

Fecha: 2009-07-31 14:52:45 Dep. 2020 NUECREACIONE
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 400 OPOSIOBSERVTER Folios: 40

FORMULARIO ÚNICO DE OPOSICIÓN

OPOSICIÓN A:

- | | |
|--|--|
| ☒ Patente de Invención. | ☐ Marca Colectiva. |
| ☐ Patente de Modelo de Utilidad. | ☐ Marca de Certificación. |
| ☐ Diseño Industrial. | ☐ Lema Comercial. |
| ☐ Esquema de Trazado para Circuitos Integrados. | |
| ☐ Marcas de Productos o Servicios. | |

**SOLICITUD
PUBLICADA**

Numero del expediente: 07-129828

Solicitante: GALAXY BIOTECH, LLC KENNEDY KRIEGER
INSTITUTE

Apoderado: DILIA MARIA RODRIGUEZ D ALEMAN

Título de la nueva creación o denominación del signo: **MÉTODOS PARA EL TRATAMIENTO DE TUMORES DE CEREBRO CON ANTICUERPOS**

Gaceta: 603

③ OPOSICIÓN PRESENTADA POR:

SOLICITANTE	Nombre: PROCAPS S.A. Dirección: CALLE 80 No. 78b-201 Nacionalidad o Domicilio: COLOMBIA Lugar de Constitución: BARRANQUILLA	IDENTIFICACIÓN C.C. ☐ NIT ☒ C.E. ☐ Otro ☐ Cual _____ Número <u>890.106.527-5</u>
	Teléfono: 371 99 00 Fax: 373 08 18 E-mail: _____	
REPRESENTANTE O APODERADO	Nombre: ELIANA ISAURA MORENO BOHORQUEZ Dirección: CARRERA 13 No. 119-95 Teléfono: (571)213 12 13 Fax: (571)612 19 79 E-mail: negocios@optimum-co.com	IDENTIFICACIÓN C.C. ☒ NIT ☐ C.E. ☐ Otro ☐ Cual _____ Número <u>51.975.664</u> TP <u>83823</u>

④ FUNDAMENTOS DE LA OPOSICIÓN:

Art.14, 16, 18, 20 y 30 de la Decisión 486 de la CAN

Causal: No cumple con los requisitos de patentabilidad.

Signo opositor: _____

Justificación:

La presente solicitud no cumple con los requisitos de patentabilidad dado que lo revelado, ya se encontraba en el estado de la técnica o es posible derivarlo de la misma tal y como se demostrará en el capítulo “Fundamento de Hechos”.

⑤ Comprobante de pago No. _____ Fecha _____

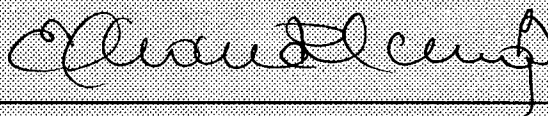
⑥ ANEXOS

- ☒ Comprobante de pago de la tasa.
- ☐ Poderes, si tuere el caso.
- ☐ Pruebas de los fundamentos de la oposición.
- ☐ Documentos que acrediten el legítimo interés, si tuere el caso.
- ☐ Documento que acredita la existencia y representación legal de las partes, cuando sean personas jurídicas.

⑦

NOMBRE: ELIANA ISAURA MORENO BOHORQUEZ

FIRMA:



C.C. 51'975.664

TP. 83823

Señora Jefe,

DIVISIÓN DE NUEVAS CREACIONES

Superintendencia de Industria y Comercio

E. S. D.

Referencia : Expediente N° 07 129828 ✓
Asunto : Oposición a la solicitud de patente de Invención titulada
"MÉTODOS PARA EL TRATAMIENTO DE TUMORES
DE CEREBRO CON ANTICUERPOS"
Solicitante : GALAXY BIOTECH, LLC KENNEDY KRIEGER
INSTITUTE
Opositora : PROCAPS S.A.

Publicada en la Gaceta de la Propiedad Industrial
N° 603 del 30 de Abril de 2009

Yo, **ELIANA ISAURA MORENO BOHORQUEZ**, abogada en ejercicio, identificada como aparece al pie de su firma, con tarjeta profesional No. 83823 del Consejo Superior de la Judicatura, en mi condición de apoderada de la sociedad **PROCAPS S.A.** conformada bajo las leyes de la República de Colombia y domiciliada en la ciudad de Barranquilla, Colombia, atentamente manifiesto que estando dentro del término de ley y por orden expresa de mi representada, presento oposición a la concesión del registro de la Patente de Invención titulada "**MÉTODOS PARA EL TRATAMIENTO DE TUMORES DE CEREBRO CON ANTICUERPOS**", cuyo titular es **GALAXY BIOTECH, LLC KENNEDY KRIEGER INSTITUTE**., y pido a

Usted declarar fundada esta oposición y en consecuencia negar el registro de la patente solicitada.

1. INTERÉS PARA ACTUAR

Conforme a lo dispuesto en el artículo 42 de la Decisión 486 de 2000 de la CAN, estando dentro del término estipulado por la ley, manifiesto el legítimo interés de nuestra representada para oponerse al registro de la patente de la referencia, atendiendo a que la solicitud pretendida no cumple con los requisitos dispuestos en la ley como se analizará más adelante y de ser concedida afectaría directamente la comercialización de ~~algunos~~ productos de mi representada, que contienen, en particular anticuerpo monoclonal (mAb). Por lo tanto, solicito respetuosamente a ese Despacho se sirva tener en cuenta la presente oposición dentro del trámite administrativo que se surte en esta Superintendencia.

2. FUNDAMENTOS DE HECHOS

- 2.1. La solicitud objeto de la presente fue presentada el 7 de diciembre de 2007 reivindicando prioridad bajo la solicitud US20050687118P del 2005-06-02; y fue publicada en Colombia, en la Gaceta de la Propiedad Industrial N° 603 del 30 de Abril de 2009, cuya publicación internacional corresponde a la WO2006130773 del 2006-12-07.
- 2.2. La solicitud colombiana 07 129828 objeto de la presente oposición, se refiere a un método para el tratamiento de un tumor de cerebro en un paciente, caracterizado porque comprende administrar un anticuerpo monoclonal (mAb) a un paciente

que tiene un tumor de cerebro y mediante esto tratar el tumor de cerebro; donde el anticuerpo monoclonal es quimérico, humanizado o humano donde el anticuerpo monoclonal es un anticuerpo monoclonal anti-HGF neutralizante; donde el anticuerpo monoclonal es un anticuerpo monoclonal L2G7 humanizado donde el anticuerpo monoclonal es administrado intravenosamente y donde el tumor del cerebro es un glioma.

2.3. En primera instancia, se debe advertir que las reivindicaciones se refieren a un método de tratamiento y al uso como tal, y no sólo a la composición farmacológica o a un método de fabricación mediante la cual se obtenga un producto tangible y según la legislación, decisión 486 de la Comunidad Andina de Naciones, Artículo 14.- *“Los Países Miembros otorgarán patentes para las invenciones, sean de producto o de procedimiento (...)”*, los usos no están contemplados como patentables; y, Artículo 20.- *“No serán patentables (...) d) los métodos terapéuticos o quirúrgicos para el tratamiento humano o animal, así como los métodos de diagnóstico aplicados a los seres humanos o a animales”*. Solo serán patentables los productos o procedimientos. Por lo tanto a luz de la norma, dicha materia referente al uso y a un método de tratamiento no sería patentable.

2.4. Ahora bien, con base en el artículo 14 de la Decisión 486 de la CAN consagra lo siguiente: *“Los países miembros otorgarán patentes para las invenciones, sean de producto o procedimiento, en todos los campos de la tecnología, siempre que sean nuevas, tengan nivel inventivo y sean susceptibles de aplicación industrial”*. Así las cosas, las invenciones objeto de patente deben ser nuevas y tener nivel inventivo. Bien, pues antes de la fecha de prioridad de esta solicitud se encuentran los siguientes documentos que afecta la patentabilidad de la presente solicitud:

- D1: US2005019327 publicada el 27 de enero de 2005.

2.5. Ahora bien, el documento D1, particularmente en la descripción revela el uso de un anticuerpo monoclonal L2G7 el cual es un anticuerpo de neutralización anti-HGF y donde el anticuerpo anti-HGF de dicha anterioridad D1 revela que opcionalmente incluye cimerito, humanizado, anti-HGF humano mAbs y más particularmente un anticuerpo humanizado L2G7 como se indica en la página 4, párrafo 0031 a página 5, párrafo 0036. Según esta anterioridad se revelan métodos para tratar el glioma de tumor cerebral, glioblastoma en pacientes humanos por vía intravenosa de un anticuerpo anti-HGF según se ilustra en la página 3 párrafo 0029, página 5 párrafo 0037, página 6 párrafo 0043 y página 9 párrafo 0068. Así las cosas, como lo evidencia dicho documento D1, además que la presente no cumple con lo que dicta la norma respecto a los métodos terapéuticos y los usos, tampoco lo ilustrado es novedoso pues lo revelado ya se encontraba en el estado de la técnica. A los ojos de la anterioridad D1, la presente carece evidentemente de NOVEDAD.

2.6. En consecuencia y de acuerdo a lo descrito anteriormente, se deriva que la solicitud colombiana 07 129828 no puede pretender un monopolio por patente toda vez que lo que pretende no cumple con dos de los tres requisitos necesarios para su protección.

2.7. Que por consiguiente, respecto a la solicitud pretendida se tiene que:

2.7.1. CARECE DE NOVEDAD: Según la decisión 486 de la Comunidad Andina de Naciones, Artículo 16.- *“Una invención se considerará nueva cuando no está comprendida en el estado de la técnica. El estado de la técnica comprenderá todo lo que haya sido accesible al público por una descripción escrita u oral, utilización, comercialización o cualquier otro medio antes de la fecha de presentación de la solicitud*

de patente o, en su caso, de la prioridad reconocida.” A la fecha de prioridad, 2 de junio de 2005 ya era conocido, pues como se demostró, era parte del arte anterior con base en el documento D1, un método para el tratamiento de un tumor de cerebro en un paciente, caracterizado porque comprende administrar un anticuerpo monoclonal (mAb), sobre el cual busca protección en el capítulo reivindicatorio.

2.7.2. CARENCIA DE APLICACIÓN INDUSTRIAL La Decisión 486 de la CAN consagra en su artículo 14 lo siguiente: “Los países miembros otorgarán patentes para las invenciones, sean de producto o procedimiento, en todos los campos de la tecnología, siempre que sean nuevas, tengan nivel inventivo y sean susceptibles de aplicación industrial”. La presente solicitud carece de aplicación Industrial ya que como se demostró anteriormente, algunas cláusulas reclaman el método terapéutico y estos no son aplicables industrialmente porque primero no se obtiene un producto o proceso; y segundo no es reproducible o repetible a escala industrial. Por lo tanto, el objeto de la presente invención referido a los usos y métodos terapéuticos, no cumplen con el requisito de la aplicación industrial.

2.8. Dado lo anterior, es posible deducir que la solicitud y lo reivindicado no es objeto de patentabilidad pues es contrario a lo que dicta la Decisión 486 de la Comunidad Andina de Naciones, Artículo 14.- “Los Países Miembros otorgarán patentes para las invenciones, sean de producto o de procedimiento, en todos los campos de la tecnología, siempre que sean nuevas, tengan nivel inventivo y sean susceptibles de aplicación industrial.”. La solicitud pretendida no cumple con dos de los tres requisitos de patentabilidad.



Derecho de la Competencia, Propiedad
Industrial y Comercio Exterior

2.9. Por todo lo anterior, atentamente solicito a usted que se sirva declarar fundada la presente oposición y negar el registro Patente de invención denominada **“MÉTODOS PARA EL TRATAMIENTO DE TUMORES DE CEREBRO CON ANTICUERPOS”**, cuyo titular es **GALAXY BIOTECH, LLC KENNEDY KRIEGER INSTITUTE**, solicitud Colombiana que apareció publicada en la Gaceta de la Propiedad Industrial N° 603 del 30 de Abril de 2009.

3. PRUEBAS

De conformidad con lo dispuesto por las normas vigentes, solicito a usted tener como prueba en este asunto los siguientes:

- D1: US2005019327 publicada el 27 de enero de 2005.

4. FUNDAMENTO DE DERECHO

Fundamento esta observación en las normas legales siguientes:

- 4.1. Numeral 1 del artículo 586 del Código de Comercio.
- 4.2. Artículo 42 de la Decisión 486 de la Comisión del Acuerdo de Cartagena.
- 4.3. En las demás normas concordantes.

5. ANEXOS



Derecho de la Competencia, Propiedad
Industrial y Comercio Exterior

Para los efectos correspondientes anexo al presente escrito los siguientes documentos:

1. Poder para actuar en el presente.
2. Documento que acredita al representante legal de **PROCAPS S.A.**
3. Recibo de caja del respectivo pago de tasa.
4. Copias de los documentos:
 - D1: US2005019327 publicada el 27 de enero de 2005.



Derecho de la Competencia, Propiedad
Industrial y Comercio Exterior

6. NOTIFICACIONES

Para efectos de notificaciones que por disposición del artículo 50 del Decreto 01 de 1984 debe hacer su Despacho, informo que mi representada y la suscrita recibiremos notificaciones en la Secretaría de su Despacho o en mis oficinas situadas en la Carrera 13 N° 119-95, Of. 104 de esta ciudad de Bogotá.

Señor Superintendente,

ELIANA ISAURA MORENO BOHORQUEZ

C.C. 51'975.664 de Bogotá

T.P.A. 83823 del C.S. de la J.



PODER ESPECIAL

Yo, **WALTER TANAKA TATEKAWA**, mayor de edad y vecino de la ciudad de Barranquilla (Colombia), identificado como aparece al pie de mi firma, quien en el presente documento actúa en calidad de Representante Legal de la sociedad **PROCAPS S.A.** sociedad legalmente constituida y domiciliada en la ciudad de Barranquilla (Colombia), por el presente documento otorgo a la doctora **ELIANA ISAURA MORENO BOHORQUEZ**, abogada en ejercicio, identificada como aparece al pie de su firma, con tarjeta profesional No. 83823 del C.S. de la J., poder especial, amplio y suficiente, para formular oposiciones u observaciones a la solicitud de patente No. 07 129828, titulada "METODOS PARA EL TRATAMIENTO DE TUMORES DE CEREBRO CON ANTICUERPOS", cuyo titular es **GALAXY BIOTECH, LLC KENNEDY KRIEGER INSTITUTE**, publicada en la gaceta No. 603 del 30 de abril de 2009.

Nuestro apoderado queda facultado, de manera expresa, para realizar todo tipo de actos necesarios para llevar a cabo el trámite de la oposición, incluyendo, sin limitación, preparar y presentar solicitudes, hacer declaraciones, pagar tasas, contribuciones e impuestos, recabar los títulos o certificados. Asimismo quedan autorizados para impugnar administrativa y judicialmente todo lo que fuere resuelto en el expediente a que se hace expresa referencia en el presente poder, con todas las facultades generales y específicas que fuera requerido para ella.

De la misma manera, además de las facultades que le confiere la naturaleza del presente mandato, se encuentran facultados para recibir, desistir, transigir, sustituir, y reasumir el presente poder, incoar acciones, interponer recursos y demás facultades conferidas por la ley.

Dado y Firmado en Barranquilla (Colombia) a los 16 días del mes de Julio de 2009.

PROCAPS S.A.

C.C. N° 16'251.923

NIT: 890.106.527-5

Representante Legal

Acepto:

ELIANA ISAURA MORENO BOHORQUEZ

C.C. 51'975.664 de Bogotá

T.P.A. 83823 del C.S. de la J.





NOTARIA TERCERA DEL CIRCULO DE BARRANQUILLA
ALFONSO LUIS AVILA FADUL
DILIGENCIA DE PRESENTACION PERSONAL Y
RECONOCIMIENTO
El suscrito Notario certifica que este escrito fue presentado
personalmente por:
WALTER TANAKA TATEKAWA
Identificado con: 16.251.923 de PALUR
Quien declaró que su contenido es cierto y que la firma puesta en
él es suya.
[Firma]
22 JUL 2009
NOTARIO TERCERO DE BARRANQUILLA
HUELLA DEL
COMPARECIENTE

[Firma]



PRESENTACION PERSONAL
El anterior escrito fue presentado ante el Notario Dieciocho del Circulo de Bogotá
personalmente por EFIRANA ISHURA
ALFONSO MORALES
quien exhibió la c.c. 1975664 de POSTA
y Tarjeta Profesional No. 83823 C.S.J.
Fecha 31 JUL 2009
[Firma]
EL NOTARIO



R. 07-129828

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 07-129828-00004-0000

Fecha: 2009-07-31 14:52:45 Dep. 2020 NUECREACION
Tra. 11 PATENTEIYII Eve: 378 FASENACIONAL
Act. 400 OPOSIOBSERVTER Folios: 40

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 09 - 59,413
FECHA : AGOSTO 11 DE 2009

**** CONSIGNACION ****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
LAB. PROCAPS	RECIBO DE CAJA			56594	31/07/2009	540,000.00
LAB. PROCAPS	RECIBO DE CAJA			56595	31/07/2009	540,000.00
LAB. PROCAPS	RECIBO DE CAJA			56596	31/07/2009	540,000.00

**** CONCEPTO ****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-04	PRESENTACION DE OBSERVACIONES	
12		MARCAS Y LEÑAS COMERCIALES	287,000.00
		TOTAL :	\$ 287,000.00

SON: DOSCIENTOS OCHENTA Y SIETE MIL PESOS

RESPONSABLE :

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____



***** Pag. 1

CAMARA DE COMERCIO
DE BARRANQUILLA

08*****

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.

PROCAPS S.A.-----

NIT: 890.106.527-5-----

EL SUSCRITO SECRETARIO DE LA CAMARA DE COMERCIO DE BARRANQUILLA, CON FUNDAMENTO EN LAS INSCRIPCIONES DEL REGISTRO MERCANTIL:

C E R T I F I C A

Que por Escritura Pública No. 1,190 del 22 de Julio de 1976, otorgada en la Notaria Tercera de Barranquilla, inscrito(as) en esta Cámara de Comercio, el 28 de Julio de 1976 bajo el No. 6,070 del libro respectivo, fue constituida la sociedad----- limitada denominada "PRODUCTORA DE CAPSULAS DE GELATINAS LIMITADA "PROCAPS LIMITADA".-----

C E R T I F I C A

Que por Escritura Pública No. 1,307 del 28 de Junio de 1979, otorgada en la Notaria Tercera de Barranquilla, inscrito(as) en esta Cámara de Comercio, el 23 de Julio de 1979 bajo el No. 10,039 del libro respectivo, la sociedad antes mencionada----- se transformo en sociedad anonima bajo la denominacion social de "PRODUCTORA DE CAPSULAS DE GELATINA S.A." "PROCAPS S.A.".-----

C E R T I F I C A

Que por Escritura Pública No. 3,810 del 31 de Dic/bre de 1980, otorgada en la Notaria Primera de Barranquilla, inscrito(as) en esta Cámara de Comercio, el 30 de Enero de 1981 bajo el No. 12,705 del libro respectivo, la sociedad antes mencionada----- se transformo en sociedad de responsabilidad limitada bajo al razon social de "PRODUCTORA DE CAPSULAS DE GELATINA LIMITADA "PROCAPS LI-MITADA".-----

C E R T I F I C A

Que por Escritura Pública No. 3,755 del 10 de Nov/bre de 1983, otorgada en la Notaria Unica de Soledad, inscrito(as) en esta Cámara de Comercio, el 22 de Nov/bre de 1983 bajo el No. 17,922 del libro respectivo, la sociedad antes mencionada----- se transformo en sociedad anonima bajo la denominacion social de "PRODUCTORA DE CAPSULAS DE GELATINA S.A." "PROCAPS S.A.".-----

C E R T I F I C A

Que por Escritura Pública No. 3,615 del 06 de Dic/bre de 1996, otorgada en la Notaria 2a. de Barranquilla, inscrito(as) en esta Cámara de Comercio, el 20 de Dic/bre de 1996 bajo el No. 67,218 del

***** C O N T I N U A *****

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.

PROCAPS S.A.-----
NIT: 890.106.527-5.

libro respectivo, la sociedad antes mencionada-----
cambio de razon social, por la denominacion LABORATORIOS PROCAPS S.A

C E R T I F I C A

Que por Escritura Pública No. 3,775 del 18 de Dic/bre de 1996,
otorgada en la Notaria 2a. de Barranquilla, inscrito(as) en esta
Cámara de Comercio, el 23 de Enero de 1997 bajo el No. 67,704 del
libro respectivo, la sociedad antes mencionada-----
cambio de razon social, por la denominacion PROCAPS S.A. -----

C E R T I F I C A

Que dicha sociedad ha sido reformada por las siguientes escrituras
y/o documentos privados:

Numero	aaaa/mm/dd	Notaria	No. Insc o Reg	aaaa/mm/dd
39	1979/01/18	Notaria 3a. de Barranquilla.	9,452	1979/01/24
992	1981/04/30	Notaria 1a. de Barranquilla.	13,112	1981/05/06
1,464	1982/08/27	Notaria Unica de Soledad	15,533	1982/09/08
2,744	1983/08/18	Notaria Unica de Soledad	17,416	1983/08/26
3,826	1984/07/16	Notaria Unica de Soledad	19,554	1984/07/27
3,835	1985/08/26	Notaria Unica de Soledad	22,230	1985/09/04
2,260	1986/09/10	Notaria Unica de Soledad	24,995	1986/09/15
1,849	1989/07/11	Notaria 2a. de Barranquilla.	33,909	1989/07/13
444	1990/02/21	Notaria 2a. de Barranquilla.	36,101	1990/03/16
3,090	1991/12/17	Notaria 2a. de Barranquilla.	43,993	1992/01/28
269	1993/02/01	Notaria 2a. de Barranquilla	48,428	1993/02/16
2,342	1993/07/28	Notaria 2a. de Barranquilla	50,582	1993/08/10
3,652	1993/11/09	Notaria 2a. de Barranquilla	51,798	1993/11/22
3,615	1996/12/06	Notaria 2a. de Barranquilla	67,218	1996/12/20
89	1997/01/16	Notaria 2a. de Barranquilla	67,704	1997/01/23
2,070	1997/07/17	Notaria 2a. de Barranquilla	70,627	1997/07/31
2,281	1997/08/04	Notaria 2a. de Barranquilla	70,813	1997/08/14
765	1999/04/22	Notaria 3a. de Barranquilla	80,628	1999/04/23
459	2001/02/21	Notaria 2a. de Barranquilla	91,502	2001/02/23
2,608	2001/10/05	Notaria 2a. de Barranquilla	95,771	2001/11/08
1,730	2004/07/29	Notaria 2. de Barranquilla	112,999	2004/08/26
416	2005/03/03	Notaria 2. de Barranquilla	116,364	2005/03/10
1,613	2005/07/28	Notaria 2. de Barranquilla	119,322	2005/08/18
710	2009/05/05	Notaria 3 a. de Barranquilla	150,130	2009/06/30
1,054	2009/06/25	Notaria 3 a. de Barranquilla	150,130	2009/06/30

C E R T I F I C A

Que de acuerdo con la(s) escritura(s) arriba citada(s), la sociedad
se rige por las siguientes disposiciones:

DENOMINACION O RAZON SOCIAL:

PROCAPS S.A.-----

SIGLA: PROCAPS.

DOMICILIO PRINCIPAL: Barranquilla.

NIT NO: 890.106.527-5.

MATRÍCULA MERCANTIL: 24,802.



***** Pag. 2

CAMARA DE COMERCIO
DE BARRANQUILLA

08*****

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.

PROCAPS S.A.-----

NIT: 890.106.527-5

C E R T I F I C A

Direccion para notificaciones judiciales:

CL 80 No 78 B - 201.

De la ciudad de Barranquilla.

C E R T I F I C A

DURACION: El término de duración de la sociedad se fijó hasta el 18 de Agosto de 2073.

C E R T I F I C A

OBJETO SOCIAL: El objeto principal de la sociedad sera: a) La fabricacion, importacion y comercializacion de alimentos procesados y suplementos dietarios. b) La fabricacion de capsulas y otros productos similares a partir de materiales tales como gelatinas u otros de distinta naturaleza, que puedan ser utilizados como envases o recipientes de productos y sustancias quimicas, farmaceuticas, alimenticias y cosmeticas, para uso y consumo humano y veterinario. c) La investigacion, desarrollo y fabricacion de toda clase de productos y sustancias quimicas, farmaceuticas, alimenticias y cosmeticas para uso y consumo humano, animal y vegetal. d) La compra y venta de materias primas, insumos y productos intermedios necesarios para los procesos de fabricacion de sustancias quimicas, farmaceuticas, alimenticias y cosmeticas, asi como la comercializacion interna y externa de los productos terminados. e) La compra, venta, importacion, exportacion y distribucion de toda clase de elementos instrumentales y equipos medicos, quirurgicos, odontologicos y hospitalarios para la salud humana y animal. f) La compra, venta, exportacion, fabricacion, importacion, distribucion de dulces, confites, galletas y todo tipo de golosinas o alimentos para el consumo humano. g) Actuar como representante o agente de personas naturales y juridicas, nacionales y extranjeras en la realizacion de operaciones o transacciones relacionadas con la industria farmaceutica y en especial con cualquiera de las actividades descritas en los literales a), b), c), d) y e) antes descritos. h) Prestar los servicios de asesorias y consultoria en las areas administrativas, de mercadeo, analisis clinico, asistencia tecnica, investigacion, costos y produccion, exportaciones, importaciones, ventas, relacionadas con iguales o similares actividades a las que constituyen el objeto de PROCAPS S.A., asi como los de publicidad y promocion de productos quimicos, alimenticios, cosmeticos y

***** C O N T I N U A *****

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.

PROCAPS S.A.-----

NIT: 890.106.527-5.

farmaceuticos para uso humano, animal o industrial. i) Adquirir, operar, administrar y explotar, a cualquier titulo, empresa o establecimientos dedicados a la investigacion, desarrollo, fabricacion o comercializacion de productos quimicos, farmaceuticos, alimenticios, hospitalarios o cosmeticos. En desarrollo de su objeto principal, la sociedad podra tomar interes social como accionista, socio o fundador de empresas que tengan igual o similar objeto, comprar, vender, importar y exportar maquinarias, insumos y repuestos requeridos para el desarrollo de sus actividades; tomar dinero en prestamo, financiar y garantizar las operaciones comerciales que celebre con terceros relativas a su objeto; celebrar y ejecutar todo tipo de actos y contratos necesarios para desarrollar sus operaciones; girar, aceptar, otorgar y protestar todo tipo de titulos valores e instrumentos negociables, comprar, vender y constituir gravámenes sobre bienes muebles e inmuebles, designar representantes apoderados en el pais y en el exterior y, en general, realizar cualquier acto o negocio que sea necesario o conveniente para el desarrollo de su objeto social y para la mejor proteccion de sus intereses corporativos o la de sus socios. La sociedad podra garantizar el cumplimiento de sus obligaciones adquiridas por terceros o por sus socios, para lo cual podra inclusive constituir gravámenes reales sobre sus bienes de cualquier naturaleza.

C E R T I F I C A

CAPITAL

Nro Acciones

Valor Acción

Autorizado

\$*****2,000,000,000 *****2,000,000 *****1,000

Suscrito

\$*****1,229,071,000 *****1,229,071 *****1,000

Pagado

\$*****1,229,071,000 *****1,229,071 *****1,000

C E R T I F I C A

ADMINISTRACION: La direccion y administracion de la sociedad corresponde a la Asamblea General de Accionistas, a la Junta Directiva, al Presidente, al Vicepresidente y a un Gerente General. Corresponde a la Junta Directiva las siguientes funciones y atribuciones entre otras: Autorizar cualquier acto o contrato cuya cuantia sea superior al equivalente en pesos Colombianos a doscientos cincuenta mil dolares de los Estados Unidos de America (US\$250,000), liquidados a la tasa de cambio representativa del mercado vigente el dia en que se surta la transaccion. Cuando se trate de operaciones en virtud de las cuales la sociedad garantice obligaciones de terceros o de los socios, se requerira que la operacion sea aprobada con el voto de por lo menos 2/3 partes de los integrantes de la Junta Directiva. La sociedad tendra un Presidente, quien ejercera la representacion legal de la sociedad y tendra las siguientes atribuciones entre otras: Ejecutar las decisiones de la Asamblea General de Accionistas y de la Junta Directiva. Ejercer la Representacion Legal de la sociedad en todos los actos y contratos que se

***** C O N T I N U A *****



***** Pag. 3

CAMARA DE COMERCIO
DE BARRANQUILLA

08*****

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.

PROCAPS S.A.-----

NIT: 890.106.527-5

requieran para el desarrollo del objeto social, de conformidad con lo previsto en las leyes y en los presentes estatutos. Constituir apoderados judiciales y extrajudiciales, recibir notificaciones, absolver interrogatorios de parte, confesar obligaciones de la sociedad, transigir, desistir y allanar a la sociedad en cualquier asunto judicial. Aprobar y celebrar en nombre de la sociedad todo acto o contrato cuya cuantia sea igual o inferior al equivalente en pesos colombianos a doscientos cincuenta mil dolares (de los Estados Unidos de America (US\$250.000), liquidados a la tasa de cambio representativa del mercado vigente el dia en que se surta la transacion o celebracion del acto. Las demas funciones que le correspondan como Representante Legal por disposicion de la ley, de estos estatutos o de la Junta Directiva. El Presidente tendra un suplente, designado por la Junta Directiva, quien reemplazara al Presidente en sus faltas temporales, absolutas o meramente accidentales, con entidades facultades y sin que sea necesario para ello acreditar la ausencia del principal. La sociedad tendra un Vicepresidente designado por la Junta Directiva, quien tambien ejercera la representacion legal de la sociedad, pudiendo ejercerla conjunta o separadamente con el Presidente. El vicepresidente tendra las siguientes facultades entre otras. Ejecutar dentro de su competencia las decisiones de la Asamblea General de Accionistas, de la Junta Directiva y del Presidente. Ejercer la representacion legal de la sociedad en todos los actos y contratos que se requieran para el desarrollo del objeto social, de conformidad con lo previsto en las leyes y en los presentes estatutos. Constituir apoderados judiciales y extrajudiciales, recibir notificaciones, absolver interrogatorios de parte, confesar obligaciones de la sociedad, transigir, desistir y allanar a la sociedad en cualquier asunto judicial. Aprobar y celebrar en nombre de la sociedad todo acto o contrato cuya cuantia sea igual o inferior al equivalente en pesos colombianos a ciento cincuenta mil dolares de los Estados Unidos de America (US\$150.000), liquidados a la tasa de cambio representativa del mercado vigente el dia en que se surta la aprobacion o celebracion del acto, siempre que no se trate de enajenar bienes inmuebles o cualquier otro activo fijo de la sociedad, inclusive de naturaleza muebles, acciones, cuotas o partes de interes inversiones y todo tipo de derechos de la sociedad, o de constituir gravámenes sobre cualquiera de los anteriores, facultades que radican exclusiva y privativamente en cabeza del Presidente. Las demas funciones que le corresponda como Representante legal por disposicion de la ley, de estos estatutos o de la Junta Directiva. El Vicepresidente tendra (1) suplente, designado por la Junta Directiva, quien lo reemplazara conjunta o separadamente en sus faltas temporales, absolutas o meramente accidentales, con identicas facultades y sin que sea necesario para

***** C O N T I N U A *****

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.

PROCAPS S.A.-----
NIT: 890.106.527-5.

ello acreditar la ausencia del principal. La sociedad tendra un (1) Gerente General, con su respectivo Suplente, designados por la Junta Directiva. El Suplente reemplazara al Gerente General, en sus faltas temporales, absolutas o meramente accidentales, quien junto con el gerente general, llevaran la representacion legal de la sociedad, con identicas facultades, pudiendolas ejercer conjunta o separadamente con el Presidente y Vicepresidente, con identicas facultades que el Gerente General y sin que sea necesario para ello acreditar la ausencia del titular. El Gerente General tendra las siguientes facultades entre otras: Ejercer la representacion legal de la sociedad, en todos los actos y contratos que se requieran para el desarrollo del objeto social, de conformidad con lo previsto en las leyes y en los presentes estatutos. Aprobar y celebrar en nombre de la sociedad todo acto o contrato cuya cuantia sea igual o inferior al equivalente en pesos Colombianos a Cincuenta Mil dolares de los Estados Unidos de America (US\$50.000), liquidados a la tasa de cambio representativa del mercado vigente el dia en que se surta la aprobacion o celebracion del acto, siempre que no se trate de enajenar a cualquier titulo bienes inmuebles o cualquier otro activo fijo de la sociedad, inclusive de naturaleza mueble, acciones, cuotas o partes de interes, inversiones y todo tipo de derechos de la sociedad o de constituir gravámenes sobre cualquiera de las anteriores facultades que radican exclusiva y privativamente en cabeza del Presidente.

C E R T I F I C A

Que según Acta No. 116 del 27 de Junio de 1997 correspondiente a la Asamblea de Accionistas en Barranquilla, de la sociedad:
PROCAPS S.A.-----

cuya parte pertinente se inscribió en esta Cámara de Comercio, el 15 de Agosto de 1997 bajo el No. 70,838 del libro respectivo, y según Acta No. 127 del 22 de Marzo de 2000 correspondiente a la Asamblea de Accionistas en Barranquilla, de la sociedad:
PROCAPS S.A.-----

cuya parte pertinente se inscribió en esta Cámara de Comercio, el 29 de Mayo de 2000 bajo el No. 87,250 del libro respectivo, fueron hechos los siguientes nombramientos:

CLASE: JUNTA DIRECTIVA

Principales

1. Minski Gontovnik Ruben	CC.*****7,463,982
2. Minski Gontovnik Meyer	CC.*****7,461,324
3. Minski Gontovnik Jose	CC.*****8,671,834

Suplentes

1. Minski Zirberblum Elliot	CC.*****72,219,541
2. Minski Deitel Daniel	*****
3. Minski Sharon	PA.*990,212,082,805

C E R T I F I C A

***** C O N T I N U A *****

Barranquilla, 15 de Julio de 2009

Hr:13:52:28



***** Pag. 4

CAMARA DE COMERCIO
DE BARRANQUILLA

08*****

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.

PROCAPS S.A.-----

NIT: 890.106.527-5.

Que según Acta No. 116 del 27 de Junio de 1997 correspondiente a la Asamblea de Accionistas en Barranquilla, de la sociedad: PROCAPS S.A. cuya parte pertinente se inscribió en esta Cámara de Comercio, el 15 de Agosto de 1997 bajo el No. 70,838 del libro respectivo, fueron hechos los siguientes nombramientos:

Cargo/Nombre	Identificación
Presidente.	
Minski Gontovnik Ruben	CC.*****7,463,982
Suplente del Presidente	
Minski Minski Abraham	CC.*****802,575
Vicepresidente	
Minski Gontovnik Meyer	CC.*****7,461,324
Suplente del vicepresidente	
Minski Gontovnik Jose	CC.*****8,671,834

C E R T I F I C A

Que según Acta No. 11 del 01 de Febrero de 2001 correspondiente a la Junta Directiva en Barranquilla, de la sociedad: PROCAPS S.A. cuya parte pertinente se inscribió en esta Cámara de Comercio, el 12 de Febrero de 2001 bajo el No. 91,273 del libro respectivo, fueron hechos los siguientes nombramientos:

Cargo/Nombre	Identificación
Gerente General.	
Uribe Ochoa Guillermo Luis	CC.*****70,073,754

C E R T I F I C A

Que según Acta No. 406 del 02 de Sep/bre de 2004 correspondiente a la Junta Directiva en Barranquilla, de la sociedad: PROCAPS S.A. cuya parte pertinente se inscribió en esta Cámara de Comercio, el 09 de Sep/bre de 2004 bajo el No. 113,263 del libro respectivo, fueron hechos los siguientes nombramientos:

Cargo/Nombre	Identificación
Suplente del Gerente.	
Tanaka Tatekawa Walter	CC.*****16,251,923

C E R T I F I C A

Que según Acta No. 124 del 29 de Marzo de 1999 correspondiente a la Asamblea de Accionistas en Barranquilla, de la sociedad: PROCAPS S.A. cuya parte pertinente se inscribió en esta Cámara de Comercio, el 04 de Agosto de 1999 bajo el No. 82,226 del libro respectivo, fueron hechos los siguientes nombramientos:

Cargo/Nombre	Identificación
--------------	----------------

***** C O N T I N U A *****

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.

PROCAPS S.A. -----
NIT: 890.106.527-5.

Revisor Fiscal Ppal.
Vargas Pertuz Ana Isabel

CC.*****32,696,645

C E R T I F I C A

Que según Acta No. 127 del 22 de Marzo de 2000 correspondiente a la Asamblea de Accionistas en Barranquilla, de la sociedad: PROCAPS S.A. cuya parte pertinente se inscribió en esta Cámara de Comercio, el 29 de Mayo de 2000 bajo el No. 87,250 del libro respectivo, fueron hechos los siguientes nombramientos:

Cargo/Nombre

Identificación

Suplente del Revisor Fiscal.

Lozada Villareal Milena

CC.*****32,748,111

C E R T I F I C A

Que por Escritura Pública No. 2,784 del 07 de Dic/bre de 2007, otorgada en la Notaria 2 a. de Barranquilla cuya parte pertinente se inscribió en esta Cámara de Comercio, el 20 de Dic/bre de 2007 bajo el No. 3,492 del libro respectivo, -----

y las inscripciones 3.492-1, 3.493, 3.494, 3.495, 3.496, 3.497, -----
3.498, 3.499, 3.500, 3.501, 3.502, 3.503, 3.504, 3.505, 3.506 -----
3.507, Consta que RUBEN MINSKI GONTOVNIK, C.C. No. 7.463.982, -----
obrando como Presidente y Representante Legal de la sociedad C.F.

NATURMEGA S.A. que obrando en su condición de Presidente y -----
Representante Legal de la sociedad PROCAPS S.A., el presidente -----
debidamente facultado por los Estatutos Sociales y por el Maximo -----
Organó Social, otorga los siguientes poderes: Otorgar poder -----
general, amplio y suficiente a los doctores JOSEFINA DEL CARMEN -----
MIELES BUELVAS CC.No.33.138.497; a MARCELA CARVAJALINO PAGANO -----

CC.No.22.579.748; CRISTINA VIOLI FRANCESCHINI CC.No.22.463.716, con -----
las facultades que se transcriben a continuación: a.- Para que -----
representen a la sociedad PROCAPS S.A., ante todo tipo de -----

autoridades administrativas y jurisdiccionales, en asuntos de -----
naturaleza civil, laboral, comercial, penal, aduanera, cambiaria, -----
tributaria, policiva, administrativa y fiscal. b.- Contestar y -----

presentar demandas, requerimientos, recursos, pedir y aportar -----
pruebas, recibir, notificarse, ejercer la acción de tutela, -----
tramitar incidente de desacato, comparecer en los procesos que -----

cursen contra la sociedad, allanarse en nombre de la sociedad en -----
los asuntos que sean necesarios, transigir, desistir, sustituir, -----
renunciar y reasumir el poder. c.- La facultad expresa de -----

conciliar por tanto pueden llegar a acuerdos conciliatorios, -----
transaccionales, judiciales o extrajudiciales o extrajudiciales, -----
cualquiera que sea su cuantía, para lo cual las apoderadas deben -----

aportar la autorización simple y escrita del Representante legal -----
de la sociedad. d.- Podrán presentar solicitudes de devolución y/o -----
compensación de impuestos, ventas y rentas, recibir los pagos por -----
las devoluciones y adelantar todo trámite o proceso administrativo -----

ante la DIAN, de tal forma que en ningún momento quede sin -----
representación legal de la sociedad. PODERES ESPECIALES: a.- Se -----
confiere poder especial amplio y suficiente a las personas que se -----

determinan seguidamente, que conforman el grupo A y B, para, para -----

***** C O N T I N U A *****

Barranquilla, 15 de Julio de 2009

Hr:13:52:28



***** Pag. 5

CAMARA DE COMERCIO
DE BARRANQUILLA

08*****

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.

PROCAPS S.A.-----

NIT: 890.106.527-5

que conjuntamente suscriban contratos de suministros de mercancías y servicios con entidades oficiales, hasta la suma de OCHOCIENTOS MIL DOLARES DE LOS ESTADOS UNIDOS DE AMERICA (US800.000.00), liquidados, a la tasa representativa del mercado vigente en el día que se realice la respectiva transacción. Grupo A. WALTER NANAKA TATEKAWA CC.No.16.251.923, CLAUDIA TANGARIFE CASTILLO CC.No.41.660.293, CARMEN ALICIA FLOREZ CC.No.22.434.044, IVAN VILLAREAL CAMACHO CC.No.91.201.749, CARMEN ALICIA HERRERA DIAZGRANADOS CC.No.22.377.540, DAVID ANTONIO CHARRIS GIRALDO CC.No.19.341.274, RICARDO DORADO HURTADO CC.No.79.715.772, JAIME FORERO LLINAS CC.No.19.300.593. b.- Se confiere poder especial amplio y suficiente al señor WILLIAM ALBERTO BARROS CERRA CC.No.8.715.941, para que en nombre y representación de PROCAPS S.A., suscriban declaraciones cambiarias, aduaneras y tributarias a que este obligada la sociedad, en el giro normal de sus negocios y operaciones sociales, por tanto quedan facultados para firmarlas, como también los demás documentos relacionados con ellas, notificarse, aportar y solicitar las pruebas que sean necesarias. c.- Otorgar poder especial amplio y suficiente al señor MARLON TATIS PAREDES CC.No.72.176.874 y al señor WILLIAM ALBERTO BARROS CERRA CC.No.8.715.941, para que en nombre y representación de PROCAPS S.A., suscriban las declaraciones, reportes y cualquier documento relacionado con los trámites o registros de Comercio Exterior y o cualquier entidad del Estado que haga sus veces: d) Otorgar poder especial amplio y suficiente a la doctora CARMEN ALICIA HERRERA DIAZGRANADOS, CC.No.22.377.540 y al doctor WALTER TANAKA TATEKAWA, CC.No.16.251.923, para que en nombre y representación de PROCAPS S.A.; endosen los títulos valores de la sociedad hasta por la suma de UN MIL MILLONES DE PESOS (1.000.000.000.00) M.L. CUARTO: El representante legal de la sociedad PROCAPS S.A.; doctor RUBEN MINSKI GONTOVNIK, manifiesta que la presente reforma fue aprobada por la Asamblea General Extraordinaria de Accionista de PROCAPS S.A., con observancia de las disposiciones legales y estatutarias.

C E R T I F I C A

Que por Escritura Pública No. 1,933 del 16 de Sep/bre de 2008, otorgada en la Notaría 2 a. de Barranquilla cuya parte pertinente se inscribió en esta Cámara de Comercio, el 06 de Octubre de 2008 bajo el No. 3,748 del libro respectivo, Consta que el señor RUBEN MINSKI GONTOVNIK, C.C. No. 7.463.982, que comparece en su condición de Presidente y Representante Legal de la Sociedad PROCAPS S.A., confiere por medio de este instrumento publico Poder especial Amplio y Suficiente a la doctora MILENA

***** C O N T I N U A *****

CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.

PROCAPS S.A.-----
NIT: 890.106.527-5.

ESCAFFI PARDO, C.C. No. 32.758.420, para que en nombre y representación de PROCAPS S.A., suscriba declaraciones cambiarias, aduaneras y tributarias a que este obligada la sociedad en el giro normal de sus negocios y operaciones sociales, por tanto queda facultada para firmar, como tambien los demas documentos relacionados con ellas, notificarse, aportar y solicitar las pruebas que sean necesarias.

C E R T I F I C A

Que en esta Cámara de Comercio no aparecen inscripciones posteriores de documentos referentes a reforma, disolución, liquidación o nombramientos de representantes legales de la expresada sociedad.

C E R T I F I C A

Que su última Renovación fue el: 27 de Marzo de 2009.

La información sobre embargos de establecimiento se suministra en Certificados de Matrícula, la de contratos sujetos a registro, en Certificados Especiales.

C E R T I F I C A

Que los actos de registro aquí certificados quedan en firme cinco días hábiles después de la correspondiente inscripción siempre que, dentro de dicho término, no sean objeto de los recursos de reposición ante esta entidad y/o de apelación para ante la Superintendencia de Industria y Comercio.

m. Rojas



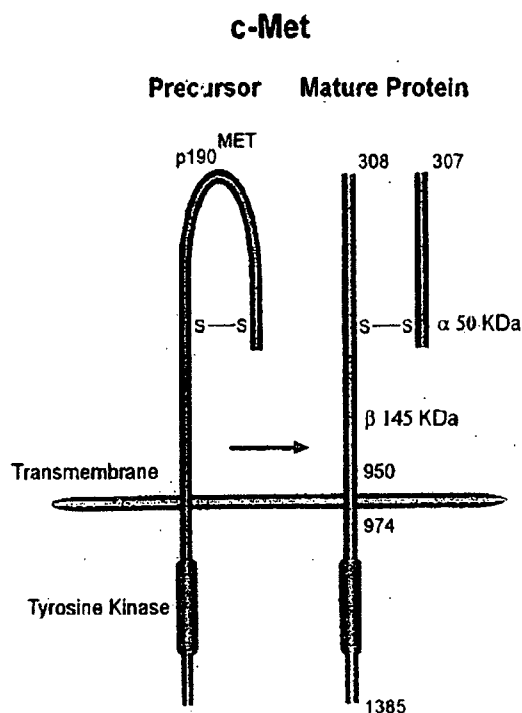
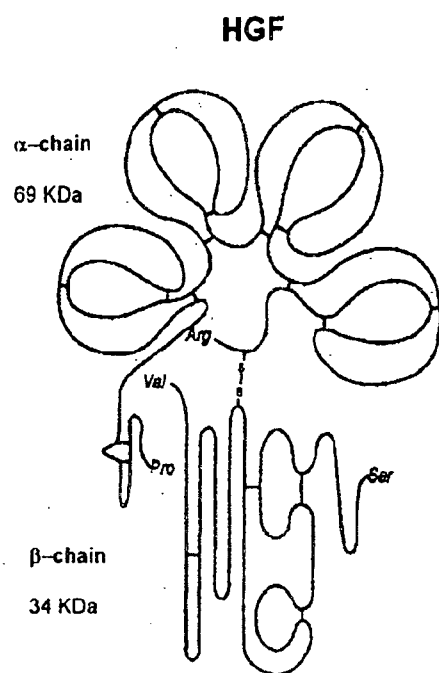
US 20050019327A1

(19) **United States**(12) **Patent Application Publication** (10) **Pub. No.: US 2005/0019327 A1****Kim et al.**(43) **Pub. Date: Jan. 27, 2005**(54) **MONOCLONAL ANTIBODIES TO
HEPATOCTE GROWTH FACTOR**(76) Inventors: **Kyung Jin Kim**, Cupertino, CA (US);
Yi-Chi Su, San Francisco, CA (US);
Lihong Wang, Palo Alto, CA (US)

Correspondence Address:

**TOWNSEND AND TOWNSEND AND CREW,
LLP
TWO EMBARCADERO CENTER
EIGHTH FLOOR
SAN FRANCISCO, CA 94111-3834 (US)**(21) Appl. No.: **10/917,915**(22) Filed: **Aug. 13, 2004****Related U.S. Application Data**(63) Continuation-in-part of application No. 10/825,060,
filed on Apr. 15, 2004.(60) Provisional application No. 60/464,061, filed on Apr.
18, 2003.**Publication Classification**(51) **Int. Cl.⁷** **A61K 39/395; C07K 16/22**(52) **U.S. Cl.** **424/145.1; 530/388.25**(57) **ABSTRACT**

The present invention is directed toward a neutralizing monoclonal antibody to hepatocyte growth factor, a pharmaceutical composition comprising same, and methods of treatment comprising administering such a pharmaceutical composition to a patient.



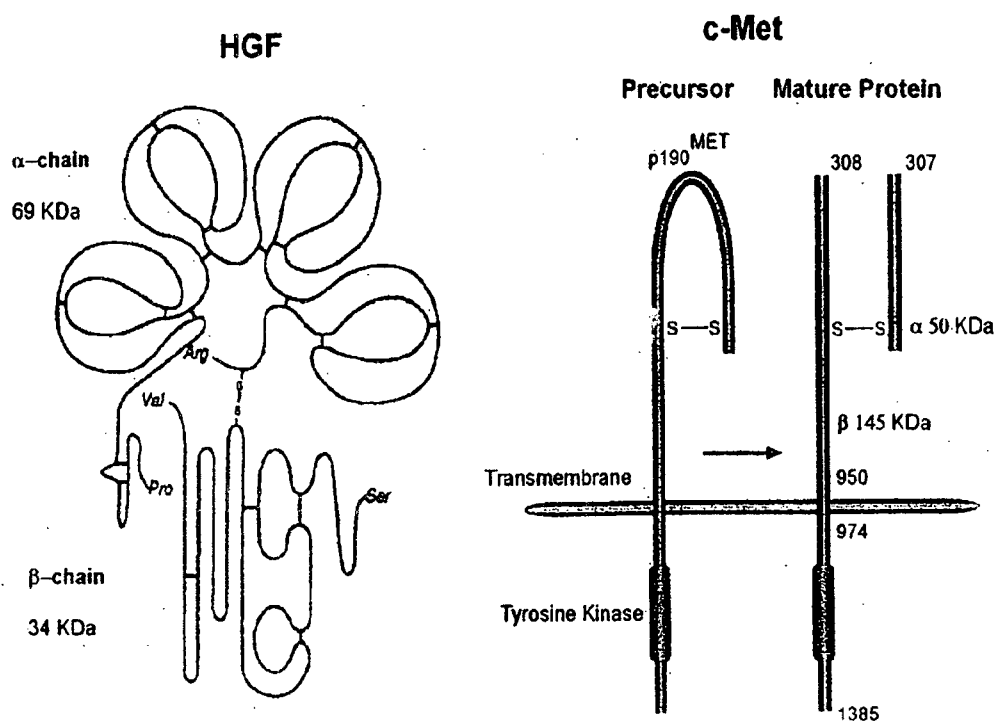


FIGURE 1

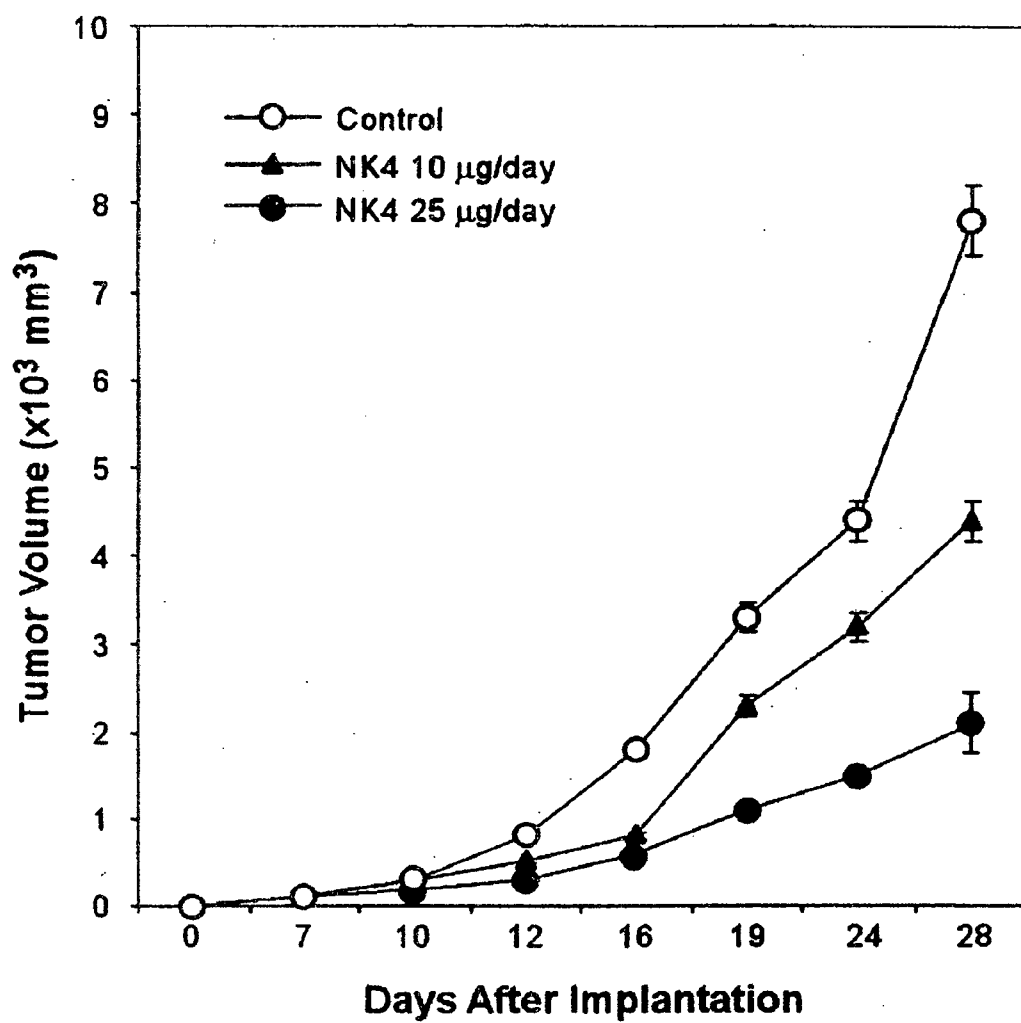


FIGURE 2

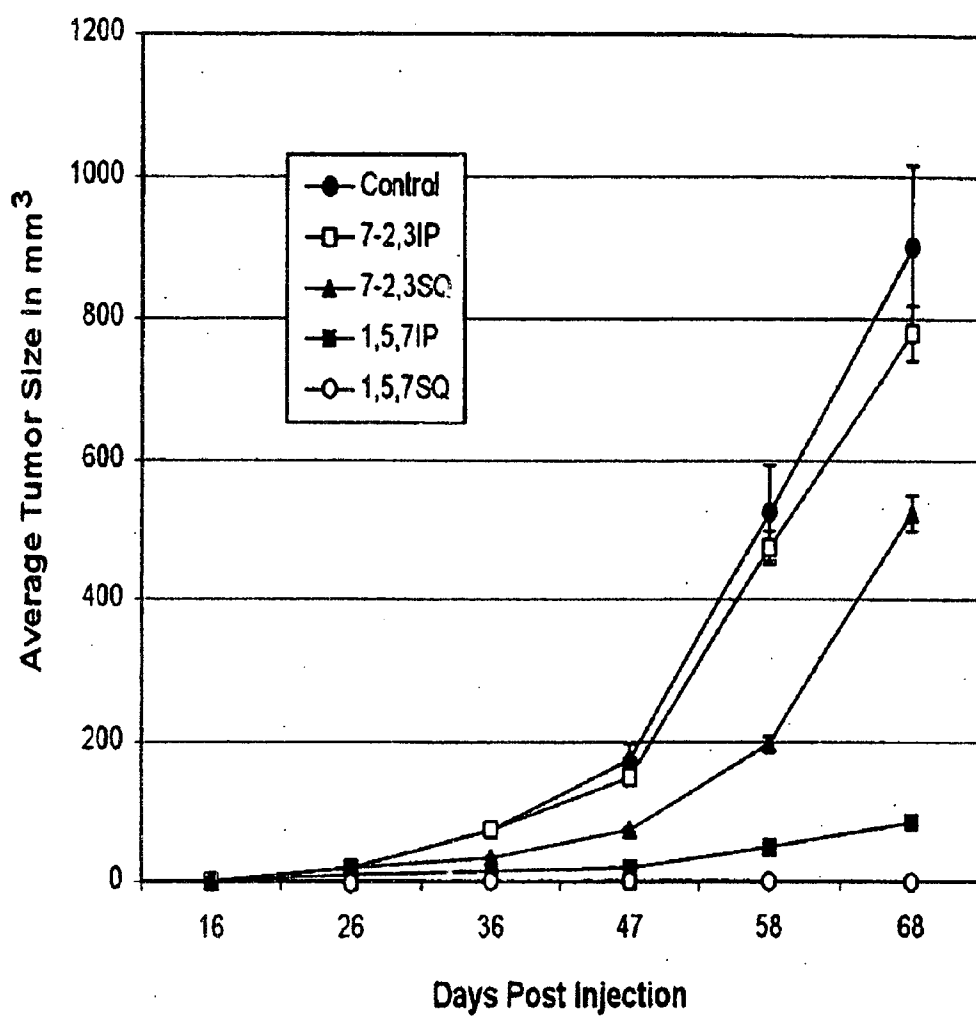


FIGURE 3

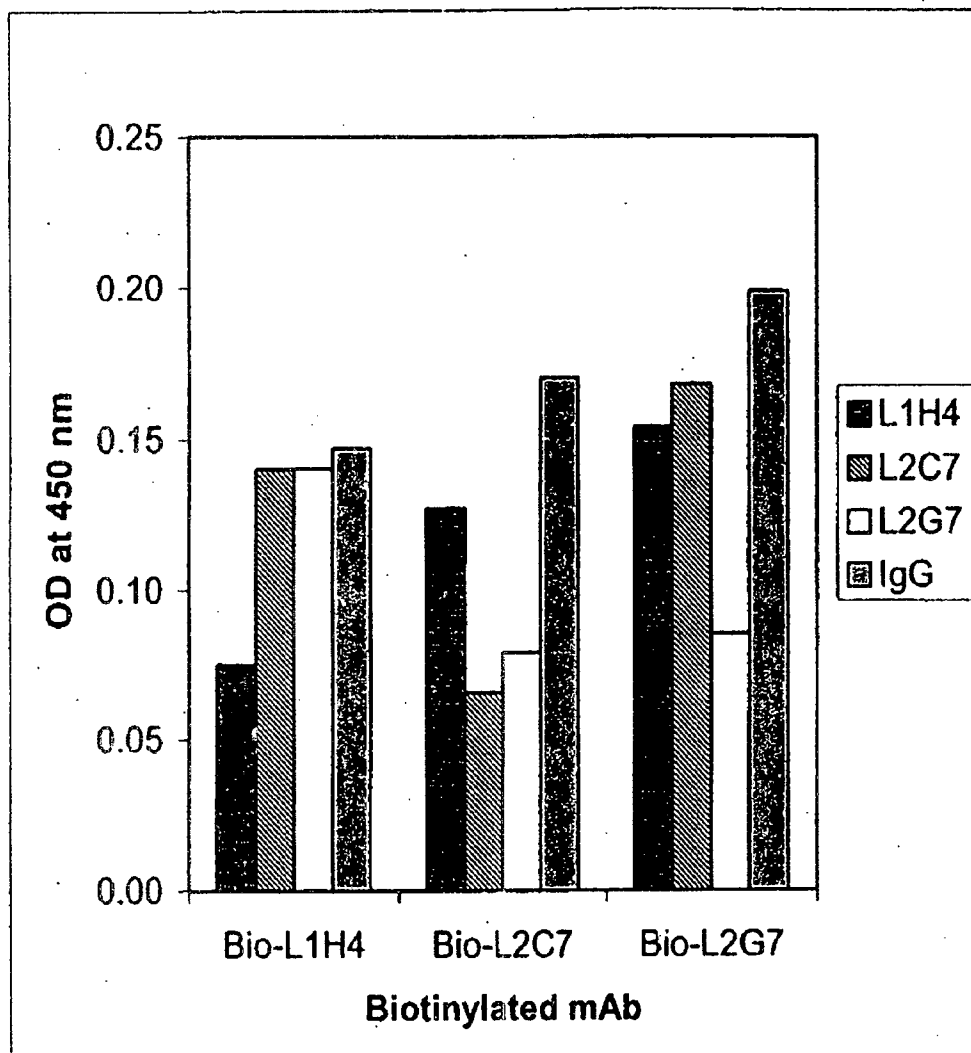


FIGURE 4

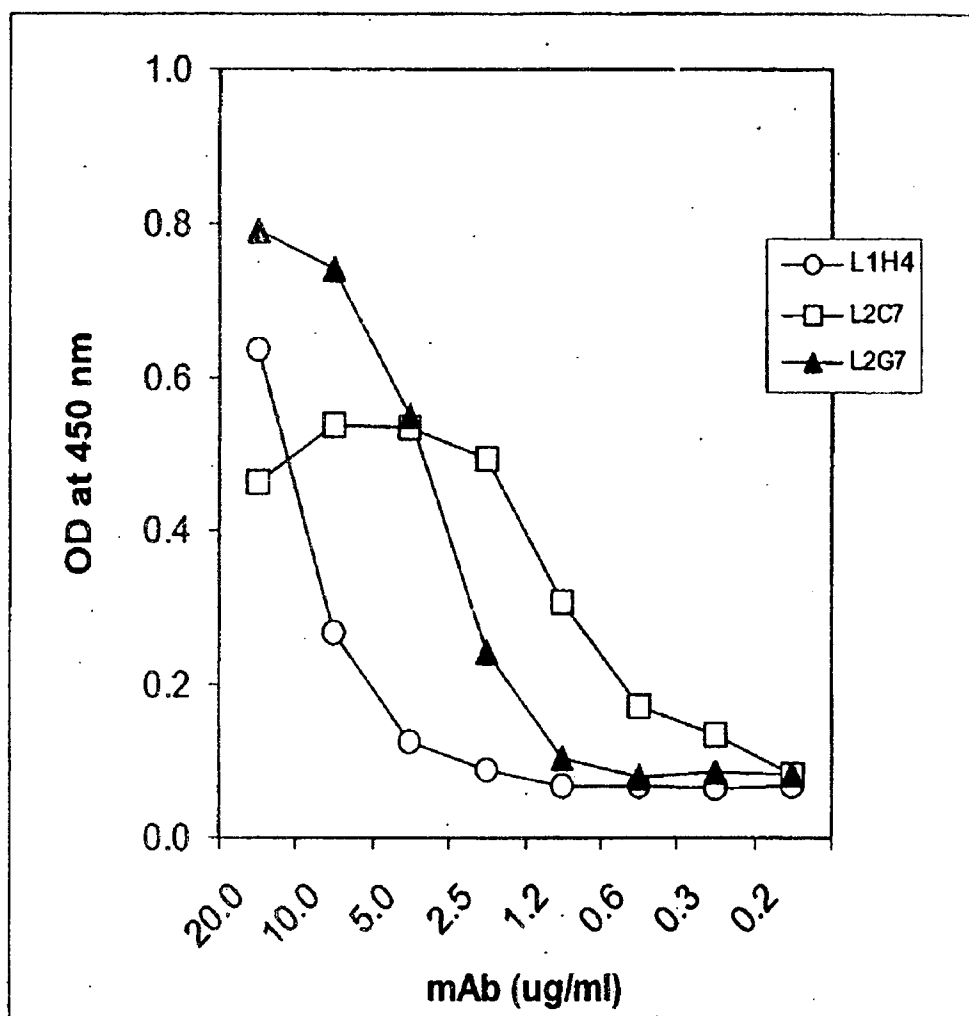


FIGURE 5

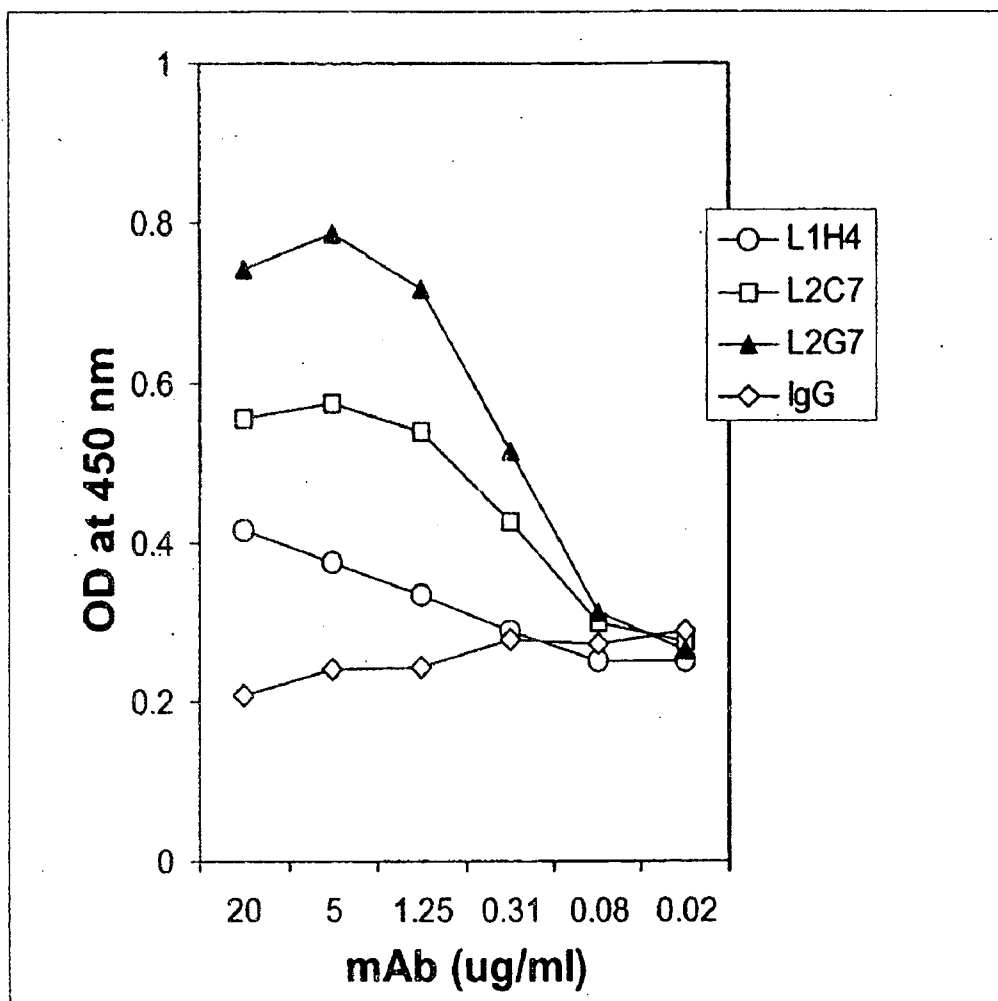


FIGURE 6

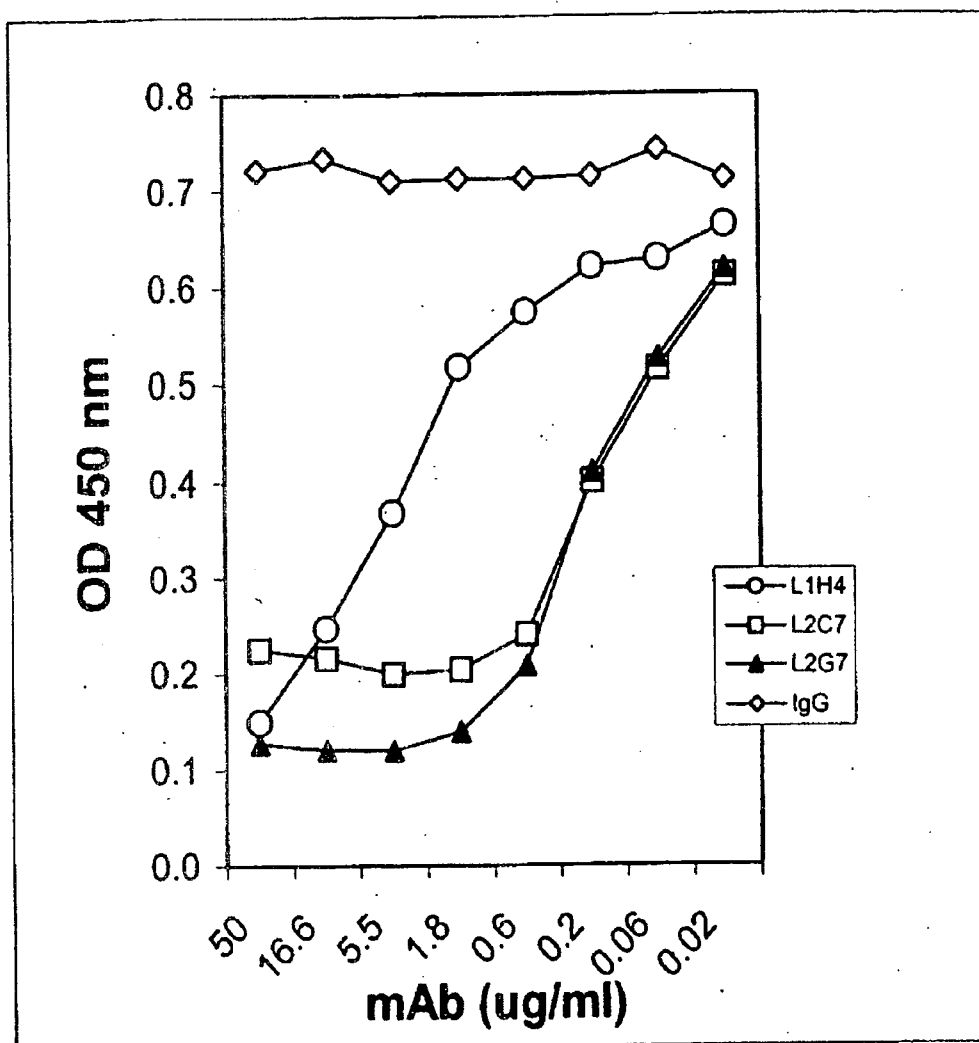


FIGURE 7

A



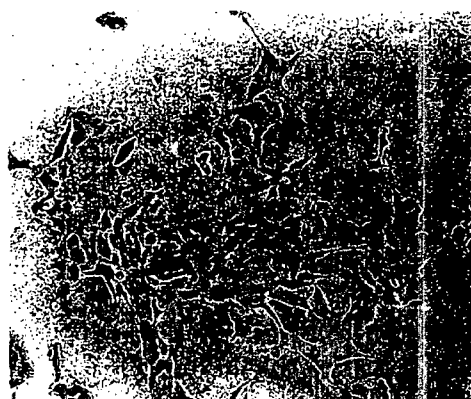
NONE

B



HGF + IgG

C



HGF + L2G7

FIGURE 8

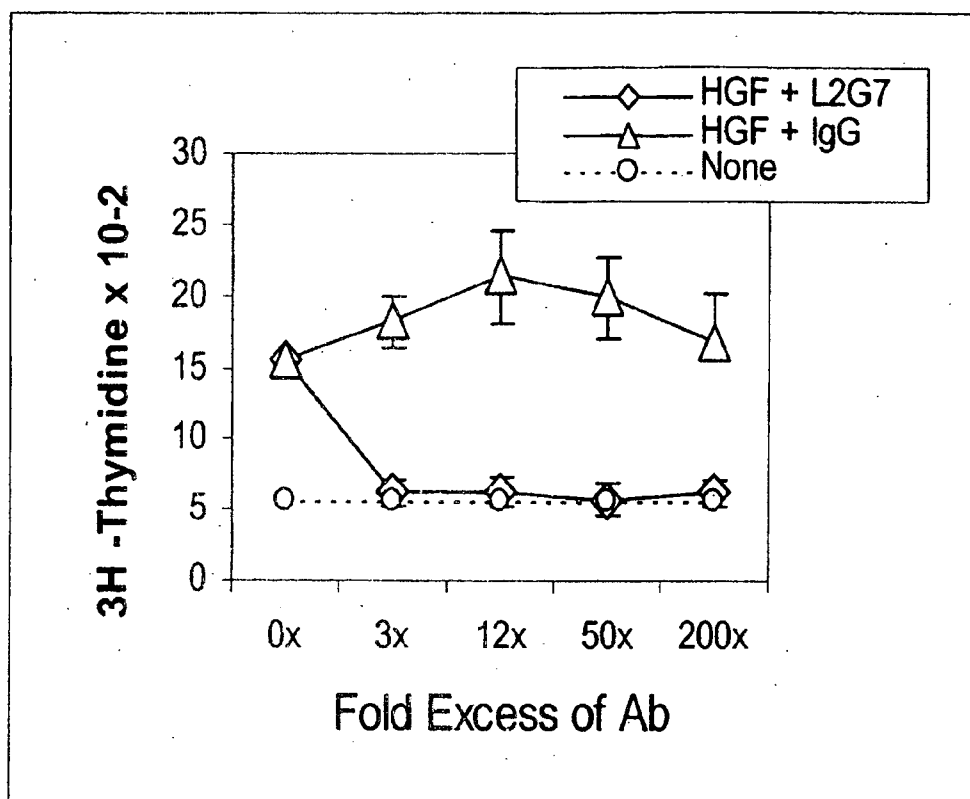


FIGURE 9

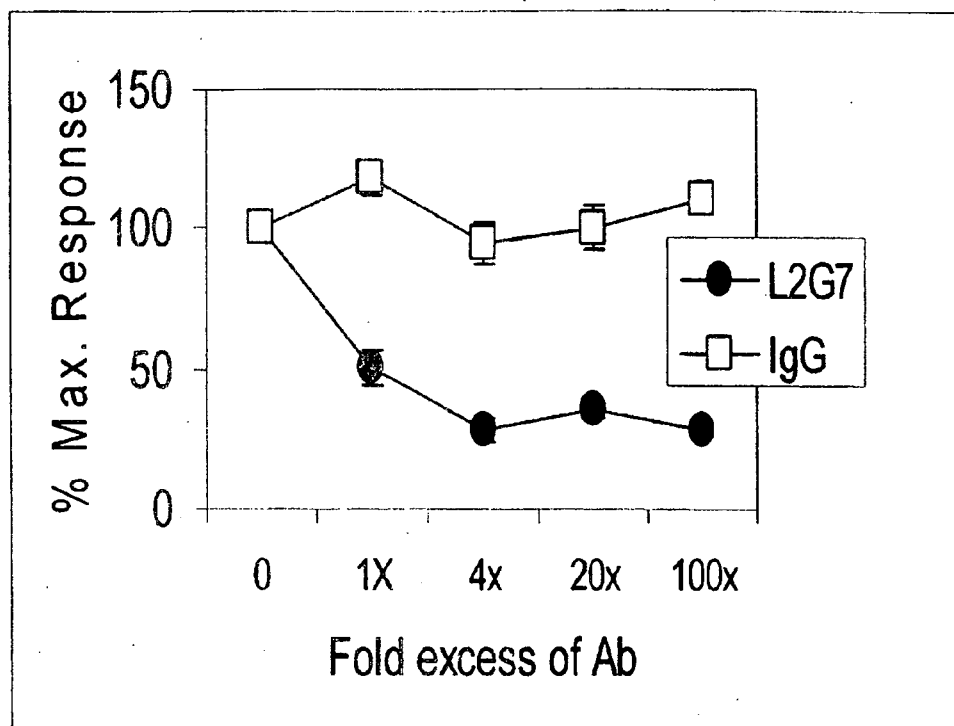


FIGURE 10

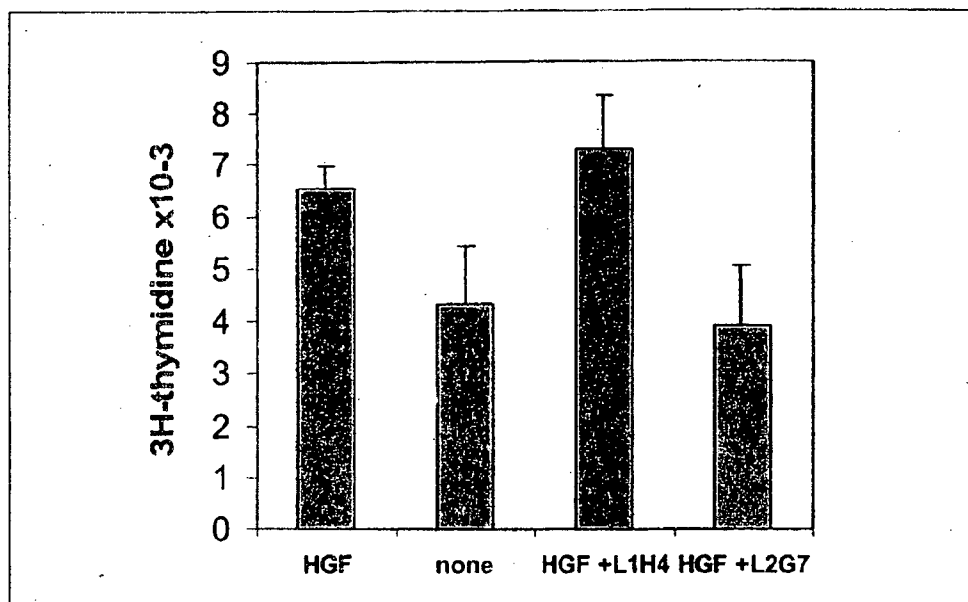


FIGURE 11

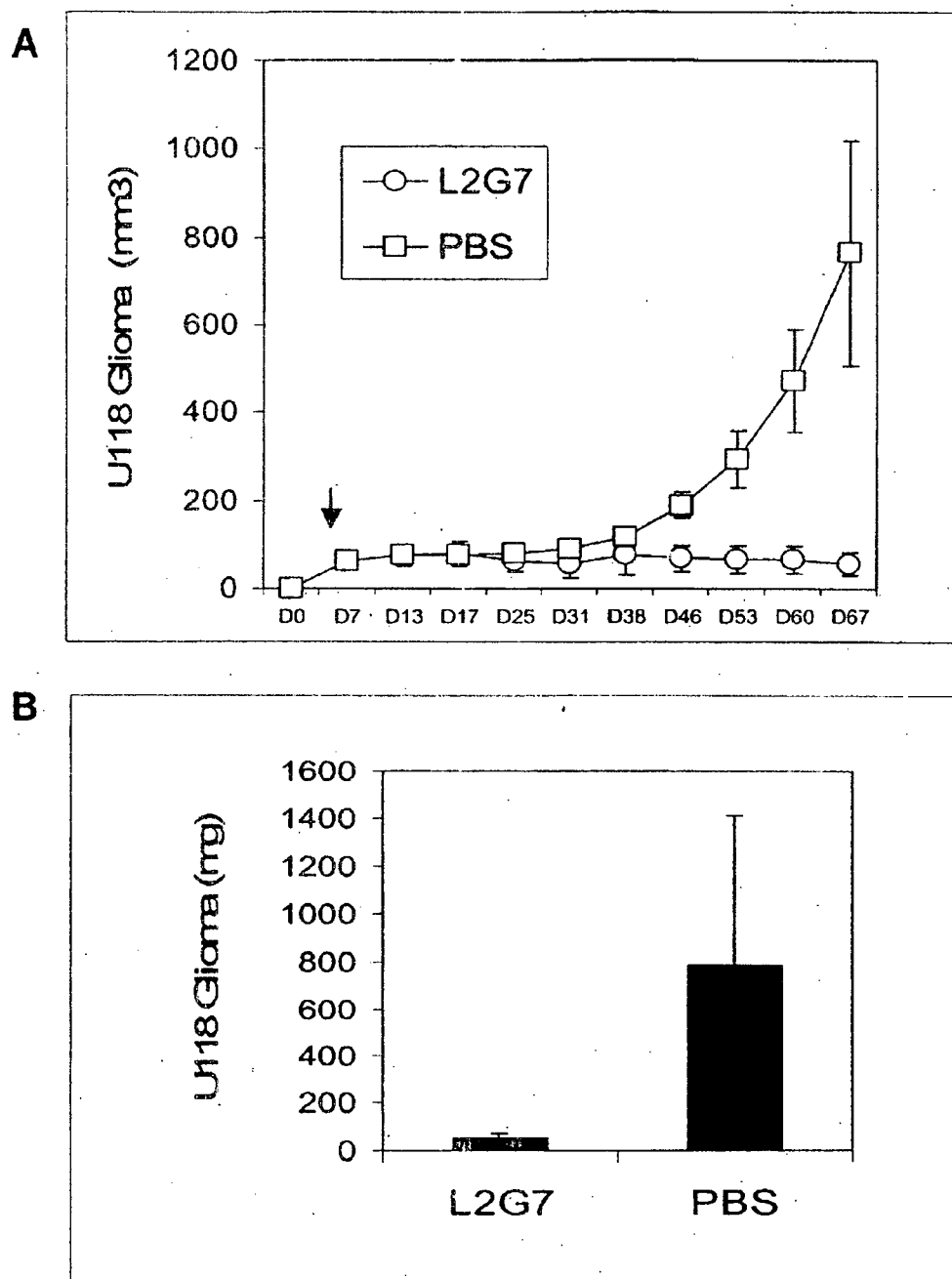


FIGURE 12

MONOCLONAL ANTIBODIES TO HEPATOCYTE GROWTH FACTOR

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. patent application Ser. No. 10/825,060 filed Apr. 15, 2004, which claims the benefit of the provisional application U.S. patent application Ser. No. 60/464,061 filed Apr. 18, 2003, both of which are incorporated by reference in their entirety for all purposes.

FIELD OF THE INVENTION

[0002] The present invention relates generally to the combination of monoclonal antibody (mAb) and recombinant DNA technologies for developing novel biologics, and more particularly, for example, to the production of monoclonal antibodies that bind to and neutralize Hepatocyte Growth Factor.

BACKGROUND OF THE INVENTION

[0003] Human Hepatocyte Growth Factor (HGF) is a multifunctional heterodimeric polypeptide produced by mesenchymal cells. HGF has been shown to stimulate angiogenesis, morphogenesis and motogenesis, as well as the growth and scattering of various cell types (Bussolino et al., *J. Cell. Biol.* 119: 629, 1992; Zarnegar and Michalopoulos, *J. Cell. Biol.* 129:1177, 1995; Matsumoto et al., *Ciba. Found. Symp.* 212:198, 1997; Birchmeier and Gherardi, *Trends Cell. Biol.* 8:404, 1998; Xin et al. *Am. J. Pathol.* 158:1111, 2001). The pleiotropic activities of HGF are mediated through its receptor, a transmembrane tyrosine kinase encoded by the proto-oncogene cMet. In addition to regulating a variety of normal cellular functions, HGF and its receptor c-Met have been shown to be involved in the initiation, invasion and metastasis of tumors (Jeffers et al., *J. Mol. Med.* 74:505, 1996; Comoglio and Trusolino, *J. Clin. Invest.* 109:857, 2002). HGF/cMet are coexpressed, often over-expressed, on various human solid tumors including tumors derived from lung, colon, rectum, stomach, kidney, ovary, skin, multiple myeloma and thyroid tissue (Prat et al., *Int. J. Cancer* 49:323, 1991; Chan et al., *Oncogene* 2:593, 1988; Weidner et al., *Am. J. Respir. Cell. Mol. Biol.* 8:229, 1993; Derksen et al., *Blood* 99:1405, 2002). HGF acts as an autocrine (Rong et al., *Proc. Natl. Acad. Sci. USA* 91:4731, 1994; Koochekpour et al., *Cancer Res.* 57:5391, 1997) and paracrine growth factor (Weidner et al., *Am. J. Respir. Cell. Mol. Biol.* 8:229, 1993) and anti-apoptotic regulator (Gao et al., *J. Biol. Chem.* 276:47257, 2001) for these tumors.

[0004] HGF is a 102 kDa protein with sequence and structural similarity to plasminogen and other enzymes of blood coagulation (Nakamura et al., *Nature* 342:440, 1989; Weidner et al., *Am. J. Respir. Cell. Mol. Biol.* 8:229, 1993, each of which is incorporated herein by reference) (FIG. 1). Human HGF is synthesized as a 728 amino acid precursor (preproHGF), which undergoes intracellular cleavage to an inactive, single chain form (proHGF) (Nakamura et al., *Nature* 342:440, 1989; Rosen et al., *J. Cell. Biol.* 127:1783, 1994). Upon extracellular secretion, proHGF is cleaved to yield the biologically active disulfide-linked heterodimeric molecule composed of an α -subunit and β -subunit (Nakamura et al., *Nature* 342:440, 1989; Naldini et al., *EMBO J.*

11:4825, 1992). The α -subunit contains 440 residues (69 kDa with glycosylation), consisting of the N-terminal hair-pin domain and four kringle domains. The β -subunit contains 234 residues (34 kDa) and has a serine protease-like domain, which lacks proteolytic activity. Cleavage of HGF is required for receptor activation, but not for receptor binding (Hartmann et al., *Proc. Natl. Acad. Sci. USA* 89:11574, 1992; Lokker et al., *J. Biol. Chem.* 268:17145, 1992). HGF contains 4 putative N-glycosylation sites, 1 in the α -subunit and 3 in the β -subunit. HGF has 2 unique cell specific binding sites: a high affinity ($K_d=2 \times 10^{-10}$ M) binding site for the cMet receptor and a low affinity ($K_d=10^{-9}$ M) binding site for heparin sulfate proteoglycans (HSPG), which are present on the cell surface and extracellular matrix (Naldini et al., *Oncogene* 6:501, 1991; Bardelli et al., *J. Biotechnol.* 37:109, 1994; Sakata et al., *J. Biol. Chem.* 272:9457, 1997). NK2 (a protein encompassing the N-terminus and first two kringle domains of the α -subunit) is sufficient for binding to cMet and activation of the signal cascade for motility, however the full length protein is required for the mitogenic response (Weidner et al., *Am. J. Respir. Cell. Mol. Biol.* 8:229, 1993). HSPG binds to HGF by interacting with the N terminus of HGF (Aoyama, et al., *Biochem.* 36:10286, 1997; Sakata, et al., *J. Biol. Chem.* 272:9457, 1997). Postulated roles for the HSPG-HGF interaction include the enhancement of HGF bioavailability, biological activity and oligomerization (Bardelli, et al., *J. Biotechnol.* 37:109, 1994; Zioncheck et al., *J. Biol. Chem.* 270:16871, 1995).

[0005] cMet is a member of the class IV protein tyrosine kinase receptor family. The full length cMet gene was cloned and identified as the cMet proto-oncogene (Cooper et al., *Nature* 311:29, 1984; Park et al., *Proc. Natl. Acad. Sci. USA* 84:6379, 1987). The cMet receptor is initially synthesized as a single chain, partially glycosylated precursor, p170^(MET) (FIG. 1) (Park et al., *Proc. Natl. Acad. Sci. USA* 84:6379, 1987; Giordano et al., *Nature* 339:155, 1989; Giordano et al., *Oncogene* 4:1383, 1989; Bardelli et al., *J. Biotechnol.* 37:109, 1994). Upon further glycosylation, the protein is proteolytically cleaved into a heterodimeric 190 kDa mature protein (1385 amino acids), consisting of the 50 kDa α -subunit (residues 1-307) and the 145 kDa β -subunit. The cytoplasmic tyrosine kinase domain of the β -subunit is involved in signal transduction.

[0006] Several different approaches have been investigated to obtain an antagonistic molecule of the HGF/cMet interaction: truncated HGF proteins such as NK1 (N terminal domain plus kringle domain 1; Lokker et al., *J. Biol. Chem.* 268:17145, 1993), NK2 (N terminal domain plus kringle domains 1 and 2; Chan et al., *Science* 254:1382, 1991) and NK4 (N-terminal domain plus four kringle domains; Kuba et al., *Cancer Res.* 60:6737, 2000), anti-cMet mAbs (Dodge, Master's Thesis, San Francisco State University, 1998) and anti-HGF mAbs (Cao et al., *Proc. Natl. Acad. Sci. USA* 98:7443, 2001, which is incorporated herein by reference).

[0007] NK1 and NK2 can compete effectively with the binding of HGF to its receptor, but have been shown to have partial agonistic activities in vitro (Cioce et al., *J. Biol. Chem.* 271:13110, 1996; Schwall et al., *J. Cell Biol.* 133:709, 1996), rather than purely antagonist activities as desired. More recently, Kuba et al., *Cancer Res.* 60:6737, 2000, demonstrated that NK4 could partially inhibit the

primary growth (FIG. 2) and metastasis of murine lung tumor LLC in a nude mouse model by continuous infusion of NK4. The fact that NK4 had to be administered continuously to obtain a partial growth inhibition of primary tumors indicates a potentially short half-life of the NK4 molecule and/or lack of potency. Compared to NK4, the approach of using antibodies will benefit from their favorable pharmacokinetics and the possibility of obtaining antibodies with much higher potency.

[0008] As another approach, Dodge (Master's Thesis, San Francisco State University, 1998) generated antagonistic anti-cMet monoclonal antibodies (mAbs). One mAb, 5D5, exhibited strong antagonistic activity in ELISA, but induced a proliferative response of cMet-expressing BAF-3 cells, presumably due to dimerization of the membrane receptors. Prat et al., *J. Cell Sci.* 111:237, 1998, also reported such agonistic activities of anti-cMet mAbs. Zaccolo et al., *Eur. J. Immunol.* 27:618, 1997, used phage display methods to develop human Fab fragments against mouse and human hepatocyte growth factor. These Fab fragments had no effect on the activity of HGF when used alone. When one of the anti-human HGF Fab fragments was combined with an antibody that bound to the Fab fragment itself, it actually enhanced the activity of HGF in a biological assay.

[0009] Cao et al., *Proc. Natl. Acad. Sci. USA* 98:7443, 2001, demonstrated that the administration of a cocktail of three anti-HGF mAbs, which were selected based upon their ability to inhibit the scattering activity of HGF in vitro, were able to inhibit the growth of human tumors in the xenograft nude mouse model (FIG. 3). They postulated that three mAbs recognizing three different binding sites on HGF were required to inhibit the bioactivities of HGF in vivo: two mAbs inhibited the binding of HGF to cMet and one mAb inhibited the binding of HGF to heparin. However, it is impractical for commercial and regulatory reasons to develop a drug combining three novel mAbs, e.g., because some clinical activity of each antibody would need to be demonstrated independently.

[0010] Thus, there is a need for a single monoclonal antibody that blocks biological activity of HGF in vitro and in vivo. The present invention fulfills this and other needs.

BRIEF SUMMARY OF THE INVENTION

[0011] In one embodiment, the invention provides a neutralizing mAb to human Hepatocyte Growth Factor (HGF). The mAb inhibits at least one, and preferably several or all biological activities of HGF including binding to its receptor cMet, inducing scattering of cells such as Madin-Darby canine kidney cells, inducing proliferation of 4MBR-5 monkey epithelial cells and/or hepatocytes and/or HUVEC, and inducing angiogenesis. The Anti-HGF mAb can inhibit such an activity when used as a single agent. A preferred anti-HGF mAb inhibits, most preferably completely inhibits, growth of a human tumor xenograft in a mouse. Preferably, the mAb of the invention is chimeric, humanized, human-like or human. Exemplary antibodies are L2G7 and its chimeric and humanized forms. Cell lines producing such antibodies are also provided. In another embodiment, a pharmaceutical composition comprising a neutralizing anti-HGF antibody, e.g., chimeric or humanized L2G7, is provided. In a third embodiment, the pharmaceutical composition is administered to a patient to treat cancer or other disease.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] FIG. 1. Schematic models of HGF and cMet.

[0013] FIG. 2. Graph showing that NK4 partially inhibits the primary growth of murine lung tumor LLC in nude mice (from Kuba et al., *Cancer Res.* 60:6737, 2000). NK4 was infused continuously for 14 days from 4th day after tumor implantation s.c. in nude mice.

[0014] FIG. 3. Graph showing that a cocktail of three anti-HGF mAbs is required to inhibit the growth of human brain tumor U-118 cells in nude mice (from Cao et al., *Proc. Natl. Acad. Sci. USA* 98:7443, 2001). U-118 tumor cells were injected s.c. into nude mice. From day 1 anti-HGF mAbs A-1, -5, and -7, or mAbs 7-2 and -3 were administered at 200 μ g/injection, twice/wk for 10 wks.

[0015] FIG. 4. Determination of relative binding epitopes of mAbs L1H4, L2C7, L2G7 blocked with skim milk and incubated with suboptimal concentration of biotinylated mAbs in the presence of 100x excess amounts of unlabeled mAbs. Biotinylated mAb bound was detected by the addition of HRP-Streptavidin.

[0016] FIG. 5. Binding of anti-HGF mAbs to rHGF as determined in a direct HGF binding ELISA. Plate was coated with the H1-F11 supernatant containing rHGF, blocked by 2% skim milk and incubated with mAbs, followed by the addition of HRP- α MlgG (as described under Examples).

[0017] FIG. 6. Abilities of anti-HGF mAbs to capture rHGF-Flag in solution. Anti-HGF mAbs were captured on a goat anti-mouse IgG coated ELISA plate. Plates were then blocked with 2% skim milk and incubated with rHGF-Flag, followed by HRP-M2 anti-Flag mAb (as described under Examples).

[0018] FIG. 7. Inhibition of rHGF-Flag binding to cMet-Fc by anti-HGF mAbs in a capture ELISA. cMet-Fc captured on goat anti-human IgG coated plate is incubated with HGF-Flag preincubated with/without mAbs. The bound rHGF-Flag was detected by the addition of HRP-M2 anti-Flag mAb (as described under Examples).

[0019] FIG. 8. Neutralization of HGF induced MDCK scattering by anti-HGF mAb L2G7. (A) Control without any treatment. (B) rHGF+IgG. (C) rHGF+mAb L2G7. MDCK cells were incubated with a 1:20 dilution of H1-F11 culture supernatant ($\sim 3 \mu$ g/ml of HGF) in the presence of 10 μ g/ml of mAbs. Photos were taken at 100x magnification.

[0020] FIG. 9. Inhibition of HGF-induced proliferation of Mv 1 LU cells by L2G7 mAb. The fold molar excess of mAb over HGF is shown on the horizontal axis, and the $\text{cpm} \times 10^{-2}$ incorporated is shown on the vertical axis. Data points were obtained in triplicate.

[0021] FIG. 10. Inhibition of HGF-induced proliferation of HUVEC by L2G7 mAb and control mouse antibody (mIgG). Data points were obtained in triplicate.

[0022] FIG. 11. Effect on HGF-induced proliferation of HCT 116 colon tumor cells by L2G7 and L1H4 antibodies. Data point were obtained in triplicate.

[0023] FIG. 12. Effect of treatment with L2G7 mAb or PBS (control) on growth of U-118 tumors in groups of NIH III Beige/Nude mice (n=6). Arrow indicates when injections

began. (A) Tumor size vs day from tumor implantation. (B) Tumor mass at end of experiment.

DETAILED DESCRIPTION OF THE INVENTION

[0024] The invention provides neutralizing anti-HGF monoclonal antibodies, pharmaceutical compositions comprising them, and methods of using them for the treatment of disease.

1. Antibodies

[0025] Antibodies are very large, complex molecules (molecular weight of ~150,000 or about 1320 amino acids) with intricate internal structure. A natural antibody molecule contains two identical pairs of polypeptide chains, each pair having one light chain and one heavy chain. Each light chain and heavy chain in turn consists of two regions: a variable ("V") region involved in binding the target antigen, and a constant ("C") region that interacts with other components of the immune system. The light and heavy chain variable regions fold up together in 3-dimensional space to form a variable region that binds the antigen (for example, a receptor on the surface of a cell). Within each light or heavy chain variable region, there are three short segments (averaging 10 amino acids in length) called the complementarity determining regions ("CDRs"). The six CDRs in an antibody variable domain (three from the light chain and three from the heavy chain) fold up together in 3-D space to form the actual antibody binding site which locks onto the target antigen. The position and length of the CDRs have been precisely defined. Kabat, E. et al., *Sequences of Proteins of Immunological Interest*, U.S. Department of Health and Human Services, 1983, 1987. The part of a variable region not contained in the CDRs is called the framework, which forms the environment for the CDRs.

[0026] A monoclonal antibody (mAb) is a single molecular species of antibody and therefore does not encompass polyclonal antibodies produced by injecting an animal (such as a rodent, rabbit or goat) with an antigen, and extracting serum from the animal. A humanized antibody is a genetically engineered (monoclonal) antibody in which the CDRs from a mouse antibody ("donor antibody", which can also be rat, hamster or other similar species) are grafted onto a human antibody ("acceptor antibody"). Humanized antibodies can also be made with less than the complete CDRs from a mouse antibody (e.g., Pascalis et al., *J. Immunol.* 169:3076, 2002). Thus, a humanized antibody is an antibody having CDRs from a donor antibody and variable region framework and constant regions from a human antibody. Thus, typically a humanized antibody comprises (i) a light chain comprising three CDRs from a mouse antibody, e.g., L2G7, a variable region framework from a human antibody, and a human constant region, and (ii) a heavy chain comprising three CDRs from a mouse antibody, e.g., L2G7, a variable region framework from a human antibody and a human constant region. In addition, in order to retain high binding affinity, at least one of two additional structural elements can be employed. See, U.S. Pat. No. 5,530,101 and 5,585,089, each of which is incorporated herein by reference, which provide detailed instructions for construction of humanized antibodies.

[0027] In the first structural element, the framework of the heavy chain variable region of the humanized antibody is

chosen to have maximal sequence identity (between 65% and 95%) with the framework of the heavy chain variable region of the donor antibody, by suitably selecting the acceptor antibody from among the many known human antibodies. Sequence identity is determined when antibody sequences being compared are aligned according to the Kabat numbering convention. In the second structural element, in constructing the humanized antibody, selected amino acids in the framework of the human acceptor antibody (outside the CDRs) are replaced with corresponding amino acids from the donor antibody, in accordance with specified rules. Specifically, the amino acids to be replaced in the framework are chosen on the basis of their ability to interact with the CDRs. For example, the replaced amino acids can be adjacent to a CDR in the donor antibody sequence or within 4-6 angstroms of a CDR in the humanized antibody as measured in 3-dimensional space.

[0028] A chimeric antibody is an antibody in which the variable region of a mouse (or other rodent) antibody is combined with the constant region of a human antibody; their construction by means of genetic engineering is well-known. Such antibodies retain the binding specificity of the mouse antibody, while being about two-thirds human. The proportion of nonhuman sequence present in mouse, chimeric and humanized antibodies suggests that the immunogenicity of chimeric antibodies is intermediate between mouse and humanized antibodies. Other types of genetically engineered antibodies that may have reduced immunogenicity relative to mouse antibodies include human antibodies made using phage display methods (Dower et al., WO91/17271; McCafferty et al., WO92/001047; Winter, WO92/20791; and Winter, *FEBS Lett.* 23:92, 1998, each of which is incorporated herein by reference) or using transgenic animals (Lonberg et al., WO93/12227; Kucherlapati WO91/10741, each of which is incorporated herein by reference).

[0029] As used herein, the term "human-like" antibody refers to a Mab in which a substantial portion of the amino acid sequence of one or both chains (e.g., about 50% or more) originates from human immunoglobulin genes. Hence, human-like antibodies encompass but are not limited to chimeric, humanized and human antibodies. As used herein, a "reduced-immunogenicity" antibody is one expected to have significantly less immunogenicity than a mouse antibody when administered to human patients. Such antibodies encompass chimeric, humanized and human antibodies as well as antibodies made by replacing specific amino acids in mouse antibodies that may contribute to B- or T-cell epitopes, for example exposed residues (Padlan, *Mol. Immunol.* 28:489, 1991). As used herein, a "genetically engineered" antibody is one for which the genes have been constructed or put in an unnatural environment (e.g., human genes in a mouse or on a bacteriophage) with the help of recombinant DNA techniques, and would therefore, e.g., not encompass a mouse mAb made with conventional hybridoma technology.

[0030] The epitope of a mAb is the region of its antigen to which the mAb binds. Two antibodies bind to the same or overlapping epitope if each competitively inhibits (blocks) binding of the other to the antigen. That is, a 1x, 5x, 10x, 20x or 100x excess of one antibody inhibits binding of the other by at least 50% but preferably 75%, 90% or even 99% as measured in a competitive binding assay compared to a control lacking the competing antibody (see, e.g., Junghans

et al., Cancer Res. 50:1495, 1990, which is incorporated herein by reference). Alternatively, two antibodies have the same epitope if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies have overlapping epitopes if some amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

2. Neutralizing Anti-HGF Antibodies

[0031] A monoclonal antibody (mAb) that binds HGF (i.e., an anti-HGF mAb) is said to neutralize HGF, or be neutralizing, if the binding partially or completely inhibits one or more biological activities of HGF (i.e., when the mAb is used as a single agent). Among the biological properties of HGF that a neutralizing antibody may inhibit are the ability of HGF to bind to its cMet receptor, to cause the scattering of certain cell lines such as Madin-Darby canine kidney (MDCK) cells; to stimulate proliferation of (i.e., be mitogenic for) certain cells including hepatocytes, 4MBR-5 monkey epithelial cells, and various human tumor cells; or to stimulate angiogenesis, for example as measured by stimulation of human vascular endothelial cell (HUVEC) proliferation or tube formation or by induction of blood vessels when applied to the chick embryo chorioallantoic membrane (CAM). Antibodies of the invention preferably bind to human HGF, i.e., to the protein encoded by the GenBank sequence with Accession number D90334 (incorporated by reference).

[0032] A neutralizing mAb of the invention at a concentration of, e.g., 0.01, 0.1, 0.5, 1, 2, 5, 10, 20 or 50 $\mu\text{g/ml}$ will inhibit a biological function of HGF (e.g., stimulation of proliferation or scattering) by about at least 50% but preferably 75%, more preferably by 90% or 95% or even 99%, and most preferably approximately 100% (essentially completely) as assayed by methods described under Examples or known in the art. Inhibition is considered complete if the level of activity is within the margin of error for a negative control lacking HGF. Typically, the extent of inhibition is measured when the amount of HGF used is just sufficient to fully stimulate the biological activity, or is 0.05, 0.1, 0.5, 1, 3 or 10 $\mu\text{g/ml}$. Preferably, at least 50%, 75%, 90%, or 95% or essentially complete inhibition will be achieved when the molar ratio of antibody to HGF is 0.5x, 1x, 2x, 3x, 5x or 10x. Preferably, the mAb will be neutralizing, i.e., inhibit the biological activity, when used as a single agent, but possible 2 mAbs will be needed together to give inhibition. Most preferably, the mAb will neutralize not just one but several of the biological activities listed above; for purposes herein, an anti-HGF mAb that used as a single agent neutralizes all the biological activities of HGF will be called "fully neutralizing", and such mAbs are most preferable. MAb of the invention will preferably be specific for HGF, that is they will not bind, or only bind to a much lesser extent (e.g., K_a at least ten-fold less), proteins that are related to HGF such as fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF). Preferred antibodies lack agonistic activity toward HGF. That is, the antibodies block interaction of HGF with cMet without stimulating cells bearing HGF directly. MAb of the invention typically have a binding affinity (K_a) for HGF of at least 10^7 M^{-1} but preferably 10^8 M^{-1} or higher, and most preferably 10^9 M^{-1} or higher or even 10^{10} M^{-1} or higher.

[0033] MAb of the invention include anti-HGF antibodies in their natural tetrameric form (2 light chains and 2 heavy chains) and may be of any of the known isotypes IgG, IgA, IgM, IgD and IgE and their subtypes, i.e., human IgG1, IgG2, IgG3, IgG4 and mouse IgG1, IgG2a, IgG2b, and IgG3. The mAbs of the invention are also meant to include fragments of antibodies such as Fv, Fab and F(ab')₂; bifunctional hybrid antibodies (e.g., Lanzavecchia et al., Eur. J. Immunol. 17:105, 1987), single-chain antibodies (Huston et al., Proc. Natl. Acad. Sci. USA 85:5879, 1988; Bird et al., Science 242:423, 1988); and antibodies with altered constant regions (e.g., U.S. Pat. No. 5,624,821). The mAbs may be of animal (e.g., mouse, rat, hamster or chicken) origin, or they may be genetically engineered. Rodent mAbs are made by standard methods well-known in the art, comprising multiple immunization with HGF in appropriate adjuvant i.p., i.v., or into the footpad, followed by extraction of spleen or lymph node cells and fusion with a suitable immortalized cell line, and then selection for hybridomas that produce antibody binding to HGF, e.g., see under Examples. Chimeric and humanized mAbs, made by art-known methods mentioned supra, are preferred embodiments of the invention. Human antibodies made, e.g., by phage display or transgenic mice methods are also preferred (see e.g., Dower et al., McCafferty et al., Winter, Lonberg et al., Kucherlapati, supra). More generally, human-like, reduced immunogenicity and genetically engineered antibodies as defined herein are all preferred.

[0034] The neutralizing anti-HGF mAbs L1H4, L2C7 and L2G7 mAbs described infra are examples of the invention, with L2G7 a preferred example. Neutralizing mAbs with the same or overlapping epitope as any of these mAbs, e.g., as L2G7, provide other examples. A chimeric or humanized form of L2G7 or with LGF is an especially preferred embodiment. A mAb (including chimeric, humanized and human antibodies) that competes with L2G7 for binding to HGF and neutralizes HGF in at least one, and preferably all, in vitro or in vivo assays described herein is also preferred. MAb that are 90%, 95%, 99% or 100% identical (determined by aligning antibody sequences according to the Kabat convention) to L2G7 in amino acid sequence, at least in the CDRs are included in the invention. Preferably such antibodies differ from L2G7 by a small number of functionally inconsequential amino acid substitutions (e.g., conservative substitutions), deletions, or insertions. Preferably such antibodies retain the functional properties of L2G7, i.e., such antibodies neutralize HGF in at least one, and preferably all, in vitro or in vivo assays described herein. For purposes of classifying amino acids substitutions as conservative or nonconservative, amino acids may be grouped as follows: Group I (hydrophobic sidechains): norleucine, met, ala, val, leu, ile; Group II (neutral hydrophilic side chains): cys, ser, thr; Group III (acidic side chains): asp, glu; Group IV (basic side chains): asn, gln, his, lys, arg; Group V (residues influencing chain orientation): gly, pro; and Group VI (aromatic side chains): trp, tyr, phe. Conservative substitutions involve substitutions between amino acids in the same class. Non-conservative substitutions constitute exchanging a member of one of these classes for a member of another.

[0035] Native mAbs of the invention may be produced from their hybridomas. Genetically engineered mAbs, e.g., chimeric or humanized mAbs, may be expressed by a variety of art-known methods. For example, genes encoding their

light and heavy chain V regions may be synthesized from overlapping oligonucleotides and inserted together with available C regions into expression vectors (e.g., commercially available from Invitrogen) that provide the necessary regulatory regions, e.g., promoters, enhancers, poly A sites, etc. Use of the CMV promoter-enhancer is preferred. The expression vectors may then be transfected using various well-known methods such as lipofection or electroporation into a variety of mammalian cell lines such as CHO or non-producing myelomas including Sp2/0 and NS0, and cells expressing the antibodies selected by appropriate antibiotic selection. See, e.g., U.S. Pat. No. 5,530,101. Larger amounts of antibody may be produced by growing the cells in commercially available bioreactors.

[0036] Once expressed, the mAbs or other antibodies of the invention may be purified according to standard procedures of the art such as microfiltration, ultrafiltration, protein A or G affinity chromatography, size exclusion chromatography, anion exchange chromatography, cation exchange chromatography and/or other forms of affinity chromatography based on organic dyes or the like. Substantially pure antibodies of at least about 90 or 95% homogeneity are preferred, and 98% or 99% or more homogeneity most preferred, for pharmaceutical uses.

3. Therapeutic Methods

[0037] In a preferred embodiment, the present invention provides a pharmaceutical formulation comprising the antibodies described herein. That is, the antibodies can be used in the manufacture of a medicament for treatment of disease. Pharmaceutical formulations (i.e., medicaments) of the antibodies contain the mAb in a physiologically acceptable carrier, optionally with excipients or stabilizers, in the form of lyophilized or aqueous solutions. Acceptable carriers, excipients or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, or acetate at a pH typically of 5.0 to 8.0, most often 6.0 to 7.0; salts such as sodium chloride, potassium chloride, etc. to make isotonic; antioxidants, preservatives, low molecular weight polypeptides, proteins, hydrophilic polymers such as polysorbate 80, amino acids, carbohydrates, chelating agents, sugars, and other standard ingredients known to those skilled in the art (Remington's Pharmaceutical Science 16th edition, Osol, A. Ed. 1980). The mAb is typically present at a concentration of 1-100 mg/ml, e.g., 10 mg/ml.

[0038] Antibodies of the invention are typically substantially pure from undesired contaminant. This means that the antibody is typically at least about 50% w/w (weight/weight) pure, as well as being substantially free from interfering proteins and contaminants. Preferably the antibodies are at least 90, 95% or 99% w/w pure. Pharmaceutical compositions for parenteral administration are usually sterile, substantially isotonic and prepared in accordance with Good Manufacturing Practices of the FDA or similar body.

[0039] In another preferred embodiment, the invention provides a method of treating a patient with a disease using an anti-HGF mAb in a pharmaceutical formulation. The mAb prepared in a pharmaceutical formulation can be administered to a patient by any suitable route, especially parentally by intravenous infusion or bolus injection, intramuscularly or subcutaneously. Intravenous infusion can be

given over as little as 15 minutes, but more often for 30 minutes, or over 1, 2 or even 3 hours. The mAb can also be injected directly into the site of disease (e.g., a tumor), or encapsulated into carrying agents such as liposomes. The dose given will be sufficient to alleviate the condition being treated ("therapeutically effective dose") and is likely to be 0.1 to 5 mg/kg body weight, for example 1, 2, 3 or 4 mg/kg, but may be as high as 10 mg/kg or even 15 or 20 mg/kg. A fixed unit dose may also be given, for example, 50, 100, 200, 500 or 1000 mg, or the dose may be based on the patient's surface area, e.g., 100 mg/m². Usually between 1 and 8 doses, (e.g., 1, 2, 3, 4, 5, 6, 7 or 8) are administered to treat cancer, but 10, 20 or more doses may be given. The mAb can be administered daily, biweekly, weekly, every other week, monthly or at some other interval, depending, e.g. on the half-life of the mAb, for 1 week, 2 weeks, 4 weeks, 8 weeks, 3-6 months or longer. Repeated courses of treatment are also possible, as is chronic administration. A regime of a dosage and intervals of administration that alleviates or at least partially arrests the symptoms of the disease (biochemical, histologic and/or clinical), including its complications and intermediate pathological phenotypes in development of the disease is referred to as a therapeutically effective regime.

[0040] The pharmaceutical compositions of the invention can also be used in prophylaxis of a patient at risk of cancer. Such patients include those having genetic susceptibility to cancer, patients who have undergone exposure to carcinogenic agents, such as radiation or toxins, and patients who have undergone previous treatment for cancer and are at risk of recurrence. A prophylactic dosage is an amount sufficient to eliminate or reduce the risk, lessen the severity, or delay the outset of the disease, including biochemical, histologic and/or clinical symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. Administration of a pharmaceutical composition in an amount and at intervals effective to effect one or more of these objects is referred to as a prophylactically effective regime.

[0041] Diseases especially susceptible to therapy with the anti-HGF mAbs of this invention include solid tumors known or suspected to require angiogenesis or to be associated with elevated levels of HGF, for example ovarian cancer, breast cancer, lung cancer (small cell or non-small cell), colon cancer, prostate cancer, pancreatic cancer, renal cancer, gastric cancer, liver cancer, head-and-neck tumors, melanoma, sarcomas, and brain tumors (e.g., glioblastomas), of children or adults. Treatment can also be administered to patients having leukemias or lymphomas. In a preferred embodiment, the anti-HGF mAb can be administered together with (i.e., before, during or after) other anti-cancer therapy. For example, the anti-HGF mAb may be administered together with any one or more of the chemotherapeutic drugs known to those of skill in the art of oncology, for example Taxol (paclitaxel) or its derivatives, platinum compounds such as carboplatin or cisplatin, anthracyclines such as doxorubicin, alkylating agents such as cyclophosphamide, anti-metabolites such as 5-fluorouracil, or etoposide. The anti-HGF mAb can be administered in combination with two, three or more of these agents in a standard chemotherapeutic regimen, for example taxol and carboplatin, e.g. for breast and ovarian cancer. Other agents with which the anti-HGF mAb can be administered include biologics such as monoclonal antibodies, including Herceptin[™] against the HER2 antigen, Avastin[™] against VEGF, or

antibodies to the EGF receptor, as well as small molecule anti-angiogenic or EGF receptor antagonist drugs. In addition, the anti-HGF mAb can be used together with radiation therapy or surgery.

[0042] Treatment (e.g., standard chemotherapy) including the anti-HGF mAb antibody may increase the median progression-free survival or overall survival time of patients with these tumors (e.g., ovarian, breast, lung, pancreas, brain and colon, especially when relapsed or refractory) by at least 30% or 40% but preferably 50%, 60% to 70% or even 100% or longer, compared to the same treatment (e.g., chemotherapy) but without anti-HGF mAb. In addition or alternatively, treatment (e.g., standard chemotherapy) including the anti-HGF mAb may increase the complete response rate, partial response rate, or objective response rate (complete+partial) of patients with these tumors (e.g., ovarian, breast, lung, pancreas, brain and colon, especially when relapsed or refractory) by at least 30% or 40% but preferably 50%, 60% to 70% or even 100% compared to the same treatment (e.g., chemotherapy) but without the anti-HGF mAb. Optionally, treatment can inhibit tumor invasion, or metastasis.

[0043] Typically, in a clinical trial (e.g., a phase II, phase II/III or phase III trial), the aforementioned increases in median progression-free survival and/or response rate of the patients treated with chemotherapy plus the anti-HGF mAb, relative to the control group of patients receiving chemotherapy alone (or plus placebo), will be statistically significant, for example at the $p=0.05$ or 0.01 or even 0.001 level. It will also be understood by one of skill that the complete and partial response rates are determined by objective criteria commonly used in clinical trials for cancer, e.g., as listed or accepted by the National Cancer Institute and/or Food and Drug Administration.

4. Other Methods

[0044] The anti-HGF mAbs of the invention also find use in diagnostic, prognostic and laboratory methods. They may be used to measure the level of HGF in a tumor or in the circulation of a patient with a tumor, and therefore to follow and guide treatment of the tumor. For example, a tumor associated with high levels of HGF would be especially susceptible to treatment with an anti-HGF mAb. In particular embodiments, the mAbs can be used in an ELISA or radioimmunoassay to measure the level of HGF, e.g., in a tumor biopsy specimen or in serum or in media supernatant of HGF-secreting cells in cell culture. The use of two anti-HGF mAbs binding to different epitopes (i.e., not competing for binding) will be especially useful in developing a sensitive "sandwich" ELISA to detect HGF. For various assays, the mAb may be labeled with fluorescent molecules, spin-labeled molecules, enzymes or radioisotopes, and may be provided in the form of kit with all the necessary reagents to perform the assay for HGF. In other uses, the anti-HGF mAbs will be used to purify HGF, e.g., by affinity chromatography.

EXAMPLES

1. Generation of Anti-HGF mAbs

[0045] To generate mAbs which bind to and block the activities of human HGF, recombinant human HGF (rHGF) was first produced in a mammalian expression system.

cDNAs encoding the recombinant human HGF (rHGF) or rHGF-Flag peptide (8 amino acid residues of Flag attached to the c-terminus of HGF) were constructed in a pIND-inducible expression vector (No et al., Proc. Natl. Acad. Sci. USA. 93:3346, 1996). These cDNAs were then transfected into EcR-293 human kidney fibroblast cells (Invitrogen) using Eugene transfection reagent (Roche). Stable cell lines, H1-F11 and 24.1, secreting HGF and HGF-Flag respectively, were selected in the presence of 600 $\mu\text{g/ml}$ of G418 and 400 $\mu\text{g/ml}$ of Zeocin (Invitrogen). H1-F11 and 24.1 were induced to secrete HGF and HGF-Flag by treatment with 4 μM of Ponasterone A (Invitrogen) for 4-5 days in serum free DMEM containing glutamine and antibiotics. After aggregates were removed by centrifugation at 15,000 rpm for 30 min at 4° C., HGF secreted into the culture supernatant was concentrated approximately 100-fold using a membrane ultrafiltration cartridge with an MW 50,000 cut-off filter [Amicon Centriprep YM-50 filter followed by microcon YM-50 filter (Millipore)]. Such concentrated H1-F11 culture supernatant contains ~100 $\mu\text{g/ml}$ of HGF and ~120 $\mu\text{g/ml}$ of bovine serum albumin.

[0046] Balb/c mice were immunized in each hind foot pad >10 times at one week intervals, with 1-2 μg of purified rHGF (Pepro Tech) or 1-2 μg of rHGF plus 1-2 μg of BSA (concentrated H1-F11 culture supernatant) resuspended in MPL-TDM (Ribi immunochem. Research). Three days after the final boost, popliteal lymph node cells were fused with murine myeloma cells, P3X63AgU.1 (ATCC CRL1597), using 35% polyethylene glycol. Hybridomas were selected in HAT medium as described (Chuntharapai and Kim, J. Immunol. 163:766, 1997, which is incorporated herein by reference). Ten days after the fusion, hybridoma culture supernatants were screened in a direct HGF binding ELISA as well as in an HGF-Flag capture ELISA. The latter assay was used to further confirm the specificity of anti-HGF mAbs selected using the direct HGF binding ELISA and to select mAbs that can bind to HGF in solution phase. Blocking activities of selected mAbs were then determined in the HGF-Flag/cMet-Fc binding ELISA and in the MDCK scatter assay as described (Jeffers et al., Proc. Natl. Acad. Sci. USA 95:14417, 1998). Selected hybridomas were cloned twice using limiting dilution techniques. The isotype of mAbs were determined using an isotyping kit (Zymed). Ascites of selected mAbs were raised and purified using ImmunoPure (A/G) IgG Purification Kit (Pierce). Also biotinylated mAbs were prepared using EZ-sulfo-NHS-LC-Biotin according to the instructions provided by Pierce. Each of the assays referred to here is described in more detail below. For the direct HGF binding ELISA, microtiter plates (Maxisorb; Nunc) are coated with 50 $\mu\text{l/well}$ of H1-F11 culture supernatants containing rHGF, diluted in PBS at a 1:2 ratio of HGF/PBS, overnight at 4° C. After washing the plate, the nonspecific binding sites are blocked with PBS containing 2% skim milk for 1 hr at room temperature (RT). After washing the plate, 50 $\mu\text{l/well}$ of purified mAbs or hybridoma culture supernatants are added to each well for 1 hr. After washing, plates are then incubated with 50 $\mu\text{l/well}$ of 1 $\mu\text{g/ml}$ of HRP-goat anti-mouse IgG (HRP-G α MlgG, Cappel) for 1 hr. The bound HRP-G α MlgG is detected by the addition of the tetramethylbenzidine substrate (Sigma). The reaction is stopped by the addition of 1N H₂SO₄ and the plates are then read at 450 nm using an ELISA plate reader. Washes are carried out 3 times in wash buffer (PBS containing 0.05% Tween 20).

[0047] For the HGF-Flag capture ELISA, microtiter plates are coated with 50 μ l/well of 2 μ g/ml of goat antibodies specific to the Fc portion of mouse IgG (G α MlgG-Fc) in PBS overnight at 4° C. and blocked with 2% skim milk for 1 hr at RT. After washing, the plates are incubated with 50 μ l/well of purified mAbs or hybridoma culture supernatants for 1 hr. After washing, plates are then incubated with 50 μ l/well of 24.1 cell culture supernatant containing rHGF-Flag. After washing, plates are then incubated with 50 μ l/well of HRP-M2 anti-Flag mAb (Invitrogen) in the presence of 15 μ g/ml of murine IgG. The bound HRP-anti-Flag M2 is detected by the addition of the substrate as described above. Washes are carried out 3 times in wash buffer.

[0048] At least three mAbs, designated L1H4, L2C7 and L2G7, obtained from hybridomas generated by immunizing the Balb/c mice with rHGF in concentrated H1-F11 culture supernatant as described above, showed binding in both the direct rHGF binding ELISA and the HGF-Flag capture ELISA and were selected for further study. These hybridomas were then cloned twice, ascites were raised in mice by standard methods, and mAbs were purified using a protein G/A column. Their isotypes were determined using an isotyping kit (Zymed Lab). The L2G7 hybridoma has been deposited on Apr. 29, 2003 with the American Type Culture Collection, P.O. Box 1549 Manassas, Va. 20108, as ATCC Number PTA-5162 under the Budapest Treaty. These deposit will be maintained at an authorized depository and replaced in the event of mutation, nonviability or destruction for a period of at least five years after the most recent request for release of a sample was received by the depository, for a period of at least thirty years after the date of the deposit, or during the enforceable life of the related patent, whichever period is longest. All restrictions on the availability to the public of these cell lines will be irrevocably removed upon the issuance of a patent from the application.

[0049] Once a single, archtypal anti-human-HGF mAb, for example L2G7, has been isolated that has the desired properties described herein of neutralizing HGF in vitro and/or inhibiting (e.g., completely) tumor growth in vivo, it is straightforward to generate other mAbs with similar properties, by using art-known methods. For example, mice may be immunized with HGF as described above, hybridomas produced, and the resulting mAbs screened for the ability to compete with the archtypal mAb for binding to HGF. Alternatively, the method of Jespers et al., *Biotechnology* 12:899, 1994, which is incorporated herein by reference, may be used to guide the selection of mAbs having the same epitope and therefore similar properties to the archtypal mAb, e.g., L2G7. Using phage display, first the heavy chain of the archtypal antibody is paired with a repertoire of (preferably human) light chains to select an HGF-binding mAb, and then the new light chain is paired with a repertoire of (preferably human) heavy chains to select a (preferably human) HGF-binding mAb having the same epitope as the archtypal mAb.

2. Characterization of Anti-HGF mAbs In Vitro

[0050] The binding epitopes of the antibodies were partially characterized by a competitive binding ELISA in which a 100x excess of unlabeled mAb was used to compete with the binding of the same or another biotinylated mAb in the HGF binding ELISA. FIG. 4 shows that the binding of the anti-HGF mAbs, L1H4 and L2G7, was inhibited only by

themselves, suggesting that they recognize unique epitopes. The binding of L2C7 was inhibited by L2G7 but not by L1H4. This suggests that the L2C7 epitope overlaps with that of L2G7 but not of L1H4. However, L2C7 was not able to inhibit the binding of L2G7, suggesting that the L2C7 and L2G7 epitopes overlap but are distinct, and/or the affinity of L2C7 is much lower than that of L2G7. The epitopes of L1H4, L2C7 and L2G7 are respectively designated A, B and C.

[0051] The relative binding abilities of the three anti-HGF mAbs were measured using purified antibodies in the direct HGF binding ELISA, in which rHGF is first bound to the plate. In this assay, L2C7 and L2G7 bound better than L1H4 (FIG. 5). The ability of the mAbs to bind rHGF-Flag in solution was also determined, using the HGF-Flag capture ELISA. All three mAbs were able to capture rHGF-Flag in solution phase but mAb L2G7 was more effective than the others (FIG. 6). These results suggest that mAb L2G7 has the highest binding affinity to HGF among the three mAbs.

[0052] One of the biological activities of HGF is the ability to bind to its receptor cMet, so the ability of the anti-HGF mAbs to inhibit binding of HGF to cMET was assayed. For this assay, cMet-Fc was first produced by transfecting human fibroblast 293 cells with cDNA encoding residues 1-929 ECD of cMet linked with the Fc portion of human IgG1 (residues 216 to 446) as described by Mark et al., *J. Biol. Chem.* 267:26166, 1992 in the pDisplay expression vector (Invitrogen). Microtiter plates are coated with 50 μ l/well of 2 μ g/ml of goat antibodies specific to the Fc portion of human IgG (G α HlgG-Fc) in PBS overnight at 4° C. and blocked with 2% BSA for 1 hr at RT. After washing the plates, 50 μ l of culture supernatant of 293 transfected with cMet-Fc cDNA is added to each well for 1 hr at RT. After washing the plates, 50 μ l/well of 24.1 cell culture supernatant containing rHGF-Flag, preincubated with various concentrations of mAbs, is added to each well for 1 hr. After washing, plates are then incubated with 50 μ l/well of HRP-M2 anti-Flag mAb (Invitrogen). The bound HRP-anti-Flag M2 is detected by the addition of the substrate as described above. Washes are carried out 3 times in wash buffer.

[0053] In this HGF-Flag/cMet-Fc binding inhibition assay, all three mAbs demonstrated some degrees of inhibition while an Ig control antibody did not (FIG. 7). mAb L2G7 at ≥ 1 μ g/ml and mAb L1H4 at 50 μ g/ml completely abolished the binding of rHGF-Flag to cMet-Fc; mAb L2C7 even at 50 μ g/ml gave only 85% inhibition. Hence, mAb L2G7 was much more potent in inhibiting the interaction of rHGF-Flag with cMet-Fc (and therefore presumably HGF with its receptor cMet) than the other antibodies, consistent with its putatively greater affinity for HGF.

[0054] Since the receptor protein used in cMet-Fc/HGF-Flag binding ELISA is a soluble receptor protein, its conformation may be different from that of the natural membrane bound receptor. Furthermore, HGF binds to HSPG in addition to cMet and it is known that the HSPG-HGF interaction enhances various HGF activities. Thus, mAbs blocking the interaction of HGF with soluble cMet may not necessarily have the capacity to neutralize HGF bioactivities on the cells. Thus, it is important to further confirm the blocking activities of mAbs in selected biological systems. HGF is known to be a potent scattering factor. Thus, the

neutralizing activity of the anti-HGF mAbs was also determined using the Madin-Darby canine kidney (MDCK cells obtained from ATCC) scatter assay as described (Jeffers et al., Proc. Natl. Acad. Sci. USA 95:14417, 1998). MDCK cells grown in DMEM supplemented with 5% FCS are plated at 10^3 cells/100 μ l/well in the presence of predetermined concentrations of rHGF with or without mAbs in DMEM with 5% FCS. After 2 days incubation at 37° C. in 5% CO₂, cells are then washed in PBS, fixed in 2% formaldehyde for 10 min at RT. After washing in PBS cells are stained with 0.5% crystal violet in 50% ethanol (v/v) for 10 min at RT. Scattering activity is determined by microscopic examination.

[0055] Culture supernatant of the H1-F11 clone secreting HGF, described above, was used as the source of HGF in the scatter assay. As little as 1:80 dilution of H1-F11 culture supernatant induced the scattering and growth of MDCK cells. However, the scattering assays were carried out using a 1:20 dilution of H1-F11 culture supernatant (~3 μ g/ml). MAb L2G7 even at a 1:5 molar ratio of HGF/mAb inhibited the HGF induced scattering of MDCK by itself (FIG. 8), conclusively demonstrating that mAb L2G7 is indeed a neutralizing mAb. mAb L1H4 at ≥ 20 μ g/ml could also neutralize scattering of MDCK induced by HGF, while mAb L2C7 even at 20 μ g/ml gave only a partial neutralizing activity (data not shown).

[0056] The various characteristics of the three anti-HGF antibodies determined in the assays above are summarized in the Table 1.

TABLE 1

Characterization of mAbs to HGF				
mAb	Isotype	Binding Epitope	Block HGF/cMet-Pc binding	Block MDCK scattering
L1H4	G1, κ	A	Weak Block	+
L2C7	G2b, κ	B	Partial Block	+/-
L2G7	G2az, κ	C	Strong Block	+++

[0057] HGF is a member of the heparin binding growth factor family including fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF). Also, HGF has ~40% overall sequence similarity with plasminogen (Nakamura et al., Nature. 342:440, 1989) and shares a similar domain structure with macrophage stimulating protein (MSP; Wang et al., Scand. J. Immunol. 56:S45, 2002). Thus, the binding specificity of the anti-HGF antibodies must be determined. The binding of anti-HGF mAbs to these HGF related proteins (available from R&D systems) is assayed using a direct binding ELISA similar to the one for HGF described above. MAb L2G7, mAb L2C7 and mAb L1H4 will not significantly bind to these proteins, demonstrating their specificity for HGF.

3. Ability of Anti-HGF MAb to Inhibit Tumor-Promoting Biological Activities of HGF

[0058] HGF has a number of biological activities that make it likely that it plays a role in the growth and invasiveness of certain human tumors. One such activity of HGF is as a powerful mitogen for hepatocytes and other epithelial cells (Rubin et al., Proc. Natl. Acad. Sci. USA. 88:415, 1991). Thus, to further prove the neutralizing activity

of the anti-HGF mAbs, the effects of the mAbs on the HGF-induced proliferation of 4MBr-5 monkey epithelial cells (ATCC) or rat hepatocytes are determined. Hepatocytes are isolated according to a method described by Garrison and Haynes, J. Biol. Chem. 269:4264, 1985. Cells are resuspended at 5×10^4 cells/ml in DMEM containing 5% FCS and stimulated with a predetermined concentration of HGF with various concentration of mAbs. After 2½ days incubation at 37° C. in 5% CO₂, the level of cell proliferation is determined by the addition of ³H-thymidine for 4 hrs. Cells are harvested using an automated cell harvester and the level of ³H-thymidine incorporated is determined on a scintillation counter. At sufficient concentrations, mAb L2G7 may largely or completely inhibit HGF-induced proliferation of the cells, and mAbs L2C7 and L1H4 may at least partially inhibit proliferation. These antibodies may also inhibit the HGF-induced proliferation of other epithelial cell lines.

[0059] For example, the inhibitory activity of L2G7 on the HGF-induced proliferation of mink lung Mv 1 Lu cells was determined (Borset et al., J. Immunol. Methods 189:59, 1996). Cells grown in DMEM containing 10% FCS are harvested by treatment with EDTA/trypsin. After washing, the cells are resuspended at 5×10^4 cells/ml in serum free DMEM with a predetermined concentration (50 ng/ml) of HGF +/- various concentrations of mAb. After 1 day incubation at 37° C. in 5% CO₂, the level of cell proliferation is determined by the addition of 1 μ Ci of ³H-thymidine for an additional 24 hr. Cells are harvested onto glass-fiber filters using an automated cell harvester and the level of ³H-thymidine incorporated is determined on a scintillation counter. FIG. 9 shows that the addition of 100-fold higher molar concentration of L2G7 mAb completely inhibited the proliferative response of Mv 1 Lu cells. Indeed, L2G7 even at a 3-fold molar ratio of mAb to HGF showed complete inhibition, while control IgG showed no inhibition even at 100-fold molar excess.

[0060] HGF is also reported to be a potent angiogenesis factor (Bussolino et al., J. Cell Biol. 119:629, 1992; Cherrington et al., Adv. Cancer Res. 79:1, 2000), and angiogenesis, the formation of new blood vessels, is believed to be essential to the growth of tumors. Therefore, the ability of the anti-HGF mAbs to inhibit the angiogenic properties of HGF is shown in three assays: (i) proliferation of human vascular endothelial cells (HUVEC), (ii) tube formation of HUVEC, and (iii) development of new blood vessels on the chick embryo chorioallantoic membrane (CAM). Since HGF has been shown to synergize with VEGF in angiogenesis (Xin et al., Am. J. Pathol. 158:1111, 2001), these assays may be performed both in the presence and absence of VEGF.

[0061] The HUVEC proliferation assay is performed as described with a modification (Conn et al., Proc. Natl. Acad. Sci. USA 87:1323, 1990). HUVEC cells obtained from Clonetics are grown in Endothelial Growth Medium (EBM-2) containing 10% FCS plus endothelial cell growth supplements provided by Clonetics. Preferably cells from passages 4 to 7 are used in this study. The cells are resuspended to be 10^5 cells/ml in medium-199 containing antibiotics, 10 mM HEPES and 10% FCS (assay medium). HUVEC cells (50 μ l/well) are added to microtiter wells containing a suitable concentration of HGF with various concentrations of anti-HGF mAbs for 1 hr at 37° C. After cells are incubated for

72 hr at 37° C. in 5% CO₂, the level of cell proliferation is determined by incorporation of ³H-thymidine for 4 hrs. At sufficient concentrations, mAb L2G7 will largely or completely inhibit HGF-induced proliferation of the HUVEC, and mAbs L2C7 and L1H4 may at least partially inhibit proliferation.

[0062] Alternatively, the level of cell proliferation may be determined by the well-known colorimetric MTT assay. The HVECs (10⁴ cells/100 μ l/well) are grown in serum free medium for 24 hr, and then incubated with 100 μ l of 50 ng/ml of HGF (predetermined to be a suboptimal amount) with various concentrations of mAb L2G7 for 72 hr. MTT solution (5 mg/ml) is added to each well (20 μ l/200 μ l medium) for 4 hr. Then 100 medium/well is removed and mixed with 100 μ l/well of acidified isopropyl alcohol (0.04N HCl in isopropyl). The plates are read on an ELISA reader at 560 nm. The % maximum response is calculated as follows: [OD of HGF+mAb treated cells-OD of untreated cells]/[OD of HGF treated cells-OD of untreated cells]x 100. **FIG. 10** shows that even a 2-fold molar excess of L2G7 mAb largely blocks the proliferation of HUVEC in response to HGF.

[0063] The endothelial tube assay is carried out essentially as described (Matsumura, et al., J. Immunol. 158:3408, 2001; Xin et al., Am. J. Pathol. 158:1111, 2001). HUVEC (Clonetics) from passage 4-7 are grown in Clonetics EGM medium supplemented with 10% FBS and endothelial cell growth supplements. Plates are coated with Matrigel (BD Biosciences) according to the manufacture's instructions at 37° C. for 30 min, and the cells are seeded as 3x10⁵ cells/ml in 1x basal medium with HGF and various concentrations of anti-HGF mAbs. Tube formation is evaluated under microscope at low-power (10x) magnification. At sufficient concentrations, mAb L2G7 will largely or completely inhibit HGF-induced endothelial tube formation, and mAbs L2C7 and L1H4 may at least partially inhibit it.

[0064] The chick embryo chorioallantoic membrane (CAM) assay is performed essentially as described (Kim et al., Nature 362:841, 1992). Three-day old chicken embryos are removed from their shells and grown in petri dishes in 5% CO₂ at 37° C. Seven days later, dried methylcellulose discs containing HGF with various concentrations of anti-HGF mAbs are layered onto the CAM. The methylcellulose discs are prepared by mixing 5 μ l of 1.5% methylcellulose in PBS with 5 μ l of HGF preincubated with mAbs. Three days later the development of blood vessels around methylcellulose discs are examined. At sufficient concentrations, mAb L2G7 will largely or completely inhibit such blood vessel formation, and mAbs L2C7 and L1H4 may at least partially inhibit it.

[0065] HGF is also reported to promote tumor growth (Comoglio and Trusolino, J. Clin. Invest. 109:857, 2002). The ability of the anti-HGF antibodies to inhibit this activity is shown in two steps. First, a number of tumor cell lines are examined for their ability to secrete HGF and proliferate in response to HGF since HGF may be an autocrine growth factor for some of these cells. These cell lines include a panel of human tumor cell lines known to express HGF and cMet (Koochekpour et al., Cancer Res. 57:5391, 1997; Wang et al., J. Cell Biol. 153:1023, 2001). Specific cell lines to be tested include U-118 glioma, HCT 116 colon carcinoma, A549 lung carcinoma and A431 epidermoid carcinoma cells, all available from the ATCC. Once such tumor cell lines are identified, the effect of anti-HGF mAbs on the proliferative response to HGF of these cells is determined, using methods similar to those described above. At sufficient concentrations, mAb L2G7 will largely or completely inhibit HGF-induced proliferation of many or all of these cell lines, and mAbs L2C7 and L1H4 may at least partially inhibit proliferation.

[0066] For example, human HCT116 tumor cells are seeded into 96-well microtiter plates at 5x10³ cells/well in 200 μ l of DMEM plus 5% FCS. After 24 hr incubation at 37° C. in 5% CO₂, cells are washed with PBS and incubated in serum free DMEM for 48 hr. Cells are then incubated with 100 ng/ml of HGF +/-20 μ g/ml of mAbs in DMEM for another 20 hr. As controls, cells grown in DMEM alone or DMEM plus 10% FCS are included. At the end of the incubation, levels of cell proliferation are determined by incorporation of ³H-thymidine for 4 hr. The result of such an experiment was carried out in triplicates is shown in **FIG. 11**. HGF induced a moderate proliferation of the HCT116 cells, which was completely abolished by addition of L2G7 antibody (but not by the less potent L1H4 antibody).

[0067] In all the assays described above, each anti-HGF antibody will neutralize or inhibit activity when used alone without other antagonists of HGF, i.e., as a single agent, but additive or synergistic effects may be achieved by administering the antibody in conjunction with other anti-HGF antibodies or other active agents.

4. Ability of Anti-HGF mAbs to Inhibit Tumor Growth In Vivo

[0068] The ability of the anti-HGF antibodies to inhibit human tumor growth is demonstrated in xenograft models in immunodeficient mice or other rodents such as rat. Illustrative but not limiting examples of immunodeficient strains of mice that can be used are nude mice such as CD-1 nude, Nu/Nu, Balb/c nude, NIH-III (NIH-bg-nu-xid BR); scid mice such as Fox Chase SCID (C.B-17 SCID), Fox Chase outbred SCID and SCID Beige; mice deficient in RAG enzyme; as well as nude rats. Experiments are carried out as described previously (Kim et al., Nature 362:841, 1992, which is incorporated herein by reference). Human tumor cells grown in complete DMEM medium are harvested in HBSS. Female immunodeficient, e.g., athymic nude mice (4-6 wks old) are injected s.c. with typically 5x10⁶ cells in 0.2 ml of HBSS in the dorsal areas. When the tumor size reaches 50-100 mm³, the mice are grouped randomly and appropriate amounts of the anti-HGF and control mAbs (typically between 0.1 and 1.0 mg, e.g. 0.5 mg) are administered i.p. once, twice or three times per week in a volume of, e.g., 0.1 ml, for e.g., 1, 2, 3, or 4 weeks or the duration of the experiment. Tumor sizes are determined typically twice a week by measuring in two dimensions [length (a) and width (b)]. Tumor volume is calculated according to $V=ab^2/2$ and expressed as mean tumor volume $\pm$ SEM. The number of mice in each treatment group is at least 3, but more often between 5 and 10, e.g., 7. Statistical analysis may be performed using, e.g., Student's t test. In a variation of this experiment, administration of the antibody begins simultaneously or shortly after injection of the tumor cells. The effect of the antibody may also be measured by prolongation of the survival of the mice, or increase in percent of the mice surviving.

[0069] Various tumor cell lines known to secrete or respond to HGF are used in separate experiments, for example U118 human glioblastoma cells, and/or HCT116 human colon tumor cells. Preferred antibodies of the invention, such as human-like and reduced-immunogenicity antibodies and the L2G7 antibody and its chimeric and humanized forms and antibodies with the same epitope as L2G7, when used as a single agent, will inhibit growth of tumors by at least 25%, but possibly 40% or 50%, and as much as 75% or 90% or greater, or even completely inhibit tumor growth after some period of time or cause tumor regression or disappearance. This inhibition will take place for at least tumor cell line such as U118 in at least one mouse strain such as NIH III Beige/Nude, but preferably will occur for 2, 3, several, many, or even essentially all HGF-expressing tumor cell lines of a particular (e.g., glioma) or any type, when tested in one or more immunodeficient mouse strains that do not generate a neutralizing antibody response against the injected antibody. Treatment with some preferred antibodies in one or more of the xenograft models leads to the indefinite survival of 50%, 75%, 90% or even essentially all mice, who would otherwise die or need to be sacrificed because of growth of their tumor.

[0070] For example, such an experiment was performed with U-118 glioblastoma cells, grown in DMEM medium with FCS and harvested in HBSS. Female NIH III Beige/Nude mice (4-6 wks old) are injected s.c. with 10^6 cells in 0.2 ml of HBSS in the dorsal areas. When the tumor size reaches $\sim 50 \text{ mm}^3$, the mice are grouped randomly into 2 groups of 6 mice each, and 200 μg of the L2G7 mAb (treatment group) or of PBS (control group) are given i.p. twice a week in a volume of 0.1 ml. Tumor sizes are determined twice a week as described above. At the end of the experiment, the tumors are excised and weighed. FIG. 12 shows that treatment with L2G7 completely inhibited tumor growth.

[0071] Similar tumor inhibition experiments are performed with the anti-HGF antibody administered in combination one or more chemotherapeutic agents such as 5-FU (5-fluorouracil) or CPT-11 (Camptosar) to which the tumor type is expected to be responsive, as described by Ashkenize et al., J. Clin. Invest. 104:155, 1999. The combination of the antibody and chemotherapeutic drug may produce a greater inhibition of tumor growth than either agent alone. The effect may be additive or synergistic, and strongly inhibit growth, e.g. by 80% or 90% or more, or even cause tumor regression or disappearance. The anti-HGF antibody may also be administered in combination with an antibody against another growth or angiogenic factor, for example anti-VEGF, and additive or synergistic growth inhibition and/or tumor regression or disappearance is expected. •

[0072] Although the invention has been described with reference to the presently preferred embodiments, it should be understood that various modifications can be made without departing from the invention. Unless otherwise apparent from the context any step, element, embodiment, feature or aspect of the invention can be used with any other.

[0073] All publications, patents and patent applications cited are herein incorporated by reference in their entirety for all purposes to the same extent as if each individual publication, patent and patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

What is claimed is:

1. A monoclonal antibody (mAb) that binds and neutralizes human Hepatocyte Growth Factor (HGF).
2. The mAb of claim 1 which is chimeric.
3. The mAb of claim 1 which is humanized.
4. The mAb of claim 1 which is human.
5. The mAb of claim 1 which inhibits binding of HGF to cMet by at least 50%.
6. The mAb of claim 1 which inhibits HGF-induced scattering of Madin-Darby canine kidney cells.
7. The mAb of claim 1 which inhibits HGF-induced proliferation of HUVEC cells.
8. The mAb of claim 1 which inhibits HGF-induced angiogenesis.
9. The mAb of claim 1 which neutralizes all biological activities of HGF.
10. The mAb of claim 1 which inhibits growth of a human tumor xenograft in a mouse.
11. The mAb of claim 10 which completely inhibits growth of a human tumor xenograft in a mouse.
12. The mAb of claim 1 which is a Fab or F(ab')_2 fragment or single-chain antibody.
13. The mAb of claim 1 which specifically binds HGF with a binding affinity of at least 10^8 M^{-1} .
14. A chimeric or humanized L2G7 mAb.
15. An antibody that competes for binding to human HGF with an antibody of claim 14.
16. A cell line producing a mAb of claim 1.
17. A pharmaceutical composition comprising a mAb of claim 14.
18. A method of treating cancer in a patient comprising administering to the patient a pharmaceutical composition comprising a neutralizing anti-HGF mAb.
19. A method of claim 18 wherein said cancer is glioblastoma.
20. The method of claim 18 wherein said mAb is a chimeric or humanized L2G7 mAb.

* * * * *

2020
Bogotá, D.C.,

Doctora:
Dilia María Rodríguez D'Aleman
Apoderada

Oficio N°: 12816

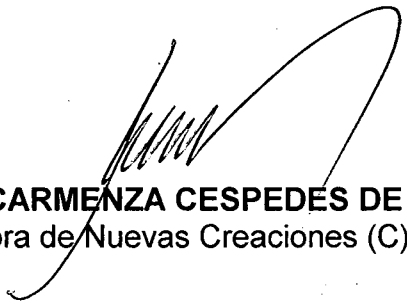
ASUNTO:

Radicación PCT N°	07-129828
Trámite	011
Evento	378
Actuación	440
Folios	009

Con fundamento en lo previsto por el artículo 43 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica que la oposición presentada por la doctora Eliana Isaura Moreno Bohórquez, en su calidad de apoderada de Procaps S.A., reúne los requisitos formales exigidos por la ley al momento de su presentación.

En cumplimiento de lo dispuesto en la citada norma, se le concede el término de sesenta (60) días hábiles siguientes a la fecha de notificación del presente oficio, para que haga valer sus argumentaciones y presente pruebas.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única N° 10 de 2001.


ALIX CARMENZA CESPEDES DE VERGEL
Directora de Nuevas Creaciones (C).

20 NOV. 2009

El anterior oficio fue notificado por fijación en lista de fecha, _____.

202021