeptide chains.
10. The binding protein of claim 1, 3, or 6, wherein the Fc region is selected from the group consisting of native sequence Fc region and a variant sequence Fc region.
11. The binding protein of claim 10, wherein the Fc region is selected from the group consisting of an Fc region from an IgG1, IgG2, IgG3, IgG4, IgA, IgM, IgE, and IgD.
12. The binding protein of claim 1, 3, or 6, wherein said VD1 of the first polypeptide chain and said VD1 of the second polypeptide chain are obtained from the same parent antibody or antigen binding portion thereof.
13. The binding protein of claim 1, 3, or 6, wherein said VD1 of the first polypeptide chain and said VD1 of the second polypeptide chain are obtained from different parent antibody or antigen binding portion thereof.
14. The binding protein of claim 1, 3, or 6, wherein said VD2 of the first polypeptide chain and said VD2 of the second polypeptide chain are obtained from the same parent antibody or antigen binding portion thereof.

15. The binding protein of claim 1, 3, or 6, wherein said VD2 of the first polypeptide chain and said VD2 of the second polypeptide chain are obtained from different parent antibody or antigen binding portion thereof.
16. The binding protein of claim 1, 3, or 6, wherein said first and said second parent antibodies
5 bind different epitopes on said antigen.
17. The binding protein of claim 1, 3, or 6, wherein said first parent antibody or antigen binding portion thereof, binds said first antigen with a potency different from the potency with which said second parent antibody or antigen binding portion thereof, binds said second antigen.
18. The binding protein of claim 1, 3, or 6, wherein said first parent antibody or antigen binding
10 portion thereof, binds said first antigen with an affinity different from the affinity with which said second parent antibody or antigen binding portion thereof, binds said second antigen.
19. The binding protein of claim 1, 3, or 6, wherein said first parent antibody or antigen binding portion thereof, and said second parent antibody or antigen binding portion thereof, are selected from the group consisting of a human antibody, a CDR grafted antibody, and a humanized
15 antibody.
20. The binding protein of claim 1, 3, or 6, wherein said first parent antibody or antigen binding portion thereof, and said second parent antibody or antigen binding portion thereof, are selected from the group consisting of a Fab fragment; a F(ab')₂ fragment; a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment consisting of
20 the VH and CH1 domains; a Fv fragment consisting of the VL and VH domains of a single arm of an antibody; a dAb fragment; an isolated complementarity determining region (CDR); a single chain antibody; and a diabody.
21. The binding protein of claim 1, 3, or 6, wherein said binding protein possesses at least one desired property exhibited by said first parent antibody or antigen binding portion thereof, or said
25 second parent antibody or antigen binding portion thereof.
22. The binding protein of claim 21, wherein said desired property is selected from one or more antibody parameters.
23. The binding protein of claim 21, wherein said antibody parameters are selected from the group consisting of antigen specificity, affinity to antigen, potency, biological function, epitope
30 recognition, stability, solubility, production efficiency, immunogenicity, pharmacokinetics, bioavailability, tissue cross reactivity, and orthologous antigen binding.
24. A DVD-Ig capable of binding two antigens comprising four polypeptide chains, wherein first and third polypeptide chains comprise VD1-(X1)_n-VD2-C-(X2)_n, wherein

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VD1 is a first heavy chain variable domain obtained from a first parent antibody or antigen binding portion thereof;

VD2 is a second heavy chain variable domain obtained from a second parent antibody or antigen binding portion thereof;

C is a heavy chain constant domain;

(X1)_n is a linker with the proviso that it is not CH1, wherein said (X1)_n is either present or absent; and

(X2)_n is an Fc region, wherein said (X2)_n is either present or absent; and

wherein second and fourth polypeptide chains comprise VD1-(X1)_n-VD2-C-

(X2)_n, wherein

VD1 is a first light chain variable domain obtained from a first parent antibody or antigen binding portion thereof;

VD2 is a second light chain variable domain obtained from a second parent antibody or antigen binding portion thereof;

C is a light chain constant domain;

(X1)_n is a linker with the proviso that it is not CH1, wherein said (X1)_n is either present or absent; and

(X2)_n does not comprise an Fc region, wherein said (X2)_n is either present or

absent, wherein the VD1 and VD2 heavy chain variable domains comprise an

amino acid sequence selected from the group consisting of SEQ ID NOs: 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73,

75, 77, 79, 81, 83, 85, 87, 89, 91, and 93 and wherein the VD1 and VD2 light

chain variable domains comprise an amino acid sequence selected from the group consisting of SEQ ID NOs: 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54,

56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, and 94.

25. A DVD-Ig capable of binding two antigens comprising four polypeptide chains, wherein first and third polypeptide chains comprise VD1-(X1)_n-VD2-C-(X2)_n, wherein

VD1 is a first heavy chain variable domain obtained from a first parent antibody or antigen binding portion thereof;

VD2 is a second heavy chain variable domain obtained from a second parent antibody or antigen binding portion thereof;

C is a heavy chain constant domain;

(X1)_n is a linker with the proviso that it is not CH1, wherein said (X1)_n is either present or absent; and

(X2)_n is an Fc region, wherein said (X2)_n is either present or absent; and

wherein second and fourth polypeptide chains comprise VD1-(X1)_n-VD2-C-

(X2)_n, wherein

VD1 is a first light chain variable domain obtained from a first parent antibody or antigen binding portion thereof;

VD2 is a second light chain variable domain obtained from a second parent antibody or antigen binding portion thereof;

C is a light chain constant domain;

(X1)_n is a linker with the proviso that it is not CH1, wherein said (X1)_n is either present or absent; and

(X2)_n does not comprise an Fc region, wherein said (X2)_n is either present or absent, wherein the DVD-Ig binds at least one antigen selected from the group consisting of CD-20, CD-19, CD-80, CD-22, CD-40, CD-3, HER-2, EGFR, IGF1,2, IGF1R, RON, HGF, c-MET, VEGF, DLL4, NRP1, PLGF, and ErbB3.

26. A method for generating a Dual Variable Domain Immunoglobulin capable of binding two antigens comprising the steps of

a) obtaining a first parent antibody or antigen binding portion thereof, capable of binding a first antigen;

b) obtaining a second parent antibody or antigen binding portion thereof, capable of binding a second antigen;

c) constructing first and third polypeptide chains comprising VD1-(X1)_n-VD2-C-(X2)_n, wherein

VD1 is a first heavy chain variable domain obtained from said first parent antibody or antigen binding portion thereof;

VD2 is a second heavy chain variable domain obtained from said second parent antibody or antigen binding portion thereof;

C is a heavy chain constant domain;

(X1)_n is a linker with the proviso that it is not CH1, wherein said (X1)_n is either present or absent; and

(X2)_n is an Fc region, wherein said (X2)_n is either present or absent;

d) constructing second and fourth polypeptide chains comprising VD1-(X1)_n-VD2-C-(X2)_n, wherein

VD1 is a first light chain variable domain obtained from said first parent antibody or antigen binding portion thereof;

VD2 is a second light chain variable domain obtained from said second parent antibody or antigen binding portion thereof;

C is a light chain constant domain;

(X1)_n is a linker with the proviso that it is not CH1, wherein said (X1)_n is either present or absent; and

(X2)_n does not comprise an Fc region, wherein said (X2)_n is either present or absent;

e) expressing said first, second, third and fourth polypeptide chains;

such that a Dual Variable Domain Immunoglobulin capable of binding said first and said second
 5 antigen is generated, wherein the binding protein is capable of binding a pair of antigens selected from the group consisting of CD-20 and CD-19; CD-20 and CD-80; CD-20 and CD-22; CD-20 and CD-40; CD-3 and HER-2; CD-3 and CD-19; EGFR and HER-2; EGFR and CD-3; EGFR and IGF1,2; EGFR and IGF1R; EGFR and RON; EGFR and HGF; EGFR and c-MET; HER-2 and IGF1,2; HER-2 and IGF1R; RON and HGF; VEGF and EGFR; VEGF and HER-2; VEGF and
 10 CD-20; VEGF and IGF1,2; VEGF and DLL4; VEGF and HGF; VEGF and RON; VEGF and NRP1; CD-20 and CD3; DLL-4 and PLGF; VEGF and PLGF, ErbB3 and EGFR, HER-2 and ErbB3, ErbB3 and HGF; HER-2 and PLGF, and HER-2 and HER-2.

27. The method of claim 26, wherein the VD1 and VD2 heavy chain variable domains comprise an amino acid sequence selected from the group consisting of SEQ ID NOs: 27, 29, 31, 33, 35,
 15 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, and 93 and wherein the VD1 and VD2 light chain variable domains comprise an amino acid sequence selected from the group consisting of SEQ ID NOs: 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, and 94.

20 28. The method of claim 26, wherein said first parent antibody or antigen binding portion thereof, and said second parent antibody or antigen binding portion thereof, are selected from the group consisting of a human antibody, a CDR grafted antibody, and a humanized antibody.

29. The method of claim 26, wherein said first parent antibody or antigen binding portion thereof, and said second parent antibody or antigen binding portion thereof, are selected from the group
 25 consisting of a Fab fragment, a F(ab')₂ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment consisting of the VH and CH1 domains; a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, a dAb fragment, an isolated complementarity determining region (CDR), a single chain antibody, and diabodies.

30 30. The method of claim 26, wherein said first parent antibody or antigen binding portion thereof possesses at least one desired property exhibited by the Dual Variable Domain Immunoglobulin.

31. The method of claim 26, wherein said second parent antibody or antigen binding portion thereof possesses at least one desired property exhibited by the Dual Variable Domain Immunoglobulin.

32. The method of claim 26, wherein the Fc region is selected from the group consisting of a native sequence Fc region and a variant sequence Fc region.
33. The method of claim 26, wherein the Fc region is selected from the group consisting of an Fc region from an IgG1, IgG2, IgG3, IgG4, IgA, IgM, IgE, and IgD.
- 5 34. The method of claim 30, wherein said desired property is selected from one or more antibody parameters.
35. The method of claim 31, wherein said desired property is selected from one or more antibody parameters.
- 10 36. The method of claim 34, wherein said antibody parameters are selected from the group consisting of antigen specificity, affinity to antigen, potency, biological function, epitope recognition, stability, solubility, production efficiency, immunogenicity, pharmacokinetics, bioavailability, tissue cross reactivity, and orthologous antigen binding.
- 15 37. The method of claim 35, wherein said antibody parameters are selected from the group consisting of antigen specificity, affinity to antigen, potency, biological function, epitope recognition, stability, solubility, production efficiency, immunogenicity, pharmacokinetics, bioavailability, tissue cross reactivity, and orthologous antigen binding.
38. The method of claim 26, wherein said first parent antibody or antigen binding portion thereof, binds said first antigen with a different affinity than the affinity with which said second parent antibody or antigen binding portion thereof, binds said second antigen.
- 20 39. The method of claim 26, wherein said first parent antibody or antigen binding portion thereof, binds said first antigen with a different potency than the potency with which said second parent antibody or antigen binding portion thereof, binds said second antigen.
40. A method for generating a Dual Variable Domain Immunoglobulin capable of binding two antigens with desired properties comprising the steps of
- 25 a) obtaining a first parent antibody or antigen binding portion thereof, capable of binding a first antigen and possessing at least one desired property exhibited by the Dual Variable Domain Immunoglobulin;
- b) obtaining a second parent antibody or antigen binding portion thereof, capable of binding a second antigen and possessing at least one desired property exhibited by the Dual Variable Domain Immunoglobulin;
- 30 c) constructing first and third polypeptide chains comprising VD1-(X1)_n-VD2-C-(X2)_n, wherein;
- VD1 is a first heavy chain variable domain obtained from said first parent antibody or antigen binding portion thereof;

VD2 is a second heavy chain variable domain obtained from said second parent antibody or antigen binding portion thereof;

C is a heavy chain constant domain;

(X1)_n is a linker with the proviso that it is not CH1, wherein said (X1)_n is either present or absent; and

(X2)_n is an Fc region, wherein said (X2)_n is either present or absent;

d) constructing second and fourth polypeptide chains comprising VD1-(X1)_n-VD2-C-(X2)_n, wherein;

VD1 is a first light chain variable domain obtained from said first parent antibody or antigen binding portion thereof;

VD2 is a second light chain variable domain obtained from said second parent antibody or antigen binding portion thereof;

C is a light chain constant domain;

(X1)_n is a linker with the proviso that it is not CH1, wherein said (X1)_n is either present or absent; and

(X2)_n does not comprise an Fc region, wherein said (X2)_n is either present or absent;

e) expressing said first, second, third and fourth polypeptide chains;

such that a Dual Variable Domain Immunoglobulin capable of binding said first and said second antigen with desired properties is generated, wherein the binding protein is capable of binding a pair of antigens selected from the group consisting of CD-20 and CD-19; CD-20 and CD-80; CD-20 and CD-22; CD-20 and CD-40; CD-3 and HER-2; CD-3 and CD-19; EGFR and HER-2; EGFR and CD-3; EGFR and IGF1,2; EGFR and IGF1R; EGFR and RON; EGFR and HGF; EGFR and c-MET; HER-2 and IGF1,2; HER-2 and IGF1R; RON and HGF; VEGF and EGFR; VEGF and HER-2; VEGF and CD-20; VEGF and IGF1,2; VEGF and DLL4; VEGF and HGF; VEGF and RON; VEGF and NRP1; CD-20 and CD3; DLL-4 and PLGF; VEGF and PLGF, ErbB3 and EGFR, HER-2 and ErbB3, ErbB3 and HGF; HER-2 and PLGF, and HER-2 and HER-2.

(19) World Intellectual Property Organization
International Bureau



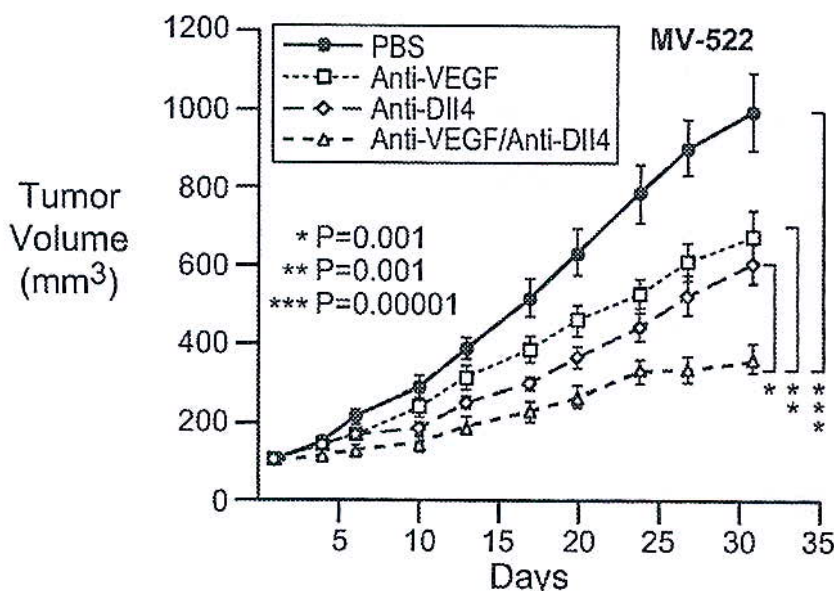
(43) International Publication Date
22 May 2008 (22.05.2008)

PCT

(10) International Publication Number
WO 2008/060705 A2

- (51) International Patent Classification:
C07K 16/00 (2006.01)
- (21) International Application Number:
PCT/US2007/070513
- (22) International Filing Date: 6 June 2007 (06.06.2007)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/811,349 6 June 2006 (06.06.2006) US
60/811,357 6 June 2006 (06.06.2006) US
60/866,772 21 November 2006 (21.11.2006) US
60/866,767 21 November 2006 (21.11.2006) US
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- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, 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, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, 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, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:
— without international search report and to be republished upon receipt of that report

(54) Title: ANTI-DLL4 ANTIBODIES AND METHODS USING SAME



(57) Abstract: The invention provides anti-DLL4 antibodies, and compositions comprising and methods of using these antibodies.

ANTI-DLL4 ANTIBODIES AND METHODS USING SAME

FIELD OF THE INVENTION

5 The present invention relates generally to the field of molecular biology. More specifically, the invention concerns anti-DLL4 antibodies, and uses of same.

BACKGROUND OF THE INVENTION

10 Development of a vascular supply is a fundamental requirement for many physiological and pathological processes. Actively growing tissues such as embryos and tumors require adequate blood supply. They satisfy this need by producing pro-angiogenic factors, which promote new blood vessel formation via a process called angiogenesis. Vascular tube formation is a complex but orderly biological event involving all or many of the following steps: a) endothelial cells (ECs) proliferate from existing ECs or differentiate
15 from progenitor cells; b) ECs migrate and coalesce to form cord-like structures; c) vascular cords then undergo tubulogenesis to form vessels with a central lumen; d) existing cords or vessels send out sprouts to form secondary vessels; e) primitive vascular plexus undergo further remodeling and reshaping; and f) peri-endothelial cells are recruited to encase the endothelial tubes, providing maintenance and modulatory functions to the vessels; such
20 cells including pericytes for small capillaries, smooth muscle cells for larger vessels, and myocardial cells in the heart. Hanahan, *Science* 277:48-50 (1997); Hogan & Kolodziej, *Nat. Rev. Genet.* 3:513-23 (2002); Lubarsky & Krasnow, *Cell* 112:19-28 (2003).

25 It is now well established that angiogenesis is implicated in the pathogenesis of a variety of disorders. These include solid tumors and metastasis, atherosclerosis, retrolental fibroplasia, hemangiomas, chronic inflammation, intraocular neovascular diseases such as proliferative retinopathies, e.g., diabetic retinopathy, age-related macular degeneration (AMD), neovascular glaucoma, immune rejection of transplanted corneal tissue and other tissues, rheumatoid arthritis, and psoriasis. Folkman et al., *J. Biol. Chem.* 267:10931-34 (1992); Klagsbrun et al., *Annu. Rev. Physiol.* 53:217-39 (1991); and Garner A., "Vascular diseases," In: *Pathobiology of Ocular Disease. A Dynamic Approach*, Garner A.,
30 Klintworth GK, eds., 2nd Edition (Marcel Dekker, NY, 1994), pp 1625-1710.

In the case of tumor growth, angiogenesis appears to be crucial for the transition from hyperplasia to neoplasia, and for providing nourishment for the growth and metastasis of the tumor. Folkman et al., *Nature* 339:58 (1989). The neovascularization

31

In one aspect, the invention provides an isolated anti-DLL4 antibody, wherein a full length IgG form of the antibody specifically binds human DLL4 with a binding affinity of about 1 nM or better, or about 500 pM or better. As is well-established in the art, binding affinity of a ligand to its receptor can be determined using any of a variety of assays, and expressed in terms of a variety of quantitative values. Accordingly, in one embodiment, the binding affinity is expressed as K_d values and reflects intrinsic binding affinity (e.g., with minimized avidity effects). Generally and preferably, binding affinity is measured in vitro, whether in a cell-free or cell-associated setting. Any of a number of assays known in the art, including those described herein, can be used to obtain binding affinity measurements, including, for example, Biacore®, radioimmunoassay (RIA) and ELISA. In some embodiments, the isolated anti-DLL4 antibody binds to both human and mouse DLL4 with similar affinity, i.e. the binding affinity for human DLL4 is no more than 100-fold more or less than the binding affinity for mouse DLL4. In some embodiments, the binding affinity for human DLL4 is no more than 10-fold more or less than the binding affinity for mouse DLL4. In some embodiments, the antibody specifically binds mouse DLL4 with a binding affinity of about 1 nM or better, or about 500 pM or better.

In one aspect, the invention provides an isolated anti-DLL4 antibody, wherein a full length IgG form of the antibody specifically binds human DLL4 with a k_{on} of about 2×10^5 or better, or about 1×10^5 or better. As is well-established in the art, the k_{on} of binding of a ligand to its receptor can be determined using any of a variety of assays and expressed in terms of a variety of quantitative values.

In one aspect, the invention provides an isolated antibody that binds a ligand binding region of DLL4. In some embodiments, the isolated antibody binds a polypeptide comprising, consisting of or consisting essentially of the DLL4 extracellular domain. In some embodiments, the isolated antibody binds a polypeptide comprising, consisting of or consisting essentially of amino acids 252-282, 1-252, 1-286, 1-324, and/or 219-286 of human DLL4.

In one aspect, the invention provides an isolated anti-DLL4 antibody that competes with Notch receptor for binding of DLL4.

In one aspect, the invention provides an isolated anti-DLL4 antibody that inhibits, reduces, and/or blocks DLL4 biological activity.

In one aspect, an anti-DLL4 antibody of the invention comprises:

(a) at least one, two, three, four, five or six hypervariable region (HVR) sequences selected from the group consisting of:

homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA*, **90**:6444-6448 (1993) has provided an alternative mechanism for making

5 bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the VH and VL domains of one fragment are forced to pair with the complementary VL and VH domains of another fragment, thereby forming two antigen-binding sites. Another strategy for

10 making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See Gruber *et al.*, *J. Immunol.*, **152**:5368 (1994).

Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt *et al. J. Immunol.* **147**: 60 (1991).

Multivalent Antibodies

15 A multivalent antibody may be internalized (and/or catabolized) faster than a bivalent antibody by a cell expressing an antigen to which the antibodies bind. The antibodies of the present invention can be multivalent antibodies (which are other than of the IgM class) with three or more antigen binding sites (e.g. tetravalent antibodies), which can be readily produced by recombinant expression of nucleic acid encoding the

20 polypeptide chains of the antibody. The multivalent antibody can comprise a dimerization domain and three or more antigen binding sites. The preferred dimerization domain comprises (or consists of) an Fc region or a hinge region. In this scenario, the antibody will comprise an Fc region and three or more antigen binding sites amino-terminal to the Fc region. The preferred multivalent antibody herein comprises (or consists of) three to about

25 eight, but preferably four, antigen binding sites. The multivalent antibody comprises at least one polypeptide chain (and preferably two polypeptide chains), wherein the polypeptide chain(s) comprise two or more variable domains. For instance, the polypeptide chain(s) may comprise VD1-(X1)_n-VD2-(X2)_n-Fc, wherein VD1 is a first variable domain, VD2 is a second variable domain, Fc is one polypeptide chain of an Fc region, X1

30 and X2 represent an amino acid or polypeptide, and n is 0 or 1. For instance, the polypeptide chain(s) may comprise: VH-CH1-flexible linker-VH-CH1-Fc region chain; or VH-CH1-VH-CH1-Fc region chain. The multivalent antibody herein preferably further comprises at least two (and preferably four) light chain variable domain polypeptides. The multivalent antibody herein may, for instance, comprise from about two to about eight

kinase 1 (ALK1), an EC specific type I TGF β receptor, resulted in a primitive EC network in the yolk sacs and arteriovenous malfunction (AVM), a phenotype shared by mice with defective Notch signaling (Urness, L. D. et al, *Nat Genet* 26, 328-31 (2000); Iso, T. et al, *Arterioscler Thromb Vasc Biol* 23, 543-53 (2003)). This led us to investigate the possible connection between these two pathways. We found that the expression of TGF β 2 (Fig. 8b) was tightly linked to the activation status of Notch, suggesting that TGF β pathway could act downstream of Notch pathway. Together, our findings support a model wherein the DLL4/Notch axis, serving as a "signaling router", integrates VEGF signaling through regulating DLL4 expression and engages TGF β pathway to promote EC differentiation.

Example 10: Treatment with anti-DLL4 antibody inhibited tumor growth *in vivo*

To directly address the possible role of DLL4/Notch signaling during tumor angiogenesis, we investigated the impact of blocking DLL4 on tumor growth in preclinical tumor models (Figs. 9a-d). In HM7, Colo205 and Calu6 xenograft tumor models (Figs. 9a-c), YW26.82 treatment was initiated after tumor establishment (≥ 250 mm³ in tumor size). In all three models, a separation in growth rates between the control and treatment groups became evident three days after dosing. The tumor volume of the treatment group remained static over two weeks of treatment. In addition to subcutaneous tumors, anti-DLL4 Mab also inhibited tumors growing in mouse mammary fat pads. In the MDA-MB-435 tumor model, treatment was started 14 days post tumor cell injection. A difference in tumor growth curves between the control and treatment groups was evident within six days after dosing and became increasingly significant as treatment continued (Fig. 9d).

We also investigated the impact of blocking DLL4 and/or VEGF on numerous tumor growth in preclinical tumor models (Figs. 9e-f; i-p). In MV-522 and WEHI3 xenograft tumor models, YW26.82 treatment and/or anti-VEGF treatment was initiated after tumor establishment (≥ 250 mm³ in tumor size). In the MV-522 model, both YW26.82 and anti-VEGF treatment inhibited tumor growth individually, but the combination of the two treatments was most effective. In the WEHI3 model, anti-VEGF treatment showed no effect on tumor growth whereas treatment with YW26.82 showed significant inhibition of tumor growth. In the SK-OV-3X1, LL2, EL4, H1299, SKMES-1, MX-1, SW620 and LS174T models, YW26.82 treatment (5 mg/kg, IP, twice weekly) and/or anti-VEGF treatment (5 mg/kg, IP, twice weekly) was administered after establishment of the tumors. In each of these models, YW26.82 treatment inhibited tumor growth alone. Furthermore,

WHAT IS CLAIMED IS:

1. An isolated anti-DLL4 antibody comprising:

(a) at least one, two, three, four or five hypervariable region (HVR) sequences selected from the group consisting of:

- 5 (i) HVR-L1 comprising sequence A1-A11, wherein A1-A11 is RASQDVSTAVA (SEQ ID NO: 10)
- (ii) HVR-L2 comprising sequence B1-B7, wherein B1-B7 is SASFLYS (SEQ ID NO:11)
- 10 (iii) HVR-L3 comprising sequence C1-C9, wherein C1-C9 is QQSYTGTVT (SEQ ID NO:18)
- (iv) HVR-H1 comprising sequence D1-D10, wherein D1-D10 is GFTFTDNWIS (SEQ ID NO:1);
- (v) HVR-H2 comprising sequence E1-E18, wherein E1-E18 is GYISPNSGFTYYADSVKG (SEQ ID NO:8) and
- 15 (vi) HVR-H3 comprising sequence F1-F15, wherein F1-F15 is VYYCARDNFGGYFDY (SEQ ID NO:9); and
- (b) at least one variant HVR, wherein the variant HVR sequence comprises modification of at least one residue of the sequence depicted in SEQ ID NOs:1-18.

20 2. An isolated anti-DLL4 antibody comprising:

(a) at least one, two, three, four or five hypervariable region (HVR) sequences selected from the group consisting of:

- (i) HVR-L1 comprising sequence A1-A11, wherein A1-A11 is RASQDVSTAVA (SEQ ID NO: 10)
- 25 (ii) HVR-L2 comprising sequence B1-B7, wherein B1-B7 is SASFLYS (SEQ ID NO:11)
- (iii) HVR-L3 comprising sequence C1-C9, wherein C1-C9 is QQSYNGPST (SEQ ID NO:15)
- (iv) HVR-H1 comprising sequence D1-D10, wherein D1-D10 is GFTFTDNWIS
- 30 (SEQ ID NO:1)
- (v) HVR-H2 comprising sequence E1-E18, wherein E1-E18 is GVINPNSGATEYADSVKG (SEQ ID NO:5) and
- (vi) HVR-H3 comprising sequence F1-F15, wherein F1-F15 is VYYCARDNFGGYFDY (SEQ ID NO:9); and

(b) at least one variant HVR, wherein the variant HVR sequence comprises modification of at least one residue of the sequence depicted in SEQ ID NOs:1-18.

3. The antibody of claim 1 or 2, wherein a HVR-L3 variant comprises 1-6 (1, 2, 3, 4, 5, or 6) substitutions in any combination of the following positions: 91 (S or W), 92 (Y or F), 93 (T, N or S), 94 (T or G), 95 (P, Q, A or T), and/or 96 (P, S, A, or V).

4. The antibody of claim 1 or 2, wherein a HVR-H2 variant comprises 1-4 (1, 2, 3, or 4) substitutions in any combination of the following positions: 50 (V, L or Y), 52 (N or S), 52a (P or S), or 53 (N, Q, T, or I).

5. An isolated anti-DLL4 antibody comprising one, two, three, four, five or six HVRs, wherein each HVR comprises, consists or consists essentially of a sequence selected from the group consisting of SEQ ID NOs: 1-18, and wherein SEQ ID NO:10 corresponds to an HVR-L1, SEQ ID NO:11 corresponds to an HVR-L2, SEQ ID NO:12, 13, 14, 15, 16, 17, or 18 correspond to an HVR-L3, SEQ ID NO:1 or 2 correspond to an HVR-H1, SEQ ID NO:3, 4, 5, 6, 7 or 8 correspond to an HVR-H2, and SEQ ID NO:9 corresponds to an HVR-H3.

6. The antibody of claim 5, wherein the antibody comprises HVR-L1, HVR-L2, HVR-L3, HVR-H1, HVR-H2, and HVR-H3, wherein each, in order, comprises SEQ ID NO:10, 11, 14, 2, 3, and 9.

7. The antibody of claim 5, wherein the antibody comprises HVR-L1, HVR-L2, HVR-L3, HVR-H1, HVR-H2, and HVR-H3, wherein each, in order, comprises SEQ ID NO:10, 11, 15, 1, 5, and 9.

8. The antibody of claim 5, wherein the antibody comprises HVR-L1, HVR-L2, HVR-L3, HVR-H1, HVR-H2, and HVR-H3, wherein each, in order, comprises SEQ ID NO:10, 11, 16, 1, 6, and 9.

9. The antibody of claim 5, wherein the antibody comprises HVR-L1, HVR-L2, HVR-L3, HVR-H1, HVR-H2, and HVR-H3, wherein each, in order, comprises SEQ ID NO:10, 11, 17, 1, 7, and 9.

10. The antibody of claim 5, wherein the antibody comprises HVR-L1, HVR-L2, HVR-L3, HVR-H1, HVR-H2, and HVR-H3, wherein each, in order, comprises SEQ ID NO:10, 11, 18, 1, 8, and 9.

5 11. The antibody of any of claims 1-10, wherein at least a portion of the framework sequence is a human consensus framework sequence.

12. The antibody of claim 1 or 2, wherein the modification is substitution, insertion or deletion.

10

13. The antibody of any of claims 1-12, wherein the antibody comprises human κ subgroup consensus framework sequence.

14. The antibody of any of claims 1-12, wherein the antibody comprises heavy
15 chain human subgroup III consensus framework sequence.

15. The antibody of claim 14, wherein the antibody comprises a substitution at one or more of position 71, 73 or 78.

20 16. The antibody of claim 15, wherein the substitution is one or more of R71A, N73T, or N78A.

17. A polynucleotide encoding an antibody of any of claims 1-16.

25 18. A vector comprising the polynucleotide of claim 17.

19. The vector of claim 18, wherein the vector is an expression vector.

20. A host cell comprising a vector of claim 18 or 19.

30

21. The host cell of claim 20, wherein the host cell is prokaryotic.

22. The host cell of claim 20, wherein the host cell is eukaryotic.

23. The host cell of claim 20, wherein the host cell is mammalian.
24. A method for making an anti-DLL4 antibody, said method comprising (a) expressing a vector of claim 19 in a suitable host cell, and (b) recovering the antibody.
- 5 25. A method for making an anti-DLL4 immunoconjugate, said method comprising (a) expressing a vector of claim 19 in a suitable host cell, and (b) recovering the antibody.
- 10 26. The method of claim 24 or 25, wherein the host cell is prokaryotic.
27. The method of claim 24 or 25, wherein the host cell is eukaryotic.
- 15 28. A method for detection of DLL4, the method comprising detecting DLL4-anti-DLL4 antibody complex in a biological sample.
29. A method for diagnosing a disorder associated with DLL4 expression, the method comprising detecting DLL4-anti-DLL4 antibody complex in a biological sample from a patient having or suspected of having the disorder.
- 20 30. The method of any of claims 28-29, wherein the anti-DLL4 antibody is detectably labeled.
31. A composition comprising an anti-DLL4 antibody of any of claims 1-16.
- 25 32. A composition comprising a polynucleotide of any of claims 17-19.
33. The composition of claim 31 or 32, wherein the composition further comprises a carrier.
- 30 34. A method for treating a tumor, cancer, or cell proliferative disorder comprising administering an effective amount of an antibody of any of claims 1-16 to a subject in need of such treatment, whereby the tumor, cancer or cell proliferative disorder is treated.

35. The method of claim 34, wherein the tumor, cancer or cell proliferative disorder is colon cancer, lung cancer or breast cancer.

5 36. The method of claim 34 or 35, further comprising administering an effective amount of an anti-angiogenic agent.

37. The method of claim 36, wherein the anti-angiogenic agent is an antagonist of vascular endothelial growth factor (VEGF).

10 38. The method of claim 37, wherein the VEGF antagonist is an anti-VEGF antibody.

39. The method of claim 38, wherein the anti-VEGF antibody is bevacizumab.

15 40. The method of any of claims 34-39 further comprising administering an effective amount of a chemotherapeutic agent.

20 41. A method of enhancing efficacy of an anti-angiogenic agent in a subject having a pathological condition associated with angiogenesis, comprising administering to the subject an effective amount of an antibody of any of claims 1-16 in addition to the anti-angiogenic agent, thereby enhancing said anti-angiogenic agent's efficacy.

25 42. The method of claim 41, wherein the pathological condition associated with angiogenesis is a tumor, a cancer, and/or a cell proliferative disorder.

43. The method of claim 41, wherein the pathological condition associated with angiogenesis is an intraocular neovascular disease.

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
14 February 2008 (14.02.2008)

PCT

(10) International Publication Number
WO 2008/019144 A2

(51) International Patent Classification:
A61K 39/39 (2006.01)

(21) International Application Number:
PCT/US2007/017546

(22) International Filing Date: 7 August 2007 (07.08.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/836,003 7 August 2006 (07.08.2006) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, 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, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, 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, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- without international search report and to be republished upon receipt of that report
- with sequence listing part of description published separately in electronic form and available upon request from the International Bureau



WO 2008/019144 A2

(54) Title: THERAPEUTIC METHODS FOR TREATING VASCULAR EYE DISORDERS WITH DLL4 ANTAGONISTS

(57) Abstract: A therapeutic method for treating ischemic or vascular disorders by administering an agent capable of inhibiting human delta-like ligand 4 (Dll4) activity to a subject in need thereof. Preferably, the agent is an anti-Dll4 antibody or antibody fragment capable of inhibiting the binding of Dll4 to a Notch receptor. In one embodiment, the ischemic or vascular disorder is ischemic injury, cerebral ischemia, cardiac ischemia, ischemic conditions affecting the limbs and other organs or tissues, arteriovenous malformations, wound healing, organ or tissue transplantation, placental insufficiency, arterial narrowing and occlusion, atherosclerosis, and systemic or pulmonary hypertension. In another embodiment, the disease treated is an eye disease or condition such as retinopathy of prematurity, ischemic retinopathy, retinal vein or artery occlusion, diabetic retinopathy, choroidal neovascularization, age related macular degeneration, corneal neovascularization, neovascular glaucoma or corneal transplantation.

Therapeutic Methods for Treating Vascular Eye Disorders with Dll4 Antagonists

Field of the Invention

[0001] This invention relates generally to methods for treating vascular and ischemic disorders by administering compositions that inhibit Dll4 interaction with Notch receptors, and/or Notch receptor activation. More specifically, this invention relates to methods of treating eye vascular and/or ischemic disorders by administering compositions that inhibit Dll4 interaction with Notch receptors.

Background

[0002] Angiogenesis is a fundamental process required for the normal development and growth of tissues and organs, and involves formation of new blood vessels from pre-existing vessels. Angiogenesis and blood vessel homeostasis are tightly controlled through a highly regulated system of angiogenic modulators. Deviation from such a tight control often leads to or is associated with disease.

[0003] While unregulated "excessive" or aberrant angiogenesis is characteristic of numerous disease states, insufficient angiogenesis, loss of blood vessels or functional or structural blood vessel occlusion can also be a serious medical problem. Promoting angiogenesis and/or preventing regression of existing blood vessels is desirable in situations where tissue becomes ischemic, for example in retinal, cerebral, cardiovascular and limb ischemia and in conditions where a vascular supply must be established, re-established, enhanced or expanded, for example in wound healing and after tissue or organ transplantation, or to stimulate establishment of collateral vessels or otherwise increase the perfusion of a tissue or organ with inadequate circulation.

[0004] The Notch-signaling pathway is a system for cell-to-cell communication used by a wide range of eukaryotes for many biological processes, such as differentiation, proliferation, and homeostasis (Artavanis-Tsakonas et al. (1999) Science 284:770-776). Notch signaling has also been implicated in the control of vascular development (Iso et al. (2003) Arterioscler Thromb Vasc Biol. 23:543-553).

[0005] Delta-like 4 (Dll4) or delta-like ligand 4 (Dll4) (hereinafter "Dll4") is a member of the Delta family of Notch ligands which exhibits highly selective expression by vascular endothelium (Shutter et al. (2000) Genes Dev. 14:1313-1318). Dll4 is a ligand for Notch receptors, including Notch1 and Notch 4. The nucleic acid and amino acid sequences for human and mouse Dll4 are shown in SEQ ID NO:1-2 and SEQ ID NO:3-4, respectively. Gene-targeted Dll4 mice have been generated (see, for example, Duarte et al. (2004) Genes Dev. 18:2474 - 2478; Krebs et al. (2004) Genes Dev. 18:2469 - 2473; Gale et al. (2004) Proc Natl Acad Sci USA 101:15949-15954). These studies showed that Dll4 was highly expressed on developing blood vessels in the mouse embryo, and that genetic deletion of even a single Dll4 allele resulted in embryonic lethality, associated with marked

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CANT.	RENTISTICO	CONCEPTO	Vr. UNIDITARIO	Vr. CONCEPTO		
1	50005-01-04	PRESENTACION DE OBSERVACIONES A SOLICITUDES	1632	OPOSICION NUEVA CRACION / PRESENTACION	315.000.00	315.000.00
					\$315.000.00	

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*** Conceptos Pagados ***

CANT.	RENTISTICO	CONCEPTO	Vr. LINDITARIO	Vr. CONCEPTO
1	50005-01-08	PRORROGA DE TERMINOS	100.000.00	100.000.00
		1608 N. CREACION PRORROGA DE TERMINOS O PLAZO ADICIONAL		\$100.000.00
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