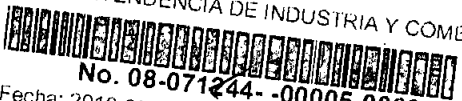


SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

No. 08-071244- -00005-0000
Fecha: 2010-05-03 16:48:21 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 400 OPOSIOBSERVTER Folios: 79

**FORMULARIO UNICO DE OPOSICION
2000-12**

1	OPOSICION 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	

2	Número del expediente: 08 071.244
SOLICITUD PUBLICADA	Solicitante: BAYER HEALTHCARE LLC.
	Apoderado: EMILIO FERRERO.
	Título de la nueva creación o denominación del signo:
	ANTICUERPOS ANTI MN Y METODOS PARA SU UTILIZACIÓN.
	Gaceta 612 del 29/01/2010, número de publicación 1231.

3	OPOSICION PRESENTADA POR:
----------	----------------------------------

OPOSITOR	Nombre: Lafrancol S.A.	IDENTIFICACION
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	Domicilio: Cali – Valle	

REPRESENTANTE O APODERADO (74)	Nombre: José Luis Reyes Villamizar	IDENTIFICACION
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	E-mail: info@reyes-abogados.com	
	Fax: 621.25.42	Número: 79'152.473 de Usaquén
	Domicilio: Bogotá, D.C.	TP 44655

4	FUNDAMENTOS DE LA OPOSICION:
Causal: Incumplimiento de requisitos de patentabilidad (Título II, Capítulo I, Decisión 486 de 2000). Se violan los artículos 14, 16 (las patentes de invención deben ser nuevas, tener nivel inventivo y ser suceptibles de aplicación industrial), 18 y 20 literal d (una invención tiene nivel inventivo si no es obvia para una persona versada en la materia).	

Justificación: VER ANEXO

5

Comprobante de pago No. 10-34335 (Oposición) Fecha Mayo 3 de 2010
(Prórroga) Fecha _____

6

Anexos:

- ☒ Comprobante de pago de la tasa
- ☒ Poderes, si fuere el caso
- ☒ Pruebas de los fundamentos de la oposición
- ☒ Certificado de existencia y representación legal de las partes, cuando sean personas jurídicas
- ☐ Documentos que acrediten el legítimo interés, si fuere el caso
- ☒ Copia de la oposición para el traslado al solicitante.

7

NOMBRE: JOSÉ LUIS REYES-VILLAMIZAR

FIRMA: _____

C.C. No. 79.152.473 T.P. No. 44655

REYES & REYES

A B O G A D O S

Señor

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

E.

S.

D.

REF: Expediente 08 071.244

TÍTULO SOLICITADO: ANTICUERPOS ANTI MN Y METODOS PARA SU UTILIZACIÓN.

PUBLICACIÓN: Gaceta 612 del 29/01/2010, número de publicación 1231.

SOLICITANTE: BAYER HEALTHCARE LLC.

APODERADO: EMILIO FERRERO.

TRÁMITE: PRESENTACIÓN DE OPOSICION

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

I. PETICIÓN

Sírvase negar el beneficio patentario a la solicitud contenida en el expediente **08 071.244**, por no cumplir con lo dispuesto por los artículos 14, 16, 18 y 20 literal d) de la Decisión 486 de 2000, con base en los fundamentos que se expondrán a continuación.

II. LEGÍTIMO INTERÉS

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

III. FUNDAMENTO DE LA OPOSICIÓN

La solicitud colombiana en trámite, que se refiere a un anticuerpo o fragmento de anticuerpo que tiene un sitio de unión a antígeno específicamente dirigido contra una proteína MN, en donde el sitio de unión a antígeno incluye por lo menos de un CDR1, CDR2 o CDR3, incumple con los requisitos indispensables para otorgársele el privilegio de patente de invención, tal y como se demuestra con los siguientes argumentos:

En primer lugar, y ante la reivindicación textual del uso de uso de la composición reivindicada en el tratamiento de trastornos asociados con la proteína MN, tales como tumores de mama, tracto respiratorio, pulmón, cerebro, órgano reproductor, tracto digestivo, colon, tracto urinario, riñón, esófago, cerviz, ojo, hígado, piel, cabeza, cuello, tiroide y paratiroide, específicamente en su reivindicación 15, es relevante señalar que dicha pretensión resulta inaceptable a la luz de la Decisión 486, en tanto los “usos” son una categoría excluida del concepto de “Productos o Procedimientos” que establece el artículo 14 de la norma citada. Lo anterior, además, en consonancia con reiteradas interpretaciones de la SIC sobre este punto.

Sin perjuicio de lo anterior me permito hacer notar que, aún si en gracia de discusión los usos o métodos terapéuticos fuesen permitidos en nuestro medio, éstos deberían cumplir necesariamente, y en forma simultánea, con los requisitos materiales de patentabilidad, es decir, novedad, nivel Inventivo y aplicación

industrial. Además de anotar que respecto del pretendido “uso”, el tercer requisito resulta de imposible cumplimiento, en tanto la reproducción industrial del uso separado, combinado o secuencial de medicamentos para tratar ciertas patologías sería del resorte de la profesión médica, y no, por supuesto, de las actividades industriales.

De la misma manera la Decisión 486 de la Comunidad Andina de Naciones en su aparte d) del artículo 20 del capítulo I, acerca de los requisitos de patentabilidad, menciona que *“no serán patentables 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”*, y si tenemos en cuenta que la solicitud en cuestión reivindica también un método de tratamiento contra trastornos asociados con tumores relacionados con la proteína MN, tal como lo señala en sus reivindicaciones 12 y 13, queda claramente expuesta la improcedencia directa para otorgar el privilegio de patente de invención a esta solicitud.

En un segundo aspecto, y esta vez refiriéndonos a la combinación farmacológica que reivindica la solicitud colombiana en cuestión, específicamente en sus reivindicaciones 12 y 13, es relevante mencionar el concepto de la Doctora en Bioquímica Silvia Susana Giarcovich¹ en su opinión escrita a cerca de “Asociaciones y Patentes” en donde afirma que las asociaciones farmacológicas están restringidas por ciertos principios y lineamientos preestablecidos por varias entidades, puesto que dichas combinaciones deben estar bajo el precepto de eficacia, seguridad y eficiencia terapéutica, veamos:

“3.3.2. Lineamientos FDA:

A continuación se mencionarán lineamientos que la agencia sanitaria de EEUU ha establecido para el caso de los llamados “*Combination Drug Products*”:

3.3.2.1. CFR

El CFR establece la política en relación a las combinaciones de drogas a dosis fija para humanos en 21CFR300.50. Allí se establece que dos o más drogas pueden ser combinadas en una única unidad posológica cuando cada componente hace una contribución al efecto propuesto y la dosis de cada componente es tal que la combinación es segura y efectiva (se consideran casos especiales cuando se busca

¹ Profesora Regular Adjunta del Departamento de Farmacología de la Facultad de Farmacia y Bioquímica de la UBA y Directora Técnica y Gerente de Estudios Clínicos de DIFFUCAP-EURAND SACIFI

aumentar la seguridad y eficacia del principal componente o minimizar su potencial abuso).

3.3.2.2. Guía "OTC Combination of Drug Products" [Docket No. 78D-0322]²:

Esta guía establece en detalle la política de la agencia para combinar dos o más ingredientes activos OTC, seguros y eficaces y caracteriza diferentes tipos de asociaciones de OTC:

- Ingredientes activos de diferentes categorías terapéuticas para tratar distintos síntomas simultáneamente: Sólo se acepta esta asociación si cada ingrediente está presente dentro de su rango de seguridad y eficacia.
- Ingredientes activos de la misma categoría terapéutica que tienen distintos mecanismos de acción para tratar los mismos síntomas o condición: Sólo se acepta esta asociación si es mejor o igual que cada ingrediente solo en su dosis terapéutica y también pueden usarse a dosis subterapéuticas si corresponde.
- Ingredientes activos de la misma categoría terapéutica que tienen el mismo mecanismo de acción: Estos no deberían ser combinados a menos que haya alguna ventaja con respecto a los ingredientes simples (mayor eficacia, seguridad, aceptación del paciente o calidad de la formulación).
- Ingredientes considerados adyuvantes, que aumenten o alteren el efecto de otro ingrediente activo, serán, considerados ingredientes activos.

3.3.2.3. Guía Draft "Current GMP for Combination Products":

Este documento provee una guía para la industria y para el *staff* de la FDA acerca de la aplicabilidad de la cGMP para las asociaciones (*combination products*) y define a un *combination product*, de acuerdo al CFR (21CFR3.2(e), de forma que no sólo involucra drogas sino *devices*:

Un producto combinado es un producto compuesto de cualquier combinación de una droga y un dispositivo; un producto biológico y un dispositivo; una droga y un producto biológico; o una droga, dispositivo, y un producto biológico. Bajo 21 CFR 3.2 (e), un producto de combinación incluye:

² Anunciada en el Federal Register (1978), Vol. 43, No. 229.

1. *Un producto que comprende dos o más componentes regulados (i.e., droga/dispositivo, biológico/dispositivo, droga/biológico, o droga/dispositivo/biológico) que son combinados físicamente, químicamente, o de otra forma, o mezclados y producidos como una entidad única;*
2. *Dos o más productos separados, empaquetados juntos en un solo paquete, o como una unidad comprendiendo droga y productos del dispositivo, dispositivo y productos biológicos, o biológico y productos de droga;*
3. *Una droga, dispositivo, o producto biológico empaquetado separadamente que, de acuerdo a su plan de investigación o el etiquetado propuesto, es destinado para utilizarse únicamente con una droga, dispositivo, o producto biológico aprobada especificada individualmente, dónde al mismo tiempo ambos son deben estar aceptados para la indicación o efecto y donde, luego de la aprobación del producto propuesto, el etiquetado del producto aprobado necesitaría ser cambiado (e.g., para reflejar un cambio en la indicación, forma farmacéutica, dosis, ruta de administración, cambio significativo en la posología); ó*
4. *Cualquier droga en investigación, dispositivo, o producto biológico empaquetado separadamente que, de acuerdo al etiquetado propuesto es para utilizarse únicamente con otra droga en investigación especificada, dispositivo, o producto biológico, dónde a la vez se requiere que ambos sean aceptados para la misma indicación, uso y efecto.*

Además, dentro de esta guía es muy interesante el concepto de "parte constitutiva de un *combination product*" porque especifica su identidad regulatoria como droga, *device* o producto biológico.

3.3.2.4. Guía Draft "Combination Products Timeliness of Premarket Reviews":

Esta guía provee información a un postulante a reclamar la resolución de una disputa acerca de la duración de la revisión de una postulación referida a un producto combinado. Es una guía referida simplemente a un problema administrativo que demuestra cuán regulado se halla este ámbito que tiene una oficina destinada, la Office of Combination Products.

3.3.3. Lineamientos EMEA: Guía "Fixed Combination Medicinal Products":

Esta guía propone evaluar las potenciales ventajas de la asociación en cuanto a mejoramiento de la relación riesgo/beneficio y a simplificación de la terapia. Asimismo, establece criterios a respetar y requisitos a cumplimentar, tanto generales como particulares. Dentro de los particulares hace especial referencia a:

- La indicación.
- Los estudios farmacodinámicos y farmacocinéticos.
- Eficacia y Seguridad: En este caso diferencia dos escenarios concretos:

a) Cuando la combinación fija corresponde exactamente a combinaciones que están ya en uso difundido, un análisis de los datos bibliográficos bien fundamentados debería ser presentado. Con tal que los datos estén bien documentados, este análisis puede ser útil en reducir la cantidad de ensayos clínicos a ser realizada y podría facilitar la selección de dosis para cada sustancia y el rango de dosis propuesto en la combinación fija.

b) Cuando la combinación fija es esencialmente nueva (las sustancias activas no son usualmente combinadas, la composición cuantitativa es inusual entre sustancias usualmente combinadas o una sustancia enteramente nueva), los datos necesarios son similares a los de una nueva entidad química en la situación donde la combinación fija será propuesta (primero línea o segunda de la línea de terapia). La existencia de experiencia con las sustancias debería ser tomada en cuenta.

y especifica Régimen de dosis y Composición, Ensayos Terapéuticos y Aspectos de Seguridad.

- Paquetes combinados de diferentes productos medicinales para administración simultánea o secuencial.

Conclusión:

Las diferentes agencias sanitarias a nivel internacional han regulado fuertemente el registro de asociaciones de forma de garantizar la seguridad y eficacia de las mismas y la buena calidad en su elaboración. Todo estudio, investigación o análisis derivados de estos requisitos representan procedimientos de rutina y no originales ideas llevadas a la práctica."

Además, se puede considerar que todo efecto sinérgico o terapéutico entre fármacos no se refiere propiamente a una invención, sino que corresponde mejor a la categoría de un descubrimiento, dado que dicho efecto sinérgico o terapéutico conseguido mediante la combinación de fármacos ocurre dentro del organismo y

se hace evidente a través de los respectivos ensayos preclínicos y/o clínicos por los que todo fármaco o combinación de fármacos se deben evaluar. Así mismo es importante volver a enfatizar en que la combinación de fármacos para tratar ciertas patologías, hace parte de las actividades de la profesión médica, y no de las actividades industriales.

Finalmente, también podemos demostrar la carencia de nivel inventivo de la conjugación entre los anticuerpos que se reivindican en la solicitud en cuestión con diversos agentes citotóxicos, veamos:

Primero que todo es importante indicar que la estrategia de conjugación entre anticuerpos y agentes anticancerígenos es una estrategia terapéutica ampliamente utilizada con anterioridad, puesto que dicha conjugación representa una mayor especificidad hacia los tejidos afectados y al mismo tiempo reduce la toxicidad de otros tejidos sanos. Esto puede evidenciarse en documentos tales como la publicación internacional WO/1987/05031 publicada el 27 de agosto de 1987, la patente europea EP 0330201 publicada el 23 de febrero de 1989 y la patente europea EP 0080401 publicada el 29 de Julio de 1992.

En segundo lugar, se debe mencionar que con anterioridad, en la publicación científica denominada "Human Tumour-Associated Cell Adhesion Proteína MN/CA IX: Identification Of M75 Epitope And Of The Region Mediating Cell Adhesión³" ya se reconocía a las proteínas MN como un bioindicador de la presencia de trastornos neoplásicos en los mismo sistemas u órganos que menciona la solicitud colombiana en trámite, específicamente en sus reivindicaciones 12 y 15 veamos:

³ J Závada, Z Zavadová, J Pastorek, Z Biesová, J Jezek and J Velek. Human tumour-associated cell adhesion protein MN/CA IX: identification of M75 epitope and of the region mediating cell adhesion. British Journal of Cancer (2000) 82(11), 1808–1813© 2000 Cancer Research Campaign DOI: 10.1054/ bjoc.2000.1111, available online at <http://www.idealibrary.com>

MN/CA IX protein (initially termed MN) was discovered in HeLa cells using monoclonal antibody (mAb) M75 (Pastorekova et al, 1992). Its possible role in oncogenesis was suggested by two facts: expression of MN/CA IX correlated with tumorigenicity of HeLa x fibroblast hybrids; it was detected in clinical specimens of cervical and certain other carcinomas, but it was absent in normal tissues of corresponding organs (Závada et al, 1993). *MN/CA9* cDNA (Pastorek et al, 1994) and gene (Opavsky et al, 1996) have been cloned; predicted protein consists of signal peptide (SP), proteoglycan-related sequence (PG), carbonic anhydrase domain (CA), transmembrane segment (TM) and short intracellular tail (IC).

MN/CA IX protein is a cell adhesion molecule; its CA is enzymatically active (Zavada et al, 1997). MN/CA IX protein is almost 100% associated with cervical carcinomas (Liao et al, 1994), with carcinomas of oesophagus (Turner et al, 1997) and with renal clear cell carcinomas (Liao et al, 1997). It was also detected in a high percentage of colorectal (Saarnio et al, 1998a) and lung (Vernylen et al, 1999) carcinomas. Therefore, MN/CA IX is a promising biomarker in tumour diagnostics. Characteristically, MN/CA IX in human carcinomas is expressed ectopically; normally it is present in stomach and bile duct mucosa (Liao et al, 1994) and in rapidly proliferating normal cells in small intestine (Saarnio et al, 1998b).

Así mismo menciona la obtención de anticuerpos específicos para dicha proteína:

An antigen specific for renal cell carcinomas was discovered using mAb G250 (Oosterwijk et al, 1986) and was found to be identical with MN/CA IX (Uemura et al, 1997). This antigen appears to be an excellent target for immunotherapy (Steffens et al, 1997; Uemura et al, 1997). Until now, molecular basis of involvement of MN/CA IX in carcinogenesis has remained unclear. One of the possible leads could be the fact that MN/CA IX is an adhesion molecule presumably engaged in cell-to-cell communication (Závada et al, 1997). In this paper we undertook the first steps towards elucidation of the role of MN/CA IX protein in communication among the cells. We attempted to identify its binding site for cell surface receptors. This was facilitated by the finding that mAb M75 blocks adhesion of cells to affinity-purified MN/CA IX protein.

Con lo anteriormente expuesto, queda claro que la conjugación entre un anticuerpo anti-MN con un agente citotóxico, es una estrategia terapéutica evidente, que cualquier persona medianamente versada en al materia, que necesitara aumentar la especificidad y reducir la toxicidad de un tratamiento de

cáncer de pulmón, tracto digestivo, renal y de cerviz, podría obtener aplicando las diversas técnicas de conjugación descritas en el estado de la técnica.

Siendo aun mas específicos, y como ya se hace evidente en la publicación científica expuesta, la obtención de anticuerpos contra este tipo de proteínas MN también se encuentran descritas en el estado de la técnica. Publicaciones tales como la publicación internacional WO/1993/18152 publicada el 16 de septiembre de 1993, la publicación internacional WO/1995/34650 publicada el 21 de diciembre de 1995, la patente americana US 6297051 publicada el 02 de octubre de 2001 y la publicación internacional WO/2004/048544 publicada el 10 junio de 2004 revelan también con anterioridad la obtención y utilización de anticuerpos contra la proteína MN en diversos tipos de cáncer.

De manera especial, la publicación internacional WO/2003/033674 publicada el 24 de Abril de 2003 y titulada "HUMAN ANTIBODIES THAT HAVE MN BINDING AND CELL ADHESION-NEUTRALIZING ACTIVITY" revela:

1. A purified preparation of a human antibody, wherein the antibody binds MN protein.
(...)
4. The preparation of claim 3 wherein the antibody binds to the human MN protein with a K_d of about 0.6 nM to about 1800 nM..
5. The preparation of claim 3 wherein the antibody binds to the human MN protein with a K_d of about 0.6 nM to about 90 nM.
6. The purified preparation of Claim 1 wherein the human antibody comprises a VH3-CDR3 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 61-80.
(...)
9. The purified preparation of Claim 1 wherein the human antibody comprises a VL λ 1-CDR3 region comprising the amino acid sequence of SEQ ID NOS: 81.
(...)
17. A nucleotide sequence that codes for a purified preparation of a human antibody, wherein the antibody binds MN protein.

18. The purified preparation of Claim 17 wherein the nucleotide sequence comprises a VH3-CDR1 region selected from the group consisting of SEQ ID NOS: 1-13.

(...)

21. The purified preparation of Claim 17 wherein the nucleotide sequence comprises a VL2-CDR1 region comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS: 37-38.

(...)

56. A method of making a human antibody, comprising the steps of:
culturing the host cell of claim 31 under conditions whereby the antibody is expressed; and purifying the human antibody from the host cell culture.

(...)

70. The method of claim 69 wherein the disorder is selected from the group consisting of renal cell carcinomas, esophagus carcinomas, cervical carcinomas, malignant colon carcinomas, and non-small cell lung carcinomas.

(...)

87. A pharmaceutical composition comprising a human antibody that binds to MN protein and a pharmaceutically acceptable carrier.

Como se puede apreciar claramente, en esta anterioridad se revelan métodos de obtención de anticuerpos humanizados específicos para proteínas MN, que involucran su codificación a partir de nucleótidos, útiles en el diagnóstico de cánceres del mismo tipo que los mencionados en la solicitud colombiana en trámite. Dichos anticuerpos, además, también poseen un CDR1 y un CDR2 y presentan un rango de valores de constante de disociación que incluyen a los valores reivindicados por la solicitud en cuestión.

En conclusión puede afirmarse que la solicitud colombiana en trámite revela anticuerpos contra las proteínas MN cuya utilidad y métodos de obtención ya se describían con anterioridad en el estado de la técnica, y busca utilizar estrategias terapéuticas igualmente reconocidas, como lo son la asociación con otros agentes anticancerígenos o la conjugación con agentes citotóxicos, lo que sugiere una simple combinación de técnicas y estrategias basadas en información anterior que irrefutablemente resulta siendo una clara guía para cualquier persona

medianamente versada en la materia que este buscando tratar de manera segura y efectiva ciertos tipos de canceres.

Así las cosas, y con fundamento en los argumentos expuestos a lo largo del presente escrito, respetuosamente reitero la solicitud para que su despacho se abstenga de conceder el privilegio de patente solicitado, y ordene en consecuencia el archivo del expediente respectivo.

IV. FUNDAMENTOS DE DERECHO

Son fundamentos de derecho el Capítulo I, artículos 14, 16, 18 y 20 literal d) de (Requisitos de Patentabilidad) y el artículo 42 (Oposiciones) de la Decisión 486 de 2000.

V. PRUEBAS

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

- ❖ Portadas y capítulos reivindicatorios de: La publicación internacional WO/1993/18152 publicada el 16 de septiembre de 1993, la publicación internacional WO/1995/34650 publicada el 21 de diciembre de 1995, la patente americana US 6297051 publicada el 02 de octubre de 2001, la publicación internacional WO/2004/048544 publicada el 10 junio de 2004 y la publicación internacional WO/2003/033674 publicada el 24 de Abril de 2003 y titulada "HUMAN ANTIBODIES THAT HAVE MN BINDING AND CELL ADHESION-NEUTRALIZING ACTIVITY".

VI. ANEXOS

Para efectos del trámite correspondiente, me permito anexar al presente escrito los siguientes anexos

1. Certificado de existencia y representación legal de LAFRANCOL S.A.

2. Poder para actuar.
3. Constancia de pago de las tasas de tramitación de oposición.
4. Copia para correr traslado al solicitante.
5. El mencionado en el acápite de pruebas.

VIII. NOTIFICACIONES

El suscrito recibirá notificaciones en la secretaría de su Despacho o en mi oficina localizada en la Carrera 17 No. 88-23, Oficina 205, Fax (1) 621.25.42 de esta ciudad. Lafrancol S.A., las recibirá en sus oficinas localizadas en la Carrera 1 N° 46-84 de Cali.

Señor Superintendente,



JOSÉ LUIS REYES VILLAMIZAR

C.C. 79'152.473 de Usaquén

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

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
24 April 2003 (24.04.2003)

PCT

(10) International Publication Number
WO 03/033674 A2(51) International Patent Classification⁷:

C12N

(74) Agent: MAHONEY, John, W.; Bayer Corporation, 800
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(21) International Application Number: PCT/US02/33470

(22) International Filing Date: 18 October 2002 (18.10.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/343,657 18 October 2001 (18.10.2001) US
60/377,716 2 May 2002 (02.05.2002) US(71) Applicant (for all designated States except US): BAYER
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Boulevard, Moraga, CA 94556 (US).(81) Designated States (national): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI,
SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VC, VN,
YU, ZA, ZW.(84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,
ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK,
TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:

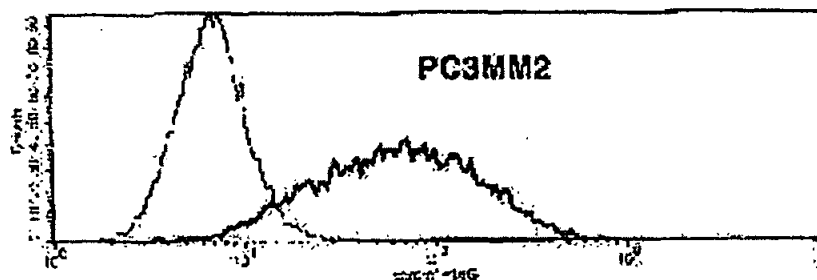
— of inventorship (Rule 4.17(iv)) for US only

Published:

— without international search report and to be republished
upon receipt of that report

[Continued on next page]

(54) Title: HUMAN ANTIBODIES THAT HAVE MN BINDING AND CELL ADHESION-NEUTRALIZING ACTIVITY



(57) Abstract: The invention is composed of monoclonal human MN antibodies or MN antibody fragments that target the GEEDLP repeat within the proteoglycan domain. The proteoglycan domain of the MN cell surface protein contains four of these identical GEEDLP repeats. Binding to the desired epitope is verified by competition ELISA, where ELISA signal can be attenuated by co-incubation with a peptide containing this repeat (PGEEDLPGEEDLP). This inhibition of binding can also be verified using Biacore assays, where binding of desired antibodies to immobilized MN or proteoglycan peptides can be inhibited by the peptide repeat. In addition to binding to the peptide repeat, human anti-MN antibodies can inhibit the cell adhesion of CGL-1 cells to MN coated plastic plates. Human anti-MN antibodies have been used to diagnose and quantify MN expression in cancer cells and tumors using FACS and immunohistochemical methods. An example is also provided where a human anti-MN IgG1 mediates tumor cell lysis through antibody-dependent cell-mediated cytotoxicity. Therefore, these antibodies will be useful for the treatment of cancers in which MN is upregulated or can be useful for the diagnosis of cancers in which MN is upregulated.

WO 03/033674 A2

We claim:

1. A purified preparation of a human antibody, wherein the antibody binds MN protein.
2. The purified preparation of Claim 1 wherein the antibody binds to the MN protein's proteoglycan domain.
3. The purified preparation of Claim 1 wherein the antibody binds to a GEEDLP repeat region within the MN protein's proteoglycan domain.
4. The preparation of claim 3 wherein the antibody binds to the human MN protein with a K_d of about 0.6 nM to about 1800 nM..
5. The preparation of claim 3 wherein the antibody binds to the human MN protein with a K_d of about 0.6 nM to about 90 nM.
6. The purified preparation of Claim 1 wherein the human antibody comprises a VH3-CDR3 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 61-80.
7. The purified preparation of Claim 1 wherein the human antibody comprises a VH3-CDR1 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 48-60.
8. The purified preparation of Claim 1 wherein the human antibody comprises a VH3-CDR3 region comprising the amino acid of SEQ ID NO: 64.
9. The purified preparation of Claim 1 wherein the human antibody comprises a VL λ 1-CDR3 region comprising the amino acid sequence of SEQ ID NOS: 81.
10. The purified preparation of Claim 1 comprising a VL λ 2-CDR1 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 82-83.

11. The purified preparation of Claim 1 wherein the human antibody comprises a VL λ 2-CDR3 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 84-89.
12. The purified preparation of Claim 1 wherein the human antibody comprises a VH3-CDR3 and VL2-CDR3 amino acid sequence pair selected from the group consisting of SEQ ID NOS: 61 and 84, SEQ ID NOS: 62 and 87, SEQ ID NOS: 63 and 89, SEQ ID NOS: 64 and 84, SEQ ID NOS: 65 and 84, SEQ ID NOS: 66 and 85, SEQ ID NOS: 67 and 88.
13. The purified preparation of Claim 1 wherein the human antibody comprises a VH3-CDR3 and VL2-CDR3 amino acid sequence pair selected from the group consisting of SEQ ID NOS: 61 and 86, SEQ ID NOS: 61 and 85, SEQ ID NOS: 61 and 87, SEQ ID NOS: 61 and 88, SEQ ID NOS: 61 and 89, SEQ ID NOS: 63 and 86, SEQ ID NOS: 63 and 85, SEQ ID NOS: 63 and 87, SEQ ID NOS: 63 and 88, and SEQ ID NOS: 63 and 84.
14. The purified preparation of Claim 1 wherein the human antibody comprises a VH3-CDR3 and VL2-CDR3 amino acid sequence pair selected from the group consisting of SEQ ID NOS: 71 and 87, SEQ ID NOS: 61 and 87, SEQ ID NOS: 72 and 87, SEQ ID NOS: 73 and 87, SEQ ID NOS: 74 and 87, SEQ ID NOS: 75 and 87, SEQ ID NOS: 76 and 87, SEQ ID NOS: 77 and 87, SEQ ID NOS: 78 and 87, SEQ ID NOS: 79 and 87, and SEQ ID NOS: 80 and 87.
15. The purified preparation of Claim 1 wherein the human antibody comprises a VH3-CDR3 and VL1-CDR-3 amino acid sequence pair selected from the group consisting of SEQ ID NOS: 61 and 81, SEQ ID NOS: 69 and 81, and SEQ ID NOS: 70 and 81.
16. The purified preparation of Claim 1 wherein the human antibody comprises VH3-CDR3, VL2-CDR3, and VH3-CDR1 amino acid sequences selected from the group consisting of SEQ ID NOS: 61 and 86 and 48, SEQ ID NOS: 61 and 86 and 49, SEQ ID NOS: 61 and 86 and 50, SEQ ID NOS: 61 and 86 and 51, SEQ ID NOS: 61 and 86 and 52, SEQ ID NOS: 61 and 86 and 53, SEQ ID NOS: 61 and 86 and 54, SEQ ID NOS: 61 and 86 and 55, SEQ ID NOS: 61 and 86 and 56, and SEQ ID NOS: 61 and 86 and 57.

17. A nucleotide sequence that codes for a purified preparation of a human antibody, wherein the antibody binds MN protein.
18. The purified preparation of Claim 17 wherein the nucleotide sequence comprises a VH3-CDR1 region selected from the group consisting of SEQ ID NOS: 1-13.
19. The purified preparation of Claim 17 wherein the nucleotide sequence comprises a VH3-CDR3 region comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS: 14-33.
20. The purified preparation of Claim 17 wherein the nucleotide sequence comprises a VL1-CDR3 region comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS: 34-36.
21. The purified preparation of Claim 17 wherein the nucleotide sequence comprises a VL2-CDR1 region comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS: 37-38.
22. The purified preparation of Claim 17 wherein the nucleotide sequence comprises a VL2-CDR3 region comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS: 39-44.
23. The purified preparation of Claim 17 wherein the nucleotide sequence comprises VH3-CDR1, VH3-CDR3 and VL2-CDR3 nucleotide sequences selected from the group consisting of SEQ ID: 1 and 14 and 41, SEQ ID: 2 and 14 and 41, SEQ ID: 3 and 14 and 41, and SEQ ID: 4 and 14 and 41.
24. The purified preparation of Claim 17 wherein the nucleotide sequence comprises VH3-CDR3, VL2-CDR1, and VL2-CDR3 nucleotide sequences selected from the group consisting of SEQ ID: 14 and 37 and 41, and SEQ ID: 14 and 38 and 41.
25. The purified preparation of Claim 17 wherein the nucleotide sequence comprises VH3-CDR3, VL2-CDR3 and VH3-CDR1 nucleotide sequences selected from the group consisting of SEQ ID: 14 and 41 and 1, SEQ ID: 14

and 41 and 2, SEQ ID: 14 and 41 and 3, SEQ ID: 14 and 41 and 4, SEQ ID: 14 and 41 and 5, SEQ ID: 14 and 41 and 6, SEQ ID: 14 and 41 and 7, SEQ ID: 14 and 41 and 8, SEQ ID: 14 and 41 and 9, and SEQ ID: 14 and 41 and 10.

26. The purified preparation of Claim 17 wherein the nucleotide sequence comprises VH3-CDR3, VL2-CDR3 nucleotide sequences selected from the group consisting of SEQ ID: 14 and 39, SEQ ID: 15 and 42, SEQ ID: 16 and 44, SEQ ID: 17 and 39, SEQ ID: 18 and 39, SEQ ID: 19 and 40, and SEQ ID: 20 and 43.
27. The purified preparation of Claim 17 wherein the nucleotide sequence comprises VH3-CDR3, VL1-CDR3 nucleotide sequences selected from the group consisting of SEQ ID: 14 and 34, SEQ ID: 22 and 34, SEQ ID: 22 and 35, SEQ ID: 22 and 36, and SEQ ID: 23 and 34.
28. The purified preparation of Claim 17 wherein the nucleotide sequence comprises VH3-CDR3, VL2-CDR3 nucleotide sequences selected from the group consisting of SEQ ID: 14 and 41, SEQ ID: 14 and 40, SEQ ID: 14 and 42, SEQ ID: 14 and 43, SEQ ID: 14 and 44, SEQ ID: 16 and 41, SEQ ID: 16 and 40, SEQ ID: 16 and 42, SEQ ID: 16 and 43, and SEQ ID: 16 and 39.
29. The purified preparation of Claim 17 wherein the nucleotide sequence comprises VH3-CDR3, VL2-CDR3 nucleotide sequences selected from the group consisting of SEQ ID: 24 and 42, SEQ ID: 14 and 42, SEQ ID: 25 and 42, SEQ ID: 26 and 42, SEQ ID: 27 and 42, SEQ ID: 28, and 42, SEQ ID: 29 and 42, SEQ ID: 30 and 42, SEQ ID: 31 and 42, SEQ ID: 32 and 42 and SEQ ID: 33 and 42.
30. An expression vector comprising the polynucleotide of claim 17, wherein said vector codes for MN binding antibody.
31. An expression vector comprising the polynucleotide of claim 18, wherein said vector codes for MN binding antibody.
32. An expression vector comprising the polynucleotide of claim 19, wherein said vector codes for MN binding antibody.

33. An expression vector comprising the polynucleotide of claim 20, wherein said vector codes for MN binding antibody.
34. An expression vector comprising the polynucleotide of claim 21, wherein said vector codes for MN binding antibody.
35. An expression vector comprising the polynucleotide of claim 22, wherein said vector codes for MN binding antibody.
36. An expression vector comprising the polynucleotide of claim 23, wherein said vector codes for MN binding antibody.
37. An expression vector comprising the polynucleotide of claim 24, wherein said vector codes for MN binding antibody.
38. An expression vector comprising the polynucleotide of claim 25, wherein said vector codes for MN binding antibody.
39. An expression vector comprising the polynucleotide of claim 26, wherein said vector codes for MN binding antibody.
40. An expression vector comprising the polynucleotide of claim 27, wherein said vector codes for MN binding antibody.
41. An expression vector comprising the polynucleotide of claim 28, wherein said vector codes for MN binding antibody.
42. An expression vector comprising the polynucleotide of claim 29, wherein said vector codes for MN binding antibody.
43. A host cell comprising the expression vector of claim 30, wherein said cell expresses MN binding antibody.
44. A host cell comprising the expression vector of claim 31, wherein said cell expresses MN binding antibody.

45. A host cell comprising the expression vector of claim 32, wherein said cell expresses MN binding antibody.
46. A host cell comprising the expression vector of claim 33, wherein said cell expresses MN binding antibody.
47. A host cell comprising the expression vector of claim 34, wherein said cell expresses MN binding antibody.
48. A host cell comprising the expression vector of claim 35, wherein said cell expresses MN binding antibody.
49. A host cell comprising the expression vector of claim 36, wherein said cell expresses MN binding antibody.
50. A host cell comprising the expression vector of claim 37, wherein said cell expresses MN binding antibody.
51. A host cell comprising the expression vector of claim 38, wherein said cell expresses MN binding antibody.
52. A host cell comprising the expression vector of claim 39, wherein said cell expresses MN binding antibody.
53. A host cell comprising the expression vector of claim 40, wherein said cell expresses MN binding antibody.
54. A host cell comprising the expression vector of claim 41, wherein said cell expresses MN binding antibody.
55. A host cell comprising the expression vector of claim 42, wherein said cell expresses MN binding antibody.
56. A method of making a human antibody, comprising the steps of:
culturing the host cell of claim 31 under conditions whereby the antibody is expressed; and purifying the human antibody from the host cell culture.

57. A method of making a human antibody, comprising the steps of:
culturing the host cell of claim 32 under conditions whereby the antibody is expressed; and purifying the human antibody from the host cell culture.
58. A method of making a human antibody, comprising the steps of:
culturing the host cell of claim 33 under conditions whereby the antibody is expressed; and purifying the human antibody from the host cell culture.
59. A method of making a human antibody, comprising the steps of:
culturing the host cell of claim 34 under conditions whereby the antibody is expressed; and purifying the human antibody from the host cell culture.
60. A method of making a human antibody, comprising the steps of:
culturing the host cell of claim 35 under conditions whereby the antibody is expressed; and purifying the human antibody from the host cell culture.
61. A method of making a human antibody, comprising the steps of:
culturing the host cell of claim 36 under conditions whereby the antibody is expressed; and purifying the human antibody from the host cell culture.
62. A method of making a human antibody, comprising the steps of:
culturing the host cell of claim 37 under conditions whereby the antibody is expressed; and purifying the human antibody from the host cell culture.
63. A method of making a human antibody, comprising the steps of:
culturing the host cell of claim 38 under conditions whereby the antibody is expressed; and purifying the human antibody from the host cell culture.
64. A method of making a human antibody, comprising the steps of:
culturing the host cell of claim 39 under conditions whereby the antibody is expressed; and purifying the human antibody from the host cell culture.
65. A method of making a human antibody, comprising the steps of:
culturing the host cell of claim 40 under conditions whereby the antibody is expressed; and purifying the human antibody from the host cell culture.
66. A method of making a human antibody, comprising the steps of:

culturing the host cell of claim 41 under conditions whereby the antibody is expressed; and purifying the human antibody from the host cell culture.

67. A method of making a human antibody, comprising the steps of:
culturing the host cell of claim 42 under conditions whereby the antibody is expressed; and purifying the human antibody from the host cell culture.
68. A method of making a human antibody, comprising the steps of:
culturing the host cell of claim 43 under conditions whereby the antibody is expressed; and purifying the human antibody from the host cell culture.
69. A method of treating a human disorder in which MN protein is expressed in certain cells, comprising the step of:
a) Providing a human having a condition in which MN protein is expressed in certain cells; and
b) Administering to said human an effective amount of a human MN antibody compound whereby, said compound includes an MN antibody and a cytotoxic agent, wherein said cytotoxic agent is capable of inducing cell death in said MN expressing cells.
70. The method of claim 69 wherein the disorder is selected from the group consisting of renal cell carcinomas, esophagus carcinomas, cervical carcinomas, malignant colon carcinomas, and non-small cell lung carcinomas.
71. The method of claim 69 wherein the antibody comprises a VH3-CDR3 region selected from the group consisting of SEQ ID NOS: 61-80.
72. The method of claim 69 wherein the antibody comprises a VH3-CDR1 region selected from the group consisting of SEQ ID NOS: 48-60.
73. The method of claim 69 wherein the antibody comprises a VL1-CDR3 region consisting of SEQ ID NOS: 81.
74. The method of claim 69 wherein the antibody comprises a VL2-CDR1 region selected from the group consisting of SEQ ID NOS: 82-83.

75. The method of claim 69 wherein the antibody comprises a VL2-CDR3 region selected from the group consisting of SEQ ID NOS: 84-89.
76. A method of detecting MN antigen in a test preparation comprising the steps of:
- a) contacting the test preparation with an antibody that specifically binds to MN antigen, and
 - b) assaying the test preparation for the presence of the antibody - MN antigen complex.
77. The method of Claim 76 wherein the antibody comprises a detectable label.
78. The method of Claim 76 wherein the antibody is bound to a solid support.
79. A method to aid in diagnosing a disorder in which MN protein level is elevated, comprising the steps of:
- a) contacting a sample from a patient suspected of having the disorder with a human antibody that binds to MN; and
 - b) assaying for the presence of an antibody-MN complex, whereby detection of an amount of the complex which is greater than an amount of the complex in a normal sample identifies the patient as likely to have the disorder.
80. The method of claim 79 wherein the antibody comprises a detectable label.
81. The method of claim 79 wherein the antibody is bound to a solid support.
82. The method of claim 79 wherein the antibody comprises a VH3-CDR3 region selected from the group consisting of SEQ ID NOS: 61-80.
83. The method of claim 79 wherein the antibody comprises a VH3-CDR1 region selected from the group consisting of SEQ ID NOS: 48-60.
84. The method of claim 79 wherein the antibody comprises a VL1-CDR3 region consisting of SEQ ID NOS: 81.

85. The method of claim 79 wherein the antibody comprises a VL2-CDR1 region selected from the group consisting of SEQ ID NOS: 82-83.
86. The method of claim 79 wherein the antibody comprises a VL2-CDR3 region selected from the group consisting of SEQ ID NOS: 84-89.
87. A pharmaceutical composition comprising a human antibody that binds to MN protein and a pharmaceutically acceptable carrier.



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : C12N 15/33, 1/20, 5/10 A61K 39/12, C12N 15/62 C12P 21/08, A61K 39/395 G01N 33/569	A1	(11) International Publication Number: WO 93/18152 (43) International Publication Date: 16 September 1993 (16.09.93)
(21) International Application Number: PCT/US93/02024 (22) International Filing Date: 8 March 1993 (08.03.93) (30) Priority data PV 709-92 11 March 1992 (11.03.92) CS 07/964,589 21 October 1992 (21.10.92) US (71) Applicants: CIBA CORNING DIAGNOSTICS CORP. [US/US]; 63 North Street, Medfield, MA 02052 (US). INSTITUTE OF VIROLOGY [SK/SK]; Slovak Academy of Sciences, Dubravska Cesta 9, 842 46 Bratislava (SK). (72) Inventors: ZAVADA, Jan ; Na pekne vyhlidce 1, 160 00 Prague 6 (CZ). PASTOREKOVA, Silvia ; PASTOREK, Jaromir ; I. Bukovcana 18, 841 07 Bratislava (SK).		(74) Agent: LAUDER, Leona, L.; 177 Post Street, Suite 800, San Francisco, CA 94108-4731 (US). (81) Designated States: AT, AU, BB, BG, BR, CA, CH, CZ, DE, DE (Utility model), DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, SN, TD, TG). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: MN GENE AND PROTEIN (57) Abstract A new gene -- MN -- and proteins/polypeptides encoded therefrom are disclosed. Recombinant nucleic acid molecules for expressing MN proteins/polypeptides and fusion proteins are provided. Expression of the MN gene is disclosed as being associated with tumorigenicity, and the invention concerns methods and compositions for detecting and/or quantitating MN antigen and/or MN-specific antibodies in vertebrate samples that are diagnostic/prognostic for neoplastic and pre-neoplastic disease. Test kits embodying the immunoassays of this invention are provided. MN-specific antibodies are disclosed that can be used diagnostically/prognostically, therapeutically, for imaging, and for affinity purification of MN proteins/polypeptides. Also provided are nucleic acid probes for the MN gene as well as test kits comprising said probes. The invention also concerns vaccines comprising MN proteins/polypeptides which are effective to immunize a vertebrate against neoplastic diseases associated with the expression of MN proteins. The invention still further concerns antisense nucleic acid sequences that can be used to inhibit MN gene expression.		

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CLAIMS

What we claim is:

1. A composition comprising an isolated MN gene and/or fragments thereof or an isolated nucleic acid sequence
5 that is substantially complementary to said MN gene or a fragment thereof.
2. A composition according to Claim 1 which comprises a nucleic acid sequence substantially complementary
10 to the cDNA sequence shown in Figure 1A-1B [SEQ ID NO.: 1] or a fragment thereof.
3. A composition according to Claim 1 wherein said MN gene or a fragment thereof or nucleic acid sequence
15 substantially complementary to said MN gene or fragment thereof is contained in a vector.
4. A composition according to Claim 3 wherein said nucleic acid sequence is operatively linked to an expression
20 control sequence in said vector.
5. A unicellular host which is either prokaryotic or eukaryotic that is transformed or transfected with the composition of Claim 4.
25
6. A composition comprising protein(s) and/or polypeptide(s) encoded by an MN gene and/or fragment(s) thereof.
7. A composition according to Claim 6 wherein said
30 protein(s) and/or polypeptide(s) comprises or comprise substantially the amino acid sequence or portion(s) thereof shown in Figures 1A-1B [SEQ ID NO.: 2], or variants of said amino acid sequence or portion(s) thereof and/or protein(s)
35 and/or polypeptide(s) which are substantially complementary to amino acid sequence(s) shown in Figure 1A-1B.

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8. A composition according to Claim 7 which is pGEX-3X-MN.

9. A composition comprising an antibody or
5 antibodies which specifically bind to an MN protein/polypeptide.

10. A composition according to Claim 9 wherein said antibodies are monoclonal.

10

11. A composition according to Claim 10 wherein said monoclonal antibodies are designated M75 and are produced by the hybridoma VU-M75 that was deposited at the ATCC under ATCC No. HB 11128.

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12. A composition according to Claim 9 wherein said antibodies are linked to a chemotherapeutic agent or toxic agent and/or to an imaging agent.

20

13. A composition comprising a hybridoma which produces a monoclonal antibody having binding specificity to an MN protein/polypeptide.

14. A method of imaging neoplastic disease in a
25 patient comprising:

a. injecting said patient with appropriately labeled antibodies according to Claim 12; and

b. detecting the binding of said labeled antibodies.

30

15. A method of delivering a chemotherapeutic agent or toxic agent to a cancer cell which comprises contacting the cell with a antibody according to Claim 12.

35

16. A method of recombinantly producing an MN protein or polypeptide comprising the steps of:

a. transforming a unicellular host with a composition according to Claim 4;

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b. culturing said unicellular host so that said MN protein and/or polypeptide is expressed; and

c. extracting and isolating said MN protein and/or polypeptide.

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17. A method of detecting and/or quantitating in a vertebrate sample MN antigen comprising the steps of:

a. contacting said sample with an antibody or antibodies according to Claim 9; and

10 b. detecting and/or quantitating binding of said antibodies to antigen in said sample.

18. A method of detecting and/or quantitating MN-specific antibodies in a vertebrate sample comprising the
15 steps of:

a. contacting and incubating the vertebrate sample with MN protein(s)/polypeptide(s); and

b. detecting and/or quantitating binding of said MN protein(s)/polypeptide(s) to antibody in said sample.

20

19. A vaccine comprising an immunogenic amount of one or more substantially pure MN proteins and/or polypeptides dispersed in a physiologically acceptable, nontoxic vehicle, which amount is effective to immunize a vertebrate against a
25 neoplastic disease associated with expression of MN proteins.

20. A method of treating neoplastic disease and/or pre-neoplastic disease comprising inhibiting the expression of MN genes by administering antisense nucleic acid sequence(s)
30 substantially complementary to mRNA transcribed from MN genes.



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WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/12, C07K 14/82, C12N 1/21, 15/62, C12Q 1/68, A61K 39/00, C12N 5/16, C07K 16/18, C12N 15/87, G01N 33/50, A61K 48/00		A2	(11) International Publication Number: WO 95/34650 (43) International Publication Date: 21 December 1995 (21.12.95)
(21) International Application Number: PCT/US95/07628 (22) International Filing Date: 15 June 1995 (15.06.95)		(72) Inventors; and (75) Inventors/Applicants (for US only): ZAVADA, Jan [CZ/CZ]; Na pekne vyhlidce 1, 160 00 Prague 6 (CZ). PAS- TOREKOVA, Silvia [SK/SK]; I. Bukovcana 18, 841 07 Bratislava (SK). PASTOREK, Jaromir [SK/SK]; I. Bukovcana 18, 841 07 Bratislava (SK).	
(30) Priority Data: 08/260,190 15 June 1994 (15.06.94) US 08/485,049 7 June 1995 (07.06.95) US 08/486,756 7 June 1995 (07.06.95) US 08/447,504 7 June 1995 (07.06.95) US 08/481,658 7 June 1995 (07.06.95) US 08/485,862 7 June 1995 (07.06.95) US 08/485,863 7 June 1995 (07.06.95) US 08/487,077 7 June 1995 (07.06.95) US		(74) Agent: LAUDER, Leona, L.; 6 Mariposa Court, Tiburon, CA 94920-2017 (US).	
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		Published Without international search report and to be republished upon receipt of that report.	
(54) Title: MN GENE AND PROTEIN			
(57) Abstract A complete genomic sequence including a full-length cDNA sequence for the MN gene, a putative oncogene, is disclosed, as well as proteins/polypeptides encoded thereby. Recombinant nucleic acid molecules for expressing MN proteins/polypeptides and recombinant proteins are also provided. Expression of the MN gene is disclosed as being associated with tumorigenicity, and the invention concerns methods and compositions for detecting and/or quantitating MN antigen and/or MN-specific antibodies in vertebrate samples that are diagnostic/prognostic for neoplastic and preneoplastic disease. Test kits embodying the immunoassays of this invention are provided. MN-specific antibodies are disclosed that can be used diagnostically/prognostically, therapeutically, for imaging, and/or for affinity purification of MN proteins/polypeptides. Also provided are nucleic acid probes for the MN gene as well as test kits comprising said probes. The invention also concerns vaccines comprising MN proteins/polypeptides which are effective to immunize a vertebrate against neoplastic diseases associated with the expression of MN proteins. The invention still further concerns antisense nucleic acid sequences that can be used to inhibit MN gene expression, and polymerase chain reaction (PCR) assays to detect genetic rearrangements in MN genes.			

CLAIMS

1. An isolated nucleic acid containing at least twenty-seven nucleotides wherein the nucleotide sequence for said nucleic acid is selected from the group consisting of:

5 (a) SEQ. ID. NOS.: 1, 5, and 27-49 and nucleotide sequences complementary to SEQ. ID. NOS.: 1, 5 and 27-49;

(b) nucleotide sequences that hybridize under stringent hybridization conditions to one or more of the following nucleotide sequences: SEQ. ID. NOS.: 1, 5, and 27-
10 49 and the respective complements of SEQ. ID. NOS.: 1, 5 and 27-49; and

(c) nucleotide sequences that differ from the nucleotide sequences of (a) and (b) in codon sequence due to the degeneracy of the genetic code.

15 2. An isolated nucleic acid according to Claim 1 wherein said nucleotide sequence is selected from the group consisting of:

(a) SEQ. ID. NO.: 5 and its complement;

(b) nucleotide sequences that hybridize under
20 stringent conditions to SEQ. ID. NO.: 5 or to its complement;

(c) nucleotide sequences that differ from the nucleotide sequences of (a) or (b) in codon sequence due to the degeneracy of the genetic code.

25 3. An isolated nucleic acid containing at least sixteen nucleotides wherein the nucleotide sequence therefor is selected from the group consisting of:

(a) the MN nucleotide sequences contained in plasmids A4a, XE1 and XE3 which were deposited at the American Type Culture Collection (ATCC) in Rockville, Maryland in the
30 United State of America under the respective ATCC Nos. 97199, 97200, and 97198;

(b) nucleotide sequences that hybridize under stringent conditions to the MN nucleotide sequences of (a);
and

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(c) nucleotide sequences that differ from the nucleotide sequences of (a) or (b) in codon sequence due to the degeneracy of the genetic code.

4. An isolated nucleic acid according to Claim 3 which functions as a polymerase chain reaction primer for MN nucleic acid sequences, and which is from 16 to about 50 nucleotides in length.

5. An isolated nucleic acid, containing at least fifty nucleotides, encoding an MN protein or polypeptide that is specifically bound either by monoclonal antibodies designated M75 secreted by the hybridoma VU-M75 deposited at the American Type Culture Collection (ATCC) in Rockville, Maryland in the United States of America under ATCC No. HB 11128, or by monoclonal antibodies designated MN12 secreted by the hybridoma MN 12.2.2 deposited at the ATCC under ATCC No. 11647, or by both of said monoclonal antibodies.

6. An isolated nucleic acid which is operatively linked to an expression control sequence within a vector wherein said nucleic acid is selected from the group consisting of:

- (a) SEQ. ID. NO.: 1 and its complement;
- (b) nucleic acids that hybridize under stringent hybridization conditions to SEQ. ID. NO.: 1 or to its complement; and
- (c) nucleic acids that differ from the nucleic acid sequences of (a) and (b) due to the degeneracy of the genetic code.

7. A unicellular host, which is either prokaryotic or eukaryotic, that is transformed or transfected with the isolated nucleic acid operatively linked to an expression control sequence in a vector according to Claim 6.

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8. A method of recombinantly producing an MN protein, MN fusion protein or MN polypeptide comprising the steps of:

- 5 (a) transforming a unicellular host with the isolated nucleic acid operatively linked to an expression control sequence in a vector according to Claim 6;
- (b) culturing said unicellular host so that said MN protein or polypeptide is expressed; and
- 10 (c) extracting and isolating said MN protein or polypeptide.

9. A recombinant nucleic acid encoding a fusion protein, that consists essentially of an MN protein or polypeptide and a non-MN protein or polypeptide, wherein the nucleotide sequence for the portion of the nucleic acid
15 encoding the MN protein or polypeptide is selected from the group consisting of:

- (a) SEQ. ID. NO.: 1;
- (b) nucleotide sequences that hybridize under stringent conditions to SEQ. ID. NO.: 1 or to its complement;
20 and

(c) degenerate variants of SEQ. ID. NO.: 1 and of the nucleotide sequences of (b);

wherein the nucleic acid encoding said MN protein or polypeptide contains at least twenty-nine nucleotides.

25 10. A method of detecting mutations in an isolated MN gene and/or fragment(s) thereof comprising the steps of:
amplifying one or more fragment(s) of said gene by the polymerase chain reaction (PCR); and
determining whether said one or more fragments
30 contain any mutations.

11. An MN protein, MN fusion protein or MN polypeptide, wherein said MN protein, MN polypeptide or the MN protein portion of said MN fusion protein is encoded by a nucleic acid of at least twenty-nine nucleotides which is
35 selected from the group consisting of:

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- (a) SEQ. ID. NO. NO.: 1;
- (b) nucleotide sequences that hybridize under stringent hybridization conditions to SEQ. ID. NO.: 1 or to its complement; and
- 5 (c) nucleotide sequences that differ from SEQ. ID. NO.: 1 or from the sequences of (b) in codon sequence due to the degeneracy of the genetic code.

12. An MN protein, MN fusion protein, or MN polypeptide wherein said MN protein or polypeptide has, and
10 wherein said MN fusion protein contains, an amino acid sequence selected from the group consisting of SEQ. ID. NOS.: 2, 6, 10-16, and 50-53.

13. A vaccine comprising an immunogenic amount of one or more MN proteins, MN fusion proteins, and/or MN
15 polypeptides according to Claims 11 or 12 dispersed in a physiologically acceptable, nontoxic vehicle, which amount is effective to immunize a vertebrate against a neoplastic disease associated with expression of MN antigen.

14. An antibody which specifically binds to an MN
20 protein, an MN fusion protein and/or an MN polypeptide according to Claims 11 or 12.

15. A monoclonal antibody according to Claim 14 which is designated MN12 and is secreted by the hybridoma MN 12.2.2 which was deposited at the American Type Culture
25 Collection (ATCC) in Rockville, Maryland in the United States of America under ATCC No. HB 11647.

16. An antibody according to Claim 14 which specifically binds to an MN antigen epitope selected from the group of epitopes represented by the following amino acid
30 sequences: SEQ. ID. NOS. 10-16.

35

17. An antibody according to Claim 14 which is linked to an imaging agent, to a chemotherapeutic agent or to a toxic agent.

18. A method of imaging pre-neoplastic or
5 neoplastic disease in a patient comprising:

- (a) injecting said patient with antibody linked to an imaging agent according to Claim 17; and
- (b) detecting the binding of said antibody.

19. A hybridoma designated MN 12.2.2 which produces
10 the monoclonal antibody MN12, and which was deposited at the American Type Culture Collection (ATCC) in Rockville Maryland in the United States of America under ATCC Accession No. HB 11647.

20. A method of delivering a chemotherapeutic agent
15 or toxic agent to a cancer cell which comprises contacting said cell with an antibody linked to a chemotherapeutic agent or to a toxic agent according to Claim 17.

21. A method of treating neoplastic disease in a patient comprising administering to said patient a
20 therapeutically effective amount of a composition comprising antibodies which specifically bind to an MN protein, an MN fusion protein and/or an MN polypeptide according to Claims 11 or 12.

22. A method of detecting and/or quantitating in a
25 vertebrate sample MN antigen comprising the steps of:

- (a) contacting said sample with one or more antibodies according to Claims 14, 15 or 16; and
- (b) detecting and/or quantitating binding of said antibody in said sample.

23. A method according to Claim 22 wherein said
30 vertebrate sample is a human tissue specimen, such as, a cell

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smear, a histological section from a biopsied tissue or organ, or an imprint preparation.

24. A method according to Claim 23 wherein said tissue specimen is ovarian, endometrial, or cervical.

5 25. A method of detecting and/or quantitating MN-specific antibodies in a vertebrate sample comprising the steps of:

 (a) contacting and incubating the vertebrate sample with an MN protein, an MN fusion protein and/or an MN
10 polypeptide according to Claims 11 or 12; and

 (b) detecting and/or quantitating binding of said MN protein, MN fusion protein and/or MN polypeptide to antibody in said sample.

26. A method of treating neoplastic disease and/or
15 pre-neoplastic disease comprising inhibiting the expression of MN genes by administering one or more antisense nucleic acid sequences that hybridize under stringent conditions to mRNA transcribed from MN genes.

27. Vectors containing an MN nucleic acid sequence
20 wherein said MN nucleic acid sequence is selected from the group consisting of:

 (a) SEQ. ID. NO.: 5 and its complement;

 (b) nucleic acids that hybridize to SEQ. ID. NO.: 5 or to its complement; and

25 (c) nucleic acids that differ from the nucleic acids of (a) or (b) due to the degeneracy of the genetic code;

 wherein said nucleic acid is at least twenty-nine nucleotides in length.

28. Vectors containing an MN nucleic acid sequence
30 according to Claim 27 selected from the group consisting of the plasmids A4a, XE1 and XE3 which are deposited at the American Type Culture Collection (ATCC) in Rockville, Maryland



US006297051B1

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(12) **United States Patent**
Zavada et al.

(10) Patent No.: **US 6,297,051 B1**
(45) Date of Patent: **Oct. 2, 2001**

(54) **MN GENE AND PROTEIN**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/177,776**

(22) Filed: **Oct. 23, 1998**

Related U.S. Application Data

(63) Continuation-in-part of application No. 08/787,739, filed on Jan. 24, 1997, now Pat. No. 6,027,887.

(51) Int. Cl.⁷ **C12N 5/00; A61K 38/00**

(52) U.S. Cl. **435/375; 424/94.1; 424/138.1; 424/139.1; 530/324; 530/388.1; 514/2; 514/17; 514/12**

(58) Field of Search **424/94.1, 138.1, 424/139.1, 146.1; 530/388.1, 324; 514/2, 17, 12; 435/375**

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ABSTRACT

Herein disclosed is a novel oncogene named MN or alternatively MN/CA IX. Abnormal expression of the MN gene is shown to signify oncogenesis, and diagnostic/prognostic methods for pre-neoplastic/neoplastic disease to detect or detect and quantitate such abnormal MN gene expression. Also disclosed are methods to treat pre-neoplastic/neoplastic disease involving the MN gene and protein, e.g., methods comprising the use of MN-specific antibodies, anti-idiotypic antibodies thereto, and anti-anti-idiotypic antibodies, and the use of MN antisense nucleic acids. Further disclosed are methods to identify and block MN binding site(s) and identify MN protein partners(s).

14 Claims, 16 Drawing Sheets

-continued

gggagagggc acagggccag acaaac

26

<210> SEQ ID NO 116
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: HUMAN

<400> SEQUENCE: 116

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1 5 10 15
Gly Gly Gly Ser
20

What is claimed is:

1. A method of inhibiting the growth of a vertebrate preneoplastic or neoplastic cell that is abnormally expressing MN protein comprising contacting that cell with a polypeptide composed of 50 or less amino acids, wherein said polypeptide specifically binds to a site on the MN protein to which vertebrate cells adhere in a cell adhesion assay; wherein said polypeptide when tested in vitro inhibits the adhesion of vertebrate cells to said MN protein; and wherein said polypeptide comprises a peptide selected by screening a phage display peptide library for specific binding to said MN protein.
2. The method according to claim 1 wherein said polypeptide that specifically binds to the MN protein comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 107-109.
3. The method according to claim 1 wherein said polypeptide specifically binds to a site within the extracellular domain of the MN protein.
4. The method according to claim 3 wherein said polypeptide specifically binds to a site within the proteoglycan-like domain of the MN protein.
5. The method according to claim 1 wherein said polypeptide specifically binds to a site within SEQ ID NO:97.

6. The method according to claim 5 wherein said site on the MN protein comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 10 and 98-103.
7. The method according to claim 1 wherein said polypeptide specifically binds to a site within the carbonic anhydrase domain of the MN protein.
8. The method according to claim 7 wherein said polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 107-109.
9. The method according to claim 1 wherein said vertebrate cell is mammalian.
10. The method according to claim 9 wherein said vertebrate cell is human.
11. The method according to claim 1 wherein said polypeptide is an oligopeptide.
12. The method according to claim 1 wherein said polypeptide is a heptapeptide.
13. The method according to claim 11 wherein said heptapeptide is selected from the group consisting of SEQ ID NOS: 107-109.
14. The method according to claim 1 wherein said phage display peptide library is a heptapeptide phage display library.

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(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
10 June 2004 (10.06.2004)

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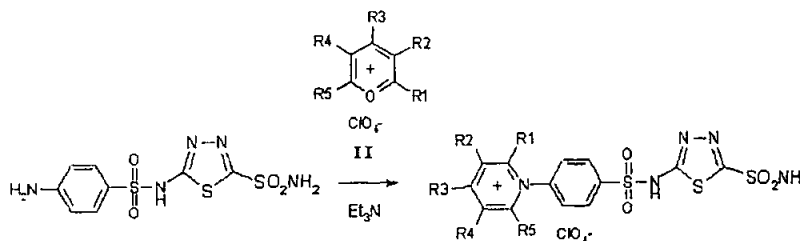
(10) International Publication Number
WO 2004/048544 A2

- (51) International Patent Classification⁷: **C12N**
- (21) International Application Number:
PCT/US2003/037783
- (22) International Filing Date:
26 November 2003 (26.11.2003)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/429,089 26 November 2002 (26.11.2002) US
60/489,473 22 July 2003 (22.07.2003) US
60/515,104 28 October 2003 (28.10.2003) US
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- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (regional): ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),

[Continued on next page]

(54) Title: CA IX-SPECIFIC INHIBITORS

Scheme 1



WO 2004/048544 A2

(57) Abstract: Therapeutic methods for inhibiting the growth of preneoplastic/ neoplastic vertebrate cells that abnormally express MN protein are disclosed. Screening assays are provided for identifying compounds, preferably membraneimpermeant compounds, which inhibit the enzymatic activity of MN protein/ polypeptides and that are useful for treating patients with preneoplastic/neoplastic disease. Further methods are disclosed for the preparation of positively-charged, membrane-impermeant heterocyclic sulfonamide CA inhibitors with high affinity for the membrane-bound carbonic anhydrase CA IX. Preferred CA IX-specific inhibitors are aromatic and heterocyclic sulfonamides, preferably that are membraneimpermeant. Particularly preferred CA IX-specific inhibitors are pyridinium derivatives of such aromatic and heterocyclic sulfonamides. The CA IX-specific inhibitors of the invention can also be used diagnostically/prognostically for preneoplastic/neoplastic disease, and for imaging use, for example, to detect precancerous cells, tumors and/or metastases. The CA IX-specific inhibitors can be labelled or conjugated to radioisotopes for radiotherapy. The CA IX-specific inhibitors may be combined with conventional therapeutic anticancer drugs, with other different inhibitors of cancer-related pathways, with bioreductive drugs, or with radiotherapy to enhance the efficiency of each treatment. The CA IX-specific inhibitors may also be combined with CA IX-specific antibodies, preferably monoclonal antibodies or biologically active antibody fragments, more preferably humanized or fully human CA IX monoclonal antibodies or biologically active fragments or such monoclonal antibodies. Still further, the CA IX-specific inhibitors can be used for gene therapy coupled to vectors for targeted delivery to preneoplastic/neoplastic cells expressing CA IX on their surfaces.

CLAIMS

1. A method of treating a mammal for a pre-cancerous or cancerous disease, wherein said disease is characterized by overexpression of MN/CA IX protein, comprising administering to said mammal a therapeutically effective amount of a composition comprising a compound, wherein said compound is selected from the group consisting of organic and inorganic molecules, and wherein said compound is determined to be a potent inhibitor of MN/CA IX enzymatic activity in a screening assay comprising:
- 5 a) preparing serial dilutions of said compound and serial dilutions of MN/CA IX protein or a fragment of the MN/CA IX protein that comprises the carbonic anhydrase domain;
- 10 b) preincubating a dilution of said compound with a dilution of said MN/CA IX protein or said MN/CA IX protein fragment for ten minutes at 20°C;
- 15 c) combining said preincubated mixture of said diluted compound and said diluted MN/CA IX protein or protein fragment with a substrate, consisting essentially of a saturated CO₂ solution, phenol red to 0.2mM, Na₂SO₄ to 0.1M, and Hepes buffer (pH 7.5) to 10mM, in a reaction vessel for a period of 10 to 100 seconds at 20°C;
- 20 d) concurrently measuring the optical density, at the absorbance maximum of 557 nm, of the contents of said reaction vessel, using a stopped flow spectrophotometer; and
- e) determining the inhibition constant K_i of said compound;
- 25 wherein if said inhibition constant K_i is determined to be less than about 50 nanomolar, said compound is determined to be a potent inhibitor of MN/CA IX enzymatic activity; and wherein said compound is not selected from the group consisting of acetazolamide, ethoxzolamide, methazolamide and cyanate.
2. The method of claim 1 wherein said mammal is a human.
- 30 3. The method of claim 1 wherein said inhibition constant K_i is determined to be less than about 35 nanomolar.

4. The method of claim 1 wherein said inhibition constant K_i is determined to be less than about 10 nanomolar.

5 5. The method of claim 1 wherein said compound is an organic compound.

6. The method of claim 1 wherein said compound is an inorganic compound.

10

7. The method of claim 5 wherein said organic compound is an aromatic compound.

8. The method of claim 5 wherein said organic compound is an aromatic sulfonamide or a heterocyclic sulfonamide.

15

9. The method of claim 8, wherein said aromatic sulfonamide is a substituted aromatic sulfonamide, wherein said aromatic sulfonamide comprises an aromatic ring structure bearing a sulfonamide moiety bonded to said ring structure and optionally bearing one or more substituents independently selected from the group consisting of halogeno, nitro, and an alkylamino group, wherein the alkyl radical of said alkylamino group comprises 1 to 4 carbon atoms.

20

10. The method of claim 8 wherein said compound is a more potent inhibitor of MN/CA IX enzymatic activity than of the enzymatic activity of a carbonic anhydrase selected from the group consisting of CA I, CA II and CA IV.

25

11. The method of claim 8 wherein said compound is a more potent inhibitor of MN/CA IX enzymatic activity than of the enzymatic activity of at least two carbonic anhydrases selected from the group consisting of CA I, CA II and CA IV.

30

12. The method of claim 8 wherein said compound is a more potent inhibitor of MN/CA IX enzymatic activity than of the enzymatic activity of each of the carbonic anhydrases in the group consisting of CA I, CA II and CA IV.

5 13. The method of claim 8 wherein said compound is a more potent inhibitor of MN/CA IX enzymatic activity than of the enzymatic activity of CA II.

14. The method of claim 13 wherein the inhibition by said compound of the enzymatic activity of CA II is tested by the method comprising the following steps:

10 a) preparing serial dilutions of said compound and serial dilutions of CA II;

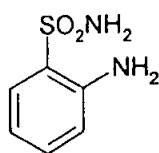
b) preincubating a dilution of said compound with a dilution of CA II for ten minutes at 20°C;

15 c) adding said preincubated mixture of said compound and said CA II to a substrate solution, comprising 4-nitrophenylacetate in anhydrous acetonitrile (pH 7.40), in a reaction vessel for a period of 1 to 3 minutes at 25°C;

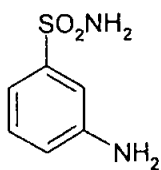
d) concurrently measuring the optical density, at the absorbance maximum of 400 nm, of the contents of said reaction vessel, using a spectrophotometer; and

20 e) determining the inhibition constant K_i of said compound.

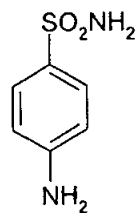
15. The method of claim 8 wherein said aromatic sulfonamide or heterocyclic sulfonamide is selected from the group consisting of:



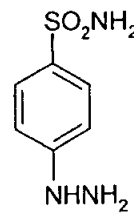
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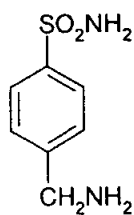
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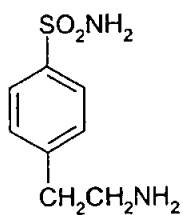
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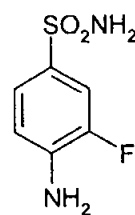
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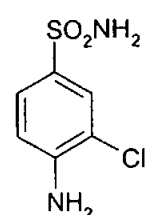
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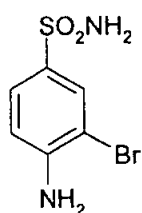
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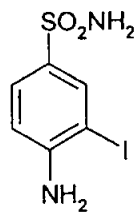
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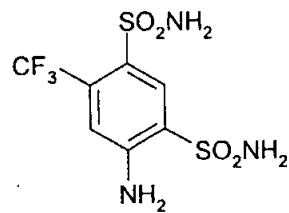
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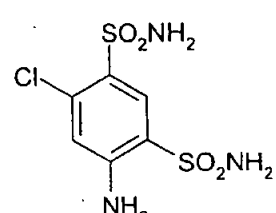
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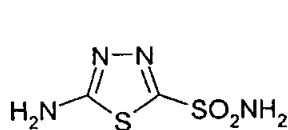
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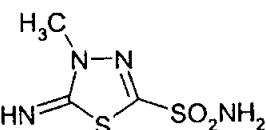
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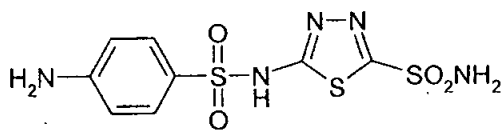
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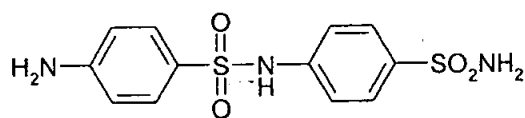
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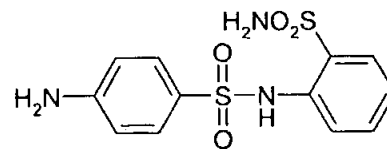
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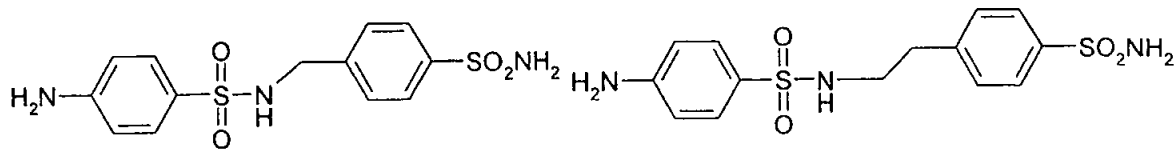
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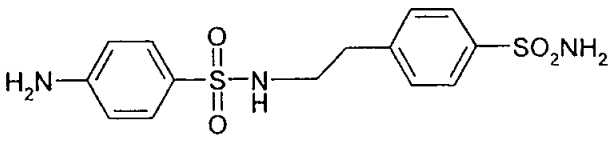
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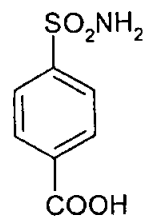
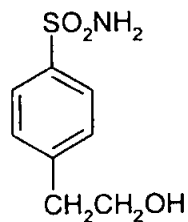
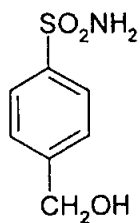
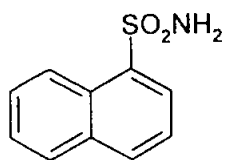
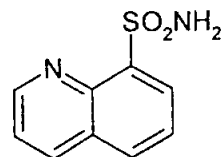
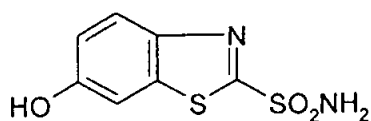
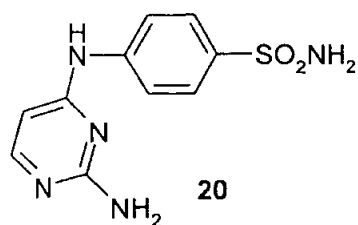
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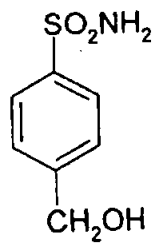
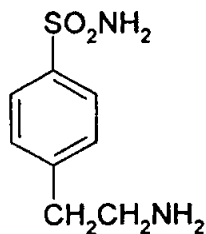
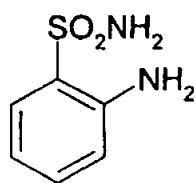
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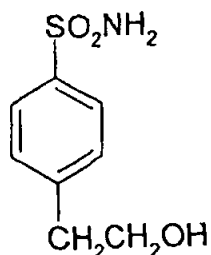


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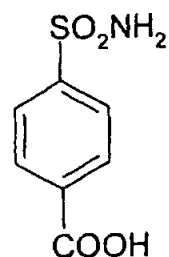


16. The method of claim 8, wherein said compound is an aromatic sulfonamide selected from the group consisting of:





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17. The method of claim 8, wherein a halogen atom is bonded to at least one carbon atom in the aromatic ring of said aromatic sulfonamide.

5

18. The method of claim 8, wherein said compound is a heterocyclic sulfonamide.

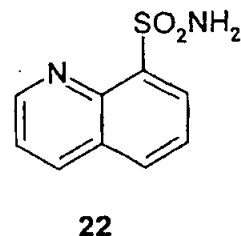
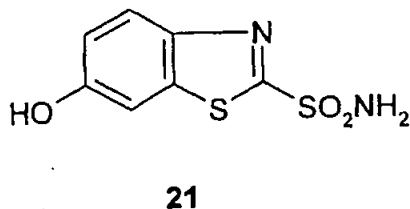
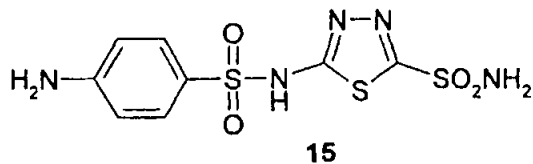
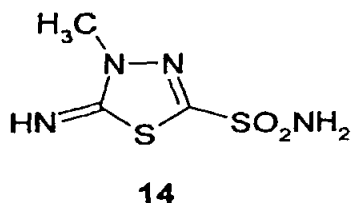
19. The method of claim 18, wherein said heterocyclic compound is a substituted heterocyclic sulfonamide, wherein said substituted heterocyclic sulfonamide comprises a heterocyclic ring structure bearing an sulfonamide moiety bonded to said ring structure and optionally bearing one or more substituents independently selected from a group consisting of halogeno, nitro, and an alkylamino group, wherein the alkyl radical of said alkylamino group comprises 1 to 4 carbon atoms.

15

20. The method of claim 18, wherein said heterocyclic sulfonamide is halogenated.

20

21. The method of claim 8, wherein said compound is a heterocyclic sulfonamide selected from the group consisting of:



22. A method of treating a mammal for a pre-cancerous or cancerous disease, wherein said disease is characterized by overexpression of MN/CA IX protein, comprising administering to said mammal a therapeutically effective amount of a composition comprising a membrane-impermeant compound, wherein said membrane-impermeant compound is selected from the group consisting of organic and inorganic molecules, and wherein said membrane-impermeant compound is determined to be a potent inhibitor of MN/CA IX enzymatic activity in a screening assay comprising:

a) preparing serial dilutions of said membrane-impermeant compound and serial dilutions of MN/CA IX protein or a fragment of the MN/CA IX protein that comprises the carbonic anhydrase domain;

b) preincubating a dilution of said membrane-impermeant compound with a dilution of said MN/CA IX protein or said MN/CA IX protein fragment for ten minutes at 20°C;

c) combining said preincubated mixture of said diluted compound and said diluted MN/CA IX protein or protein fragment with a substrate, consisting essentially of a saturated CO₂ solution, phenol red to 0.2mM, Na₂SO₄ to 0.1M, and Hepes buffer (pH 7.5) to 10mM, in a reaction vessel for a period of 10 to 100 seconds at 20°C;

d) concurrently measuring the optical density, at the absorbance maximum of 557 nm, of the contents of said reaction vessel, using a stopped flow spectrophotometer; and

e) determining the inhibition constant K_i of said membrane-impermeant compound,

wherein if said inhibition constant K_i is determined to be less than about 50 nanomolar, said membrane-impermeant compound is determined to be a potent inhibitor of MN/CA IX enzymatic activity.

23. The method of claim 22 wherein said mammal is a human.

24. The method of claim 22 wherein said inhibition constant K_i is determined to be less than about 35 nanomolar.

25. The method of claim 22 wherein said inhibition constant K_i is determined to be less than about 10 nanomolar.

26. The method of claim 22 wherein said membrane-impermeant compound is an organic compound.

27. The method of claim 22 wherein said membrane-impermeant compound is an inorganic compound.

28. The method of claim 26 wherein said membrane-impermeant organic compound is a pyridinium derivative of an aromatic sulfonamide or a pyridinium derivative of a heterocyclic sulfonamide.

29. The method of claim 28 wherein said membrane-impermeant compound is a more potent inhibitor of MN/CA IX enzymatic activity than of the enzymatic activity of a carbonic anhydrase selected from the group consisting of CA I, CA II and CA IV.

30. The method of claim 28 wherein said membrane-impermeant compound is a more potent inhibitor of MN/CA IX enzymatic activity than of the enzymatic activity of at least two carbonic anhydrases selected from the group consisting of CA I, CA II and CA IV.

5

31. The method of claim 28 wherein said membrane-impermeant compound is a more potent inhibitor of MN/CA IX enzymatic activity than of the enzymatic activity of each of the carbonic anhydrases in the group consisting of CA I, CA II and CA IV.

10

32. The method of claim 28 wherein said membrane-impermeant compound is a more potent inhibitor of MN/CA IX enzymatic activity than of the enzymatic activity of CA IV.

15

33. The method of claim 32 wherein the inhibition by said membrane-impermeant compound of the enzymatic activity of CA IV is tested by the method comprising the following steps:

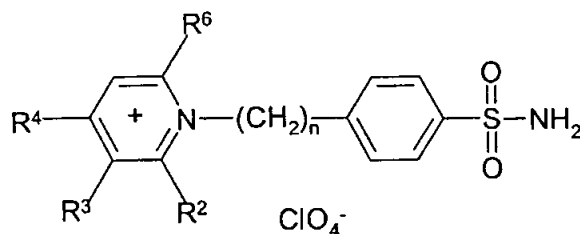
- a) preparing serial dilutions of said membrane-impermeant compound and serial dilutions of CA IV;
- 20 b) preincubating a dilution of said membrane-impermeant compound with a dilution of CA IV for ten minutes at 20°C;
- c) adding said preincubated mixture of said compound and said CA IV to a substrate solution, comprising 4-nitrophenylacetate in anhydrous acetonitrile (pH 7.40), in a reaction vessel for a period of 1 to 3 minutes at 25°C;
- 25 d) concurrently measuring the optical density, at the absorbance maximum of 400 nm, of the contents of said reaction vessel using a spectrophotometer; and
- e) determining the inhibition constant K_i of said membrane-impermeant compound.

30

34. The method of claim 28, wherein said membrane-impermeant compound is a pyridinium derivative of an aromatic sulfonamide.

35. The method of claim 34, wherein said membrane-impermeant compound is a pyridinium derivative of an aromatic sulfonamide that is selected from the group consisting of sulfanilamide, homosulfanilamide and 4-aminoethyl-benzenesulfonamide.

36. The method of claim 34, wherein said pyridinium derivative of an aromatic sulfonamide has the general formula of:



wherein

n is 0, 1, or 2;

R₂, R₃, R₄ and R₆ are each independently selected from the group consisting of hydrogen, alkyl moieties comprising from 1 to 12 carbon atoms, and aryl moieties.

37. The method of claim 36 wherein

R₂ is selected from the group consisting of methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *tert*-butyl and phenyl;

R₃ is selected from the group consisting of hydrogen and methyl;

R₄ is selected from the group consisting of hydrogen, methyl and phenyl; and

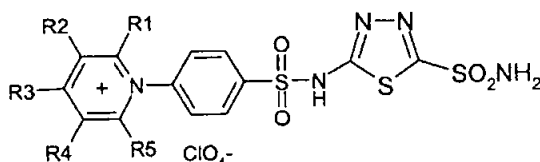
R₆ is selected from the group consisting of methyl, ethyl, *n*-propyl, *iso*-propyl, and phenyl.

38. The method of claim 37, wherein
R3 is hydrogen;
R4 and R6 are phenyl;
5 when n is 0, R2 is selected from the group consisting of methyl, ethyl,
n-propyl, iso-propyl, n-butyl, and phenyl; and
when n is 1 or 2, R2 is selected from the group consisting of methyl,
ethyl, n-propyl, iso-propyl, n-butyl, tert-butyl, and phenyl.
- 10 39. The method of claim 37, wherein
R3 is hydrogen;
R4 is phenyl; and
when n is 0, R2 and R6 are the same and are selected from the group
consisting of methyl, ethyl, n-propyl, and iso-propyl; and
15 when n is 1 or 2, R2 and R6 are the same and are selected from the
group consisting of methyl, ethyl, n-propyl, iso-propyl and phenyl.
40. The method of claim 37, wherein R2, R3, R4 and R6 are methyl.
- 20 41. The method of claim 37, wherein
when n is 0, 1 or 2, R2, R4 and R6 are methyl, and R3 is hydrogen; or
when n is 1 or 2, R2 is iso-propyl, R3 is hydrogen, R4 is methyl, and
R6 is methyl or iso-propyl; or
when n is 2, R2 and R6 are phenyl, and R3 and R4 are hydrogen.
- 25 42. The method of claim 37, wherein
when n is 2, R2 and R6 are methyl, R3 is hydrogen, and R4 is phenyl;
or
when n is 2, R2 and R6 are ethyl, R3 is hydrogen, and R4 is phenyl; or
30 when n is 2, R2, R3, R4 and R6 are methyl.

43. The method of claim 28, wherein said membrane-impermeant compound is a pyridinium derivative of a heterocyclic sulfonamide.

44. The method of claim 43, wherein said membrane-impermeant compound is a pyridinium derivative of aminobenzolamide.

45. The method of claim 43, wherein said pyridinium derivative of a heterocyclic sulfonamide has the general formula of:



wherein R1, R2, R3, R4 and R5 are each independently selected from the group consisting of hydrogen, alkyl moieties comprising from 1 to 12 carbon atoms, and aryl moieties.

46. The method of claim 45, wherein

R1 is selected from the group consisting of methyl, ethyl, iso-propyl, n-propyl, n-butyl, *tert*-butyl and phenyl;

R2 is selected from the group consisting of hydrogen and methyl;

R3 is selected from the group consisting of hydrogen, methyl, *n*-nonyl, and phenyl;

R4 is hydrogen; and

R5 is selected from the group consisting of methyl, ethyl, iso-propyl, n-propyl, n-butyl, *tert*-butyl, *n*-nonyl, and phenyl.

47. The method of claim 46, wherein

R2 and R4 are hydrogen;

R3 is methyl; and

R1 and R5 are the same and selected from the group consisting of methyl, *iso*-propyl, and *tert*-butyl.

48. The method of claim 46, wherein
R2 and R4 are hydrogen;
R3 is phenyl; and
5 R1 and R5 are the same and selected from the group consisting of
methyl, ethyl, iso-propyl, n-propyl, n-butyl, and phenyl.

49. The method of claim 46, wherein
R1 is selected from the group consisting of methyl, ethyl, iso-propyl, n-
10 propyl, and n-butyl;
R2 and R4 are hydrogen; and
R3 and R5 are phenyl.

50. The method of claim 46, wherein
15 R2 and R4 are hydrogen, R3 is hydrogen or methyl, and R1 and R5
are phenyl; or
R1, R2, and R5 are methyl, R3 is phenyl, and R4 is hydrogen; or
R1 is methyl, R2 and R4 are hydrogen, and R3 and R5 are n-nonyl.

20 51. The method of claim 46, wherein
R1 is methyl or iso-propyl, R3 and R5 are methyl, and R2 and R4 are
hydrogen; or
R1 and R5 are the same and are methyl or ethyl, R2 and R4 are
25 hydrogen, and R3 is phenyl; or
R1, R2, R3 and R5 are methyl and R4 is hydrogen.

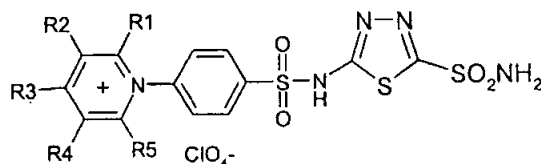
52. A method of inhibiting tumor growth in a patient having a tumor, the
cells of which tumor are characterized by overexpression of MN/CA IX protein,
30 comprising administering to said patient a therapeutically effective amount of a
composition comprising a compound, wherein said compound is selected from the
group consisting of organic and inorganic molecules, and wherein said compound is

determined to be a potent inhibitor of MN/CA IX enzymatic activity in a screening assay comprising:

- a) preparing serial dilutions of said compound and serial dilutions of MN/CA IX protein or a fragment of the MN/CA IX protein that comprises the carbonic anhydrase domain;
 - b) preincubating a dilution of said compound with a dilution of said MN/CA IX protein or protein fragment for ten minutes at 20°C;
 - c) combining said preincubated mixture of said diluted compound and said diluted MN/CA IX protein or protein fragment with a substrate, consisting essentially of a saturated CO₂ solution, phenol red to 0.2mM, Na₂SO₄ to 0.1M, and Hepes buffer (pH 7.5) to 10mM, in a reaction vessel for a period of 10 to 100 seconds at 20°C;
 - d) concurrently measuring the optical density, at the absorbance maximum of 557 nm, of the contents of said reaction vessel, using a stopped flow spectrophotometer; and
 - e) determining the inhibition constant K_i of said compound;
- wherein if said inhibition constant K_i is determined to be less than about 50 nanomolar, said compound is determined to be a potent inhibitor of MN/CA IX enzymatic activity; and wherein said compound is not selected from the group consisting of acetazolamide, ethoxzolamide, methazolamide and cyanate.

53. The method of claim 52 wherein said patient is a human.

54. A pyridinium derivative of a heterocyclic sulfonamide with the general formula of:



wherein

R1 is selected from the group consisting of methyl, ethyl, iso-propyl, *n*-propyl, *n*-butyl, *tert*-butyl and phenyl;

R2 is selected from the group consisting of hydrogen and methyl;

R3 is selected from the group consisting of hydrogen, methyl, *n*-nonyl
5 and phenyl;

R4 is hydrogen; and

R5 is selected from the group consisting of methyl, ethyl, iso-propyl, *n*-propyl, *n*-butyl, *tert*-butyl, *n*-nonyl and phenyl, except that

R1 cannot be methyl when R2 and R4 are hydrogen and R3 and R5
10 are methyl; and

R1 cannot be methyl when R2 and R4 are hydrogen, R3 is phenyl and
R5 is methyl; and

R1 cannot be phenyl when R2 and R4 are hydrogen and R3 and R5
are phenyl.

15

55. The pyridinium derivative of a heterocyclic sulfonamide of claim 54,
wherein

R2 and R4 are hydrogen;

R3 is methyl; and

R1 and R5 are the same and selected from the group consisting of *iso*-
20 propyl and *tert*-butyl.

56. The pyridinium derivative of a heterocyclic sulfonamide of claim 54,
wherein

25 R2 and R4 are hydrogen;

R3 is phenyl; and

R1 and R5 are the same and selected from the group consisting of
ethyl, *iso*-propyl, *n*-propyl, and *n*-butyl.

30

57. The pyridinium derivative of a heterocyclic sulfonamide of claim 54,
wherein

R1 is selected from the group consisting of methyl, ethyl, iso-propyl, n-propyl, n-butyl, and *tert*-butyl;

R2 and R4 are hydrogen; and

R3 and R5 are phenyl.

5

58. The pyridinium derivative of a heterocyclic sulfonamide of claim 54, wherein

R1 is iso-propyl, R3 and R5 are methyl, and R2 and R4 are hydrogen;

or

10

R2 and R4 are hydrogen, R3 is hydrogen or methyl, and R1 and R5 are phenyl; or

R1, R2, and R5 are methyl, R3 is phenyl, and R4 is hydrogen; or

R1, R2, R3 and R5 are methyl and R4 is hydrogen; or

R1 is methyl, R3 and R5 are *n*-nonyl, and R2 and R4 are hydrogen.

15

59. The method of claim 1 further comprising conjugating a radioisotope to said compound before administering said compound to said mammal.

60. The method of claim 1 further comprising administering to said mammal radiation and/or a therapeutically effective amount in a physiologically acceptable formulation of one or more of the following compounds selected from the group consisting of: conventional anticancer drugs, chemotherapeutic agents, different inhibitors of cancer-related pathways, bioreductive drugs, CA IX-specific antibodies and CA IX-specific antibody fragments that are biologically active.

25

61. The method of claim 60 wherein said CA IX-specific antibodies and/or CA IX-specific antibody fragments are humanized or fully human.

62. The method of claim 60 wherein said CA IX-specific antibodies and/or CA IX-specific antibody fragments are attached to a cytotoxic entity.

30

63. A method of treating a mammal for a precancerous or cancerous disease, wherein said disease is characterized by overexpression of MN/CA IX protein, comprising administering to said mammal a therapeutically effective amount in a physiologically acceptable formulation of a vector conjugated to a potent CA IX-specific inhibitor, wherein said vector expresses a wild-type gene that is absent from
5 or mutated in a CA IX expressing cell, that is precancerous or cancerous, and wherein the wild type gene product has an anticancer effect in said cell; or wherein said vector comprises a gene that expresses a cytotoxic protein.

10

64. The method according to claim 63 wherein said vector comprises a MN/CA IX promoter or a MN/CA IX promoter fragment, wherein said promoter or promoter fragment comprises one or more hypoxia response elements, and wherein said promoter or promoter fragment is operably linked to said wild-type gene or to
15 said gene that expresses a cytotoxic protein.

65. The method of claim 63 wherein said potent CA IX-specific inhibitor is a compound determined to inhibit CA IX enzymatic activity in a screening assay comprising:

- 20 a) preparing serial dilutions of said compound and serial dilutions of MN/CA IX protein or a MN/CA IX protein fragment that comprises the carbonic anhydrase domain;
- b) preincubating a dilution of said compound with a dilution of said MN/CA IX protein or said MN/CA IX protein fragment for ten minutes at 20°C;
- 25 c) combining said preincubated mixture of said diluted compound and said diluted MN/CA IX protein or protein fragment with a substrate, consisting essentially of a saturated CO₂ solution, phenol red to 0.2mM, Na₂SO₄ to 0.1M, and Hepes buffer (pH 7.5) to 10mM, in a reaction vessel for a period of 10 to 100 seconds at 20°C;

d) concurrently measuring the optical density, at the absorbance maximum of 557 nm, of the contents of said reaction vessel, using a stopped flow spectrophotometer; and

e) determining the inhibition constant K_i of said compound;

5 wherein if said inhibition constant K_i is determined to be less than about 50 nanomolar, said compound is determined to be a potent inhibitor of MN/CA IX enzymatic activity.

66. The method of claim 65 wherein said potent MN/CA IX inhibitor is not selected from the group consisting of acetazolamide, ethoxzolamide,
10 methazolamide and cyanate.

67. A method that is diagnostic or diagnostic and prognostic for precancer or cancer comprising contacting a mammalian sample with a CA IX-specific inhibitor conjugated to a label or a visualizing means, and detecting or
15 detecting and quantifying binding of said CA IX-specific inhibitor to cells in said sample by detecting or detecting and quantifying said label or said visualizing means on cells in said sample, wherein said detection or said detection and quantitation at a level above that for a control sample is indicative of precancerous or cancerous cells that overexpress CA IX in said sample.

20 68. The method of claim 67 wherein CA IX activated by hypoxic conditions is detected or detected and quantitated, and the mammal from whom the sample was taken is considered to have a poor prognosis, and decisions on treatment for said mammal are made in view of the presence of said hypoxic
25 conditions.

69. A method for imaging tumors and/or metastases that express CA IX in a patient comprising the administration of a CA IX-specific inhibitor linked to an imaging agent to said patient.

(51) International Patent Classification 4 : C07K 15/00, 17/06, 17/10 A61K 31/705, 39/44		A1	(11) International Publication Number: WO 87/ 05031 (43) International Publication Date: 27 August 1987 (27.08.87)
(21) International Application Number: PCT/US87/00406 (22) International Filing Date: 25 February 1987 (25.02.87) (31) Priority Application Number: 833,204 (32) Priority Date: 25 February 1986 (25.02.86) (33) Priority Country: US (60) Parent Application or Grant (63) Related by Continuation US 833,204 (CIP) Filed on 25 February 1986 (25.02.86) (71) Applicant (for all designated States except US): CENTER FOR MOLECULAR MEDICINE AND IMMUNOLOGY [US/US]; 1 Bruce Street, Newark, NJ 07103 (US).		(72) Inventors; and (75) Inventors/Applicants (for US only) : SHIH, Lisa, B. [CN/US]; 31 Ridge Court, Cedar Grove, NJ 07009 (US). PRIMUS, F., James [US/US]; R.D. 1, Box 6, Pittstown, NJ 08867 (US). GOLDENBERG, M., David [US/US]; 397 Long Hill Drive, Short Hills, NJ 07078 (US). (74) Agent: SAXE, Bernhard, D.; CMMI, 1 Bruce Street, Newark, NJ 07103 (US). (81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), DK, FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), NO, SE (European patent), US. Published With international search report.	
(54) Title: DIAGNOSTIC AND THERAPEUTIC ANTIBODY CONJUGATES			
(57) Abstract Conjugates of diagnostic or therapeutic principles, such as drugs, toxins, chelators, boron compounds and detectable labels, to an antibody, in which the diagnostic or therapeutic principle is first loaded onto a polymer carrier such as an aminodextran or a polypeptide of at least 50 amino acids in length, and this intermediate is in turn site-specifically conjugated to a targeting antibody such as an antitumor antibody. The resultant conjugate substantially retains the immunoreactivity of the antibody, and targets the diagnostic or therapeutic principle to a target tissue or organ where the diagnostic or therapeutic effect is realized.			

(19)



Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number:

0 330 201
A2

(12)

EUROPEAN PATENT APPLICATION

(21) Application number: 89103189.0

(51) Int. Cl. 4: **A61K 47/00 , A61K 39/395**

(22) Date of filing: 23.02.89

(30) Priority: 26.02.88 US 161174
04.04.88 US 176851

(43) Date of publication of application:
30.08.89 Bulletin 89/35

(84) Designated Contracting States:
CH DE FR GB IT LI SE

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(54) **Method of enhancing the effect of cytotoxic agents.**

(57) The activity of cytotoxic agents on target cells is enhanced through administration of a sensitizing agent to a patient prior to or simultaneously with administration of the cytotoxic agent. One or both of the two types of agents are attached to antibodies specific for the desired target cells, thereby enhancing the cytotoxic effect on target cells compared to non-target cells. The sensitizer and/or the cytotoxic agent may be attached to monoclonal antibodies specific for cancer cells to selectively eradicate the cancer cells while minimizing toxicity toward normal tissues.

EP 0 330 201 A2

(19)



Europäisches Patentamt
European Patent Office
Office européen des brevets



(11) Numéro de publication:

0 080 401 B1

(12)

FASCICULE DE BREVET EUROPEEN(45) Date de publication de fascicule du brevet: 29.07.92 (51) Int. Cl.⁵: **A61K 39/44**(21) Numéro de dépôt: **82402078.8**(22) Date de dépôt: **15.11.82**

Le dossier contient des informations techniques
présentées postérieurement au dépôt de la
demande et ne figurant pas dans le présent
fascicule.

(54) **Nouveaux médicaments anticancéreux pour le traitement des leucémies T constitués de la chaîne A de la ricine et d'un anticorps monoclonal spécifique.**

(30) Priorité: 20.11.81 **FR 8121836**(43) Date de publication de la demande:
01.06.83 Bulletin 83/22(45) Mention de la délivrance du brevet:
29.07.92 Bulletin 92/31(64) Etats contractants désignés:
AT BE CH DE FR GB IT LI LU NL SE

(56) Documents cités:
EP-A- 0 023 401
EP-A- 0 044 167
FR-A- 2 437 213
FR-A- 2 466 252

NATURE, vol. 294, no. 5837, 12 novembre
1981, pages 171-173, Macmillan Journals,
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EP 0 080 401 B1

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Human tumour-associated cell adhesion protein MN/CA IX: identification of M75 epitope and of the region mediating cell adhesion

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Summary MN/CA IX is a cell surface protein, strongly associated with several types of human carcinomas. It exerts activity of carbonic anhydrase and capacity of binding to cell surface receptors. In the present work, we used affinity purified MN/CA IX protein to demonstrate that the cells adhere to immobilized MN/CA IX and that the monoclonal antibody M75 abrogates cell attachment to MN/CA IX. Using synthetic oligopeptides, we identified M75 epitope and located it in the proteoglycan domain, which contains a sixfold tandem repeat of six amino acids GEEDLP. From phage display library of random heptapeptides we identified and chemically synthesized those which compete for the epitope with M75 and inhibit adhesion of cells to MN/CA IX. These heptapeptides might serve as lead compounds for drug design. © 2000 Cancer Research Campaign

Keywords: cell adhesion molecules; carbonic anhydrase; tumour immunology; phage display; drug design

MN/CA IX protein (initially termed MN) was discovered in HeLa cells using monoclonal antibody (mAb) M75 (Pastorekova et al, 1992). Its possible role in oncogenesis was suggested by two facts: expression of MN/CA IX correlated with tumorigenicity of HeLa × fibroblast hybrids; it was detected in clinical specimens of cervical and certain other carcinomas, but it was absent in normal tissues of corresponding organs (Závada et al, 1993). MN/CA IX cDNA (Pastorek et al, 1994) and gene (Opavsky et al, 1996) have been cloned; predicted protein consists of signal peptide (SP), proteoglycan-related sequence (PG), carbonic anhydrase domain (CA), transmembrane segment (TM) and short intracellular tail (IC). MN/CA IX protein is a cell adhesion molecule; its CA is enzymatically active (Závada et al, 1997). MN/CA IX protein is almost 100% associated with cervical carcinomas (Liao et al, 1994), with carcinomas of oesophagus (Turner et al, 1997) and with renal clear cell carcinomas (Liao et al, 1997). It was also detected in a high percentage of colorectal (Saarnio et al, 1998a) and lung (Vermeylen et al, 1999) carcinomas. Therefore, MN/CA IX is a promising biomarker in tumour diagnostics. Characteristically, MN/CA IX in human carcinomas is expressed ectopically; normally it is present in stomach and bile duct mucosa (Liao et al, 1994) and in rapidly proliferating normal cells in small intestine (Saarnio et al, 1998b). An antigen specific for renal cell carcinomas was discovered using mAb G250 (Oosterwijk et al, 1986) and was found to be identical with MN/CA IX (Uemura et al, 1997). This antigen appears to be an excellent target for immunotherapy (Steffens et al, 1997; Uemura et al, 1997). Until now, molecular basis of involvement of MN/CA IX in carcinogenesis has remained unclear. One of the possible leads

could be the fact that MN/CA IX is an adhesion molecule presumably engaged in cell-to-cell communication (Závada et al, 1997). In this paper we undertook the first steps towards elucidation of the role of MN/CA IX protein in communication among the cells. We attempted to identify its binding site for cell surface receptors. This was facilitated by the finding that mAb M75 blocks adhesion of cells to affinity-purified MN/CA IX protein.

MATERIALS AND METHODS

Affinity chromatography of MN/CA IX

MN/CA IX was purified by a single cycle of adsorption–elution on sulphonamide-agarose, as described for other CAs (Falkbrink et al, 1972). We used columns of p-aminomethylbenzene-sulphonamide-agarose (Sigma). Columns with adsorbed MN/CA IX were extensively washed with phosphate-buffered saline (PBS) sodium chloride (NaCl) 8.0 g l⁻¹, potassium chloride (KCl) 0.2 g l⁻¹, KH₂PO₄ 0.2 g l⁻¹, Na₂HPO₄ 1.15 g l⁻¹, pH = 7.2) and eluted with 0.1 mM acetazolamide (Sigma). All steps of purification were carried out at 0–5°C, pH 7.2, at physiological concentration of salts. Complete MN/CA IX+ was extracted with 1% Triton X-100 in PBS from Vero cells infected with vaccinia virus containing an insert of complete coding region of MN/CA IX as described (Závada et al, 1997). Before chromatography, the extract was diluted 1:6 with PBS and centrifuged for 1 h at 15 000 g. Truncated MN/CA IX ΔTMΔIC was produced from an analogous construct except that the 3' downstream primer for polymerase chain reaction (PCR) was: 5'-CGT CTA GAA GGA ATT CAG CTA GAC TGG CTC AGC A-3'. MN/CA IXΔ was shed into the medium, from which it was affinity purified after centrifugation as above. All steps of purification were monitored by dot-blots.

Received 16 September 1999

Accepted 27 January 2000

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Cells and media

The following cell lines were used: HeLa, CGL1 = non-tumorigenic hybrid HeLa × fibroblast, CGL3 = tumorigenic segregant from this hybrid. The origin of the cells and growth media were described previously (Závada et al, 1992, 1997). In addition, we used also HT29, a cell line derived from colorectal carcinoma (ATCC No. HBT-38).

Cell adhesion test

The conditions of the test were described previously (Závada et al, 1997). Briefly, $1 \mu\text{g ml}^{-1}$ of purified MN/CA IX in 50 mM mono/bicarbonate buffer, pH 9.2, was adsorbed in 30- μl drops on the bottom of bacteriological 5-cm Petri dishes for 1.5 h. Then the drops were removed by aspiration and the dishes were 3 × rinsed with PBS and blocked with 50% fetal calf serum (FCS) in culture medium for 30 min. There were two variants of the test. In the first one (Figure 2), the whole bottom of the Petri dish was blocked with 50% of FCS, and the dishes were seeded with 5 ml of cell suspension ($10^5 \text{ cells ml}^{-1}$). After overnight incubation, the cultures were rinsed with PBS, fixed and stained. In the other variant (Figure 4), only the area of adsorbed MN/CA IX was blocked and on top of MN/CA IX dots were added 30- μl drops of cell suspension in growth medium, containing added oligopeptides (or control without peptides). After incubation, rinsing and fixation, the cultures were stained with 0.5% Trypan blue in 50 mM Tris buffer pH 8.5 for 1 h, rinsed with water and dried. Stained areas of attached cells were extracted with 10% acetic acid, the extracts transferred to 96-well plates and absorbance was measured at 630 nm on a microplate reader.

ELISA

Purified GST-MN (Závada et al, 1993) at concentration 10 ng ml^{-1} in carbonate buffer pH 9.2 was adsorbed for 3 h in Maxisorb strips (NUNC). After washing and blocking (1 h) with 0.05% Tween-20 in PBS, 50 $\mu\text{l well}^{-1}$ of the antibody + antigen mixtures were added. Final dilution of mAb M75 ascites fluid was 10^{-6} ; concentration of the peptides varied according to their affinity for M75 so as to allow determination of 50% end point. These mixtures were adsorbed for 1.5 h, followed by washing with Tween-PBS. Bound antibody was detected by antimouse IgG conjugate with peroxidase (SwAM-Px, SEVAC, Prague), diluted 1:1000. In the colour reaction OPD (o-phenylenediamine dihydrochloride, Sigma) 1 mg ml^{-1} in 0.1 M citrate buffer pH 5.0 was used. To this hydrogen peroxide (H_2O_2) was added to final concentration 0.03%. This system is balanced so as to allow assay for antigen competing for M75 (Figure 3) as well as for peptides binding to the epitope of immobilized GST-MN (Figure 4).

Peptides

The peptides used in this study were prepared by the solid-phase method (Merrifield, 1995) using the Boc/Bzl strategy. The peptide acids were prepared on PAM-resin and peptide amides on MeBHA resin. Deprotection and splitting from the resin was done by liquid hydrogen fluoride. The peptides were purified by C18 reverse phase high-performance liquid chromatography (RP HPLC) and characterized by amino acid analysis and FAB MS spectroscopy.

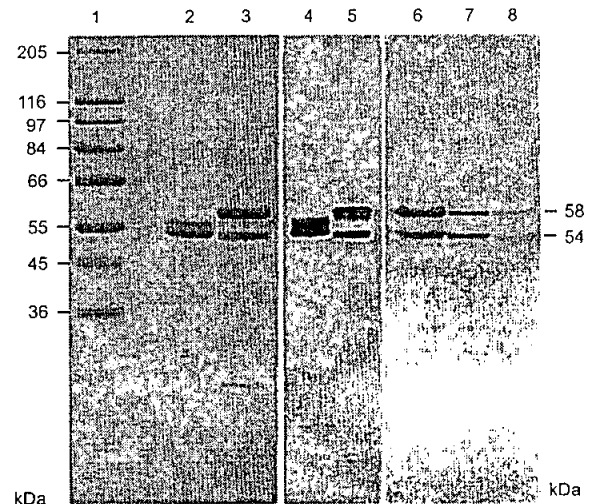


Figure 1 PAGE and Western blot analysis of MN/CA IX protein. Lanes 1–3 = gel stained with Coomassie Blue; lanes 4–8 = blots developed with mAb M75 and immunoperoxidase. Lane 1 = M_r reference proteins; lane 2 = purified MN/CA IX ΔTM , IC, 1.25 μg ; lane 3 = purified MN/CA IX $^+$, 2 μg ; lane 4 = purified MN/CA IX ΔTM , IC, 0.125 μg ; lane 5 = purified MN/CA IX $^+$, 0.2 μg ; lane 6 = extract from HT29 cells, 50 μl ; lane 7 = extract from CGL3 cells, 50 μl ; lane 8 = extract from HeLa cells, 50 μl

Western blots

MN/CA IX antigens from polyacrylamide gel electrophoresis (PAGE) gels were transferred to polyvinylidene difluoride (PVDF) membranes (Immobilon P, Millipore) and developed with M75, followed by SwAM-Px (see above) and diaminobenzidine (Sigma) with H_2O_2 . For dot-blots we used nitrocellulose membranes.

Phage display

Ph.D.-7 Phage Display Peptide Library kit was used for screening as recommended by manufacturer (New England Biolabs). A 96-well plate was coated with peptide F (legend to Figure 3). Biopanning was carried out by incubating 2×10^{11} phage with target coated plate for 1 h. Unbound phages were washed away with TBST (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.1% Tween-20) and specifically bound phages were eluted with M75 antibody (2 μg in 100 μl of TBS per well). Eluted phage was amplified and used for additional binding and amplification cycles to enrich the pool in favour of binding sequence. After 5 rounds, individual clones were picked, amplified and sequenced using T7 sequencing kit (Pharmacia).

RESULTS

Affinity chromatography of MN/CA IX protein

For purification of MN/CA IX protein we decided to use affinity chromatography on sulphonamide-agarose column, described previously for other CAs (Falkbrink et al, 1972). The advantages of this method are simplicity and the fact that the whole procedure is carried out under non-denaturing conditions. Vaccinia virus vector with an insert of the complete MN/CA9 cDNA, or with truncated cDNA (lacking transmembrane and intracellular domains) was employed as a source of MN/CA IX protein.

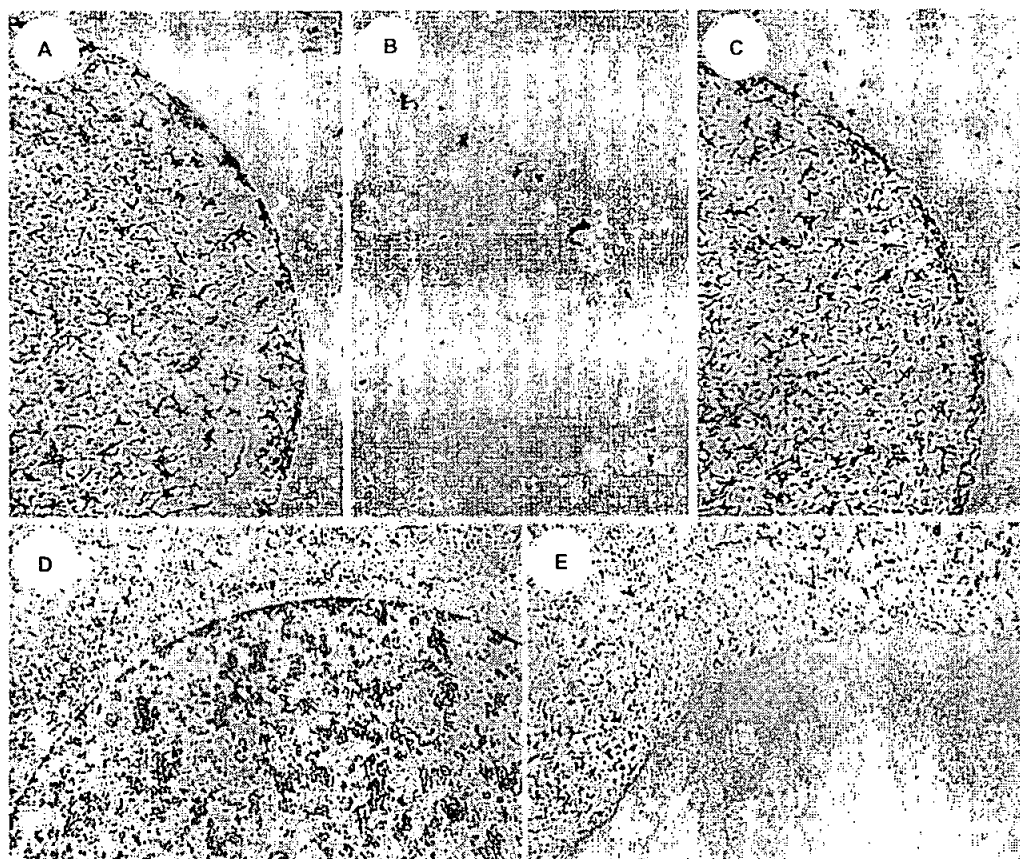


Figure 2 Adhesion of cells to the dots of MN/CA IX protein immobilized in bacteriological Petri dishes. This plasticware (not treated for use with tissue cultures) allows no adhesion of CGL1 cells and a limited adhesion of HeLa. (A) CGL1 cells do attach and spread to the dot of adsorbed MN/CA IX. (B) Treatment of the dot with MAb M75 abrogates its binding capacity. (C) Treatment with MAb M16 (irrelevant for MN/CA IX) has no effect on cell attachment. (D) HeLa cells attach to complete MN/CA IX⁺. (E) HeLa cells do not attach to truncated MN/CA IX Δ .

A single cycle of adsorption – elution yielded relatively pure proteins (Figure 1): MN/CA IX⁺ gave 2 bands of 54 and 58 kDa, MN/CA IX Δ of 54.5 and 56 kDa. These proteins strongly reacted with mAb M75 on Western blots. In extracts from HeLa, CGL3 and HT29 the blot revealed 2 bands of the same size as MN/CA IX⁺ purified from vaccinia virus construct.

Adhesion of cells to MN/CA IX protein

MN/CA IX immobilized on hydrophobic plastic enabled attachment, spreading and growth of cells (Figure 2). Extremely low concentrations of MN/CA IX corresponding to 1 $\mu\text{g ml}^{-1}$ of purified protein in adsorption buffer were sufficient to cause this effect; other cell adhesion molecules are used in 10–50 $\times$ higher concentrations. Only complete MN/CA IX protein was active in cell adhesion test, truncated MN/CA IX did not support cell adhesion at all or it showed only a low adhesion activity and in some instances it even acted as a cell 'repellent'.

Treatment of the dots of immobilized MN/CA IX with mAb M75 abrogated its capacity to attach the cells, but the control mAb M16, irrelevant for MN/CA IX had no effect. Blocking of cell attachment by M75 shows that the epitope is identical to or overlapping with the binding site of MN/CA IX for cell receptors.

Identification of the epitope recognized by Mab M75

Preliminary mapping of M75 epitope employing partial sequences of extracellular parts of MN/CA9 cDNA expressed from bacterial vectors and tested on Western blots located it in PG region. For exact mapping, our strategy was to synthesize partially overlapping oligopeptides of 15–25 aa covering the PG domain and test them in competition ELISA with M75. According to the results, this was followed by a series of 6–12 aa oligopeptides. A major part of the PG domain consists of a sixfold tandem repeat of six aa (aa 61–96); four repeats are identical (GEEDLP) and two contain two aa exchanged (SEEDSP and REEDPP) (Opavsky et al, 1996).

Figure 3 shows the result of competition ELISA with recombinant MN/CA IX and oligopeptides synthesized according to partial sequences of the PG region. MN/CA IX⁺ and Δ produced in mammalian cells (proteins A and B) possessed a higher serological activity than any other protein or peptide included in this experiment; protein C – fusion protein GST-MN synthesized in bacteria was less active. Peptides D, E, F and G are spanning the PG region. Peptides D and F caused 50% inhibition at 1 ng ml⁻¹. These two oligopeptides are mutually non-overlapping, thus the epitope is repeated in both of them. Peptide E was 1000 $\times$ less active, probably due to a different conformation. Peptide G was inactive; thus it does not contain the M75 epitope.

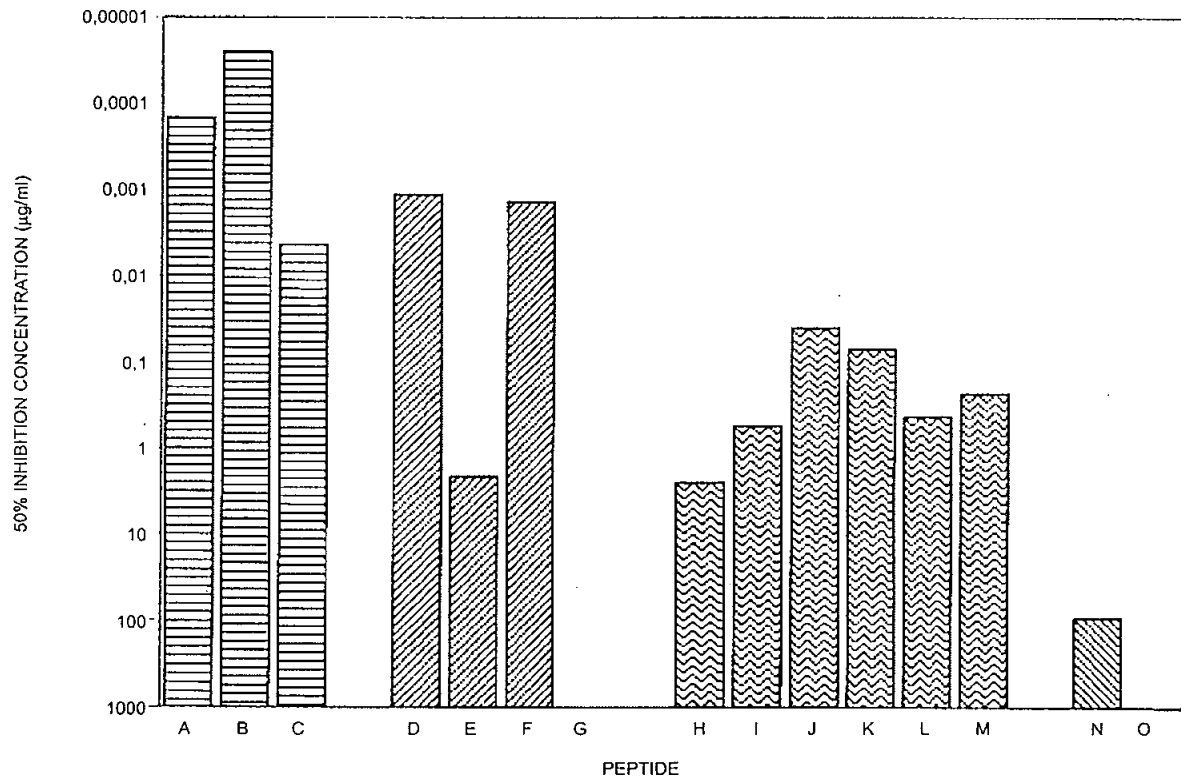


Figure 3 Competition ELISA with proteins or oligopeptides related to MN/CA IX protein. Competing proteins/peptides: (A–C) Recombinant proteins. (A) MN/CA IX+; (B) MN/CA IXΔ; (C) GST-MN; (D–G) overlapping proteins covering the PG region: (D) GGSSGEDDPLGEEDLPSEEDSPC (aa 51–72); (E) GEEDLPSEEDSPREEDPPGEEDLPGE (aa 61–85); (F) EDPPGEEDLPGEEDLPGEEDLPVC (aa 75–98); (G) EVKPKSEEEGSLKLE (aa 97–111); (H–M) dodecapeptides: (H) GEEDLPGEEDLP; (I) EEDLPGEEDLP; (J) EDLPGEEDLPGE; (K) DLPGEEDLPGE; (L) LPGEEDLPGEED; (M) PGEEDLPGEEDL; (N, O) oligopeptides 7 + 2 aa: (N) APGEEDLPA; (O) AGEEDLPGE. Other oligopeptides tested which proved negative in competition at 100 µg ml⁻¹ were oligopeptides 7 + 2 aa: AEEDLPGEA, AEDLPGEAA, ADLPGEEDA, ALPGEEDLA and all of the 6 + 2 aa permutations (AGEEDLPA, AEEDLPGEA, AEDLPGEAA, ADLPGEEDA and APGEEDLA). In oligopeptides D, E, F and G the C-terminal amino acid was present as acid, in all others as amide

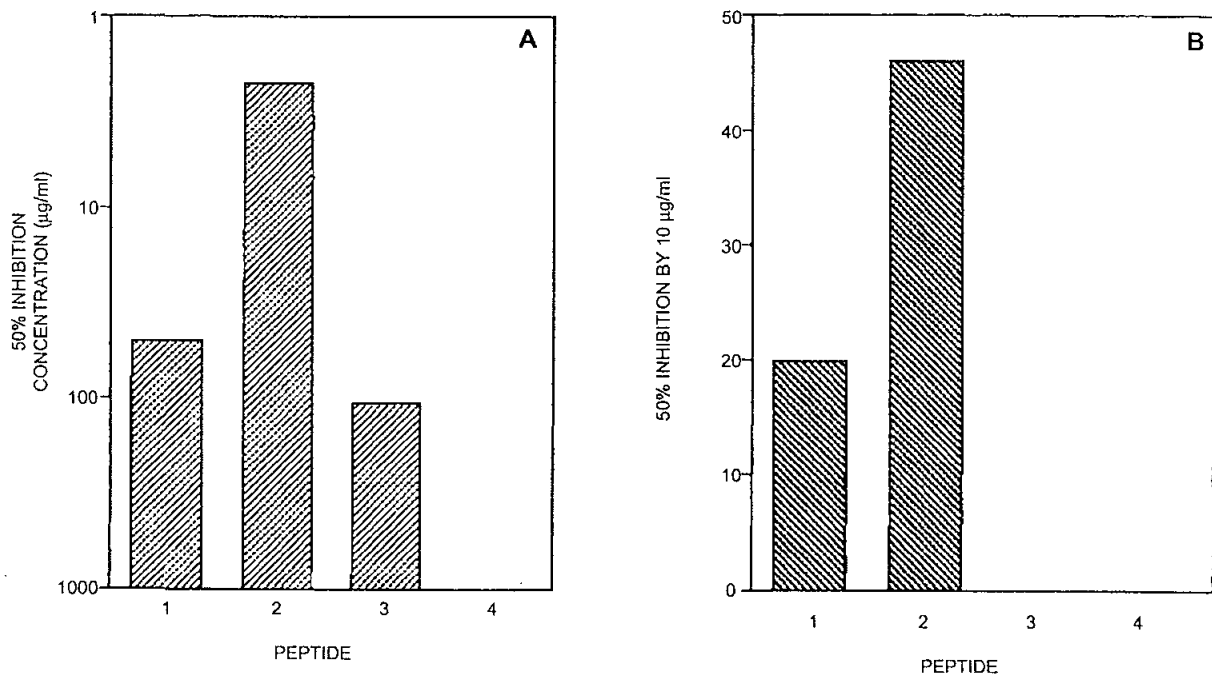


Figure 4 Competition ELISA (A) and inhibition of cell attachment (B) by oligopeptides selected from phage display library. Peptides: 1 = AKKMKRRKA, 2 = AITFNAQYA, 3 = ASASAPVSA, 4 = AGQTRSPLA. In all 4 peptides the C-terminal alanine was present as amide

The next step for identifying the epitope was to synthesize oligopeptides containing all circular permutations of the motif GEEDLP repeated twice (peptides H–M), all six dodecapeptides were serologically active (two more and four less so). Following series of seven aa sequences, flanked by alanine on both ends finally showed that the minimum serologically active sequence is the oligopeptide N = APGEEDLPA. None of still shorter oligopeptides (6 + 2 aa) competed in ELISA for M75.

It is clear that the affinity between these oligopeptides and mAb M75 very strongly increases with the size of peptide molecule.

Attempts to demonstrate adhesion of cells to immobilized oligopeptides

Our initial plan was to follow the pioneering work of Piersbacher and Ruoslahti (1984). They linked tested oligopeptides to adsorbed bovine serum albumin by cross-linking agent SPDP (N-succinimidyl 3[pyridylhydro] propionate). This is why we added onto the C-end of oligopeptides D, E and F (Figure 3) cysteine, which would enable oriented linking to adsorbed albumin. We demonstrated linking of the peptides directly in Petri dishes by immunoperoxidase staining with M75. Unfortunately, CGL1 or CGL3 cells adhered to control albumin treated with SPDP and blocked with ethanolamine (in place of oligopeptides) as strongly as to bovine serum albumin (BSA) dots with linked oligopeptides. We were unable to abrogate this non-specific adhesion. Oligopeptides D, E and F adsorb only very poorly to bacteriological Petri dishes, not allowing the cell adhesion test.

Alternatively, we tested inhibition of cell adhesion to MN/CA IX dots by oligopeptides added to the media together with the cell suspension, as described (Piersbacher and Ruoslahti, 1984). All peptides listed in the legend to Figure 3 were tested at concentrations 100 and 10 $\mu\text{g ml}^{-1}$. None of them inhibited reproducibly the adhesion of CGL1 cells.

Oligopeptides with affinity to M75 epitope which inhibit cell adhesion to MN/CA IX

As an alternative to monoclonal antibodies, we set out to select oligopeptides exerting affinity to M75 epitope as well as to MN/CA IX receptor binding site from a phage display library of random heptapeptides – Ph.D.-7. Our aim was to select phages containing the desired heptapeptides by panning on immobilized peptide F (see legend to Figure 3) and subsequent elution with M75. Eluted phage was multiplied in appropriate bacteria and subjected to four more cycles of panning and elution. From the selected phage population, ten plaques were picked, amplified and the heptapeptide-coding region was sequenced. Only three heptapeptides were represented: these, after adding alanine on both sides, are nonapeptides Nos 1, 2 and 3 from Figure 4. The last heptapeptide, synthesized again with added terminal alanines as nonapeptide No. 4, was identified by panning on GST-MN and eluted with acetazolamide. This last peptide has affinity to the active site of MN/CA IX carbonic anhydrase. We synthesized these peptides of 7 + 2 aa and tested them in competition ELISA and in cell adhesion inhibition. Both tests yielded essentially consistent results: peptide No. 2 showed the highest activity, peptide No. 1 was less active, peptide No. 3 was marginally positive only in ELISA, and peptide No. 4 was inactive.

DISCUSSION

Purification of transmembrane proteins like MN/CA IX often poses technical problems because they tend to form aggregates with other membrane proteins due to their hydrophobic TM segments. To avoid this, we engineered truncated MN/CA IX ΔICATM , which is secreted into the medium. Indeed, truncated MN/CA IX was obtained in higher purity than MN/CA IX+ (Figure 1). Unfortunately, this protein was of little use for our purposes, since it was inactive in the cell adhesion test (Figure 2). Such a situation has also been described for other cell adhesion molecules: their shed, shortened form either assumes an inactive conformation, or it adsorbs to hydrophobic plastic 'upside down', while complete proteins adsorb by hydrophobic TM segments in the 'correct' position.

MN/CA IX protein forms oligomers of 150 kDa, linked by disulphidic bonds (Pastorekova et al, 1992; Závada et al, 1993). It was not known whether these are homo- or hetero-oligomers. Figure 1 suggests that these are probably homo-oligomers, most likely trimers, since on the gel stained with Coomassie blue no additional bands of intensity comparable to two bands specific for MN/CA IX appeared. It is also unlikely that there could exist an additional protein co-migrating with one of the two major MN/CA IX bands, since the intensity of their staining on the gel and on Western blots is well comparable.

There can be no doubt on the specificity of cell attachment to purified MN/CA IX+. It is abrogated by specific mAb M75, at a dilution 1:1000 of ascites fluid. This is a correction to our previous report (Závada et al, 1997) in which we observed that MN/CA IX produced by vaccinia virus vector and fusion protein GST-MN support cell adhesion, but we did not realize that GST anchor itself contains another binding site, which is not blocked by M75.

mAb M75 reacts excellently with MN/CA IX under any circumstances – with native antigen on the surface of living cells, with denatured protein on Western blots and with antigen in paraffin sections of biopsies fixed with formaldehyde, suggesting that the epitope is small and contiguous. In competition ELISA (Figure 3) the smallest sequence reactive with M75 was 7 + 2 aa, but the affinity between M75 and tested peptides strongly depended on their molecular weight. Complete MN/CA IX was 1 000 000 $\times$ more active than the smallest serologically active peptide in terms of weight/volume concentration. In terms of molar concentration this difference would be 150 000 000 $\times$. Oligopeptides of intermediate size also showed intermediate activities. It remains to be elucidated whether such differences in activity are due to the conformation depending on the size of the molecule, or to the fact that complete MN/CA IX contains several copies of the epitope, but the smallest molecule only one.

Considering the possibility that the epitope is identical with the cell adhesion structure in MN/CA IX, we can understand why we failed to detect inhibition of cell adhesion by the oligopeptides. The binding site is just not as simple as the prototype peptide, RGD (Piersbacher and Ruoslahti, 1984).

Naturally, one can argue that the size of MN/CA IX is about the same as of immunoglobulin molecule, and that binding of M75 to its epitope may sterically hinder a different sequence of cell attachment site. This objection has been made unlikely by blocking of both M75 epitope and of cell binding site by nonapeptides 7 + 2 aa. This result (Figure 4) strongly suggests that the epitope and the binding site are indeed identical.

MN/CA IX and its PG region in particular appears to be a potential 'target molecule for therapy for the following reasons: (i) it is exposed on the cell surface; (ii) it is present in high percentage of certain human carcinomas; (iii) it is normally expressed MN/CA IX in the mucosa of alimentary tract which is not accessible to circulating antibodies, in contrast with the tumours; (iv) it is not shed (or only minimally) into the body fluids; (v) the motif GEEDLP is repeated 18 × on the surface of every MN/CA IX molecule. Oligopeptide display libraries are being employed in the first steps to develop new drugs (Winter, 1996). Selected oligopeptides can serve as lead compounds for the computerized design of new molecules, with additional properties required from a drug (DeCamp et al, 1996).

ACKNOWLEDGEMENTS

This research was supported by grant No. 301/99/0356 from Grant Agency of the Czech Republic, grant No. 2/4103/98 from Slovak Grant Agency, by Chiron-Bayer and by Volkswagen research grant No. 1/71 392. The authors are grateful to Drs O Machoň, L Kutinová and Š Němcčková for the construction of vaccinia virus vectors with MN/CA9 inserts and to Prof R Weiss and to Dr Z Palková for their help in preparing this paper.

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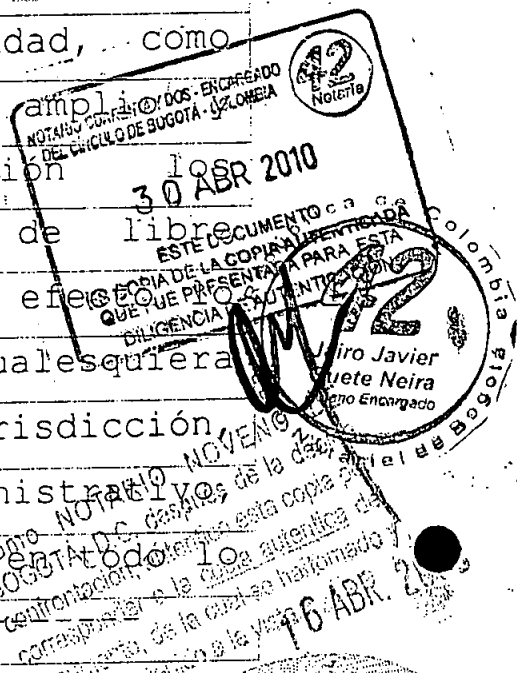
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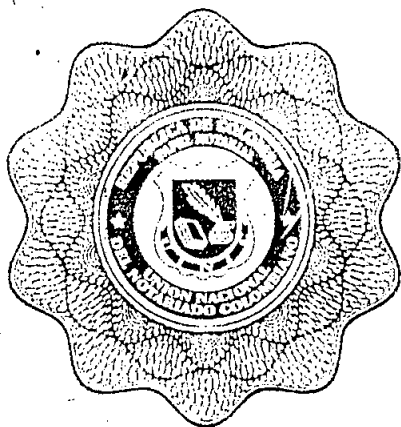
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prendas, levantamiento de prendas, inscribir
derechos de retención y en general todos los asuntos
que se deban tramitar ante la Superintendencia de
Industria y Comercio.-----

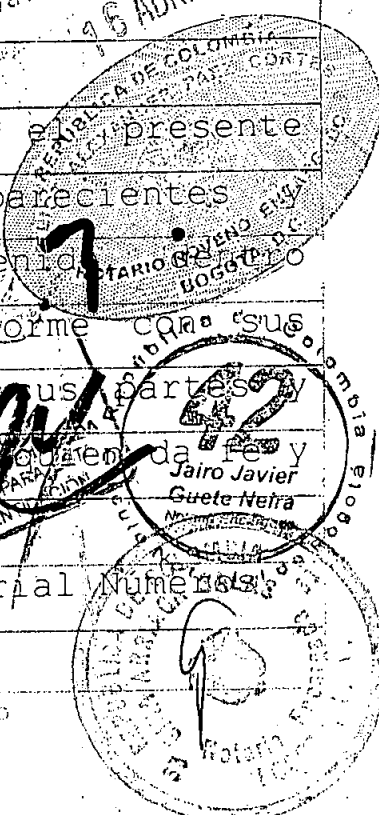
Manifestamos que de la veracidad de las
declaraciones del contenido y autenticidad de los
documentos anexos en éste instrumento público sólo
respondemos los comparecientes como NOTARIO NOVENO DE

HASTA AQUI LA MINUTA PRESENTADA BOGOTÁ, D.C. después de la lectura
confrontación, autentica esta copia por
corresponder a la copia auténtica del
documento, de la cual se ha tomado y
que se ha tenido a la vista
Bogotá, D.C. 6 ABR. 2008

OTORGAMIENTO Y AUTORIZACION: Leído en presente
instrumento Público por los comparecientes
advertidos de la formalidad de su contenido,
del término legal, lo hallaron conforme con sus
intenciones y lo aprobaron en todas sus partes y
firmaron junto con la Suscrita Notaria Pública y
lo autoriza. -----

Se utilizaron las hojas de papel Notarial Números:
AA 8567146, 8567152.

30 ABR 2010
ESTE DOCUMENTO
ES COPIA DE LA COPIA
QUE FUE PRESENTADA PARA
SUSENCIA DE AUTENTICACION



DERECHOS NOTARIALES: \$ 49.600.00

RESOLUCION No. 4188 del 28 de Diciembre del 2001

JUAN MARIA RENDON

C.C. No. 17.125.100 de Bogotá

LAFRANCOL S.A.

Nit. No. 890.301.463-8

TEL: 622-04-66.

ACEPTO :

JOSE LUIS REYES VILLAMIZAR

C.C. No. 79.152.473

TEL: 621-2631

DIANA BEATRIZ LOPEZ

DIANA BEATRIZ LOPEZ

NOTARIA 33 DE BOGOTA D.C.

n.c.a.

Es segunda COPIA (FOTOCOPIA)

tomada de su original que se expide conforme al

Artículo 79 del Decreto 960 de 1970 en 06

hojas útiles con destino a Jose Luis Reyes

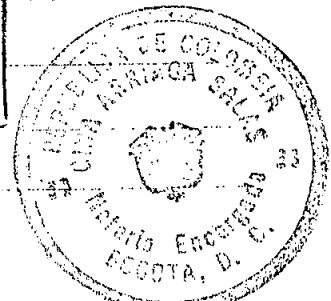
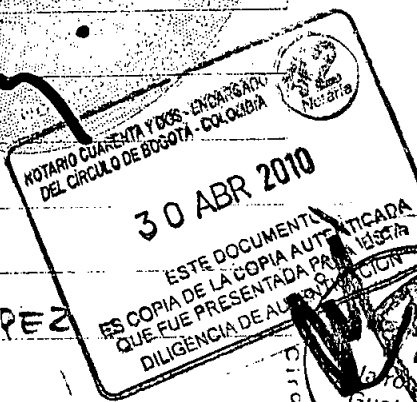
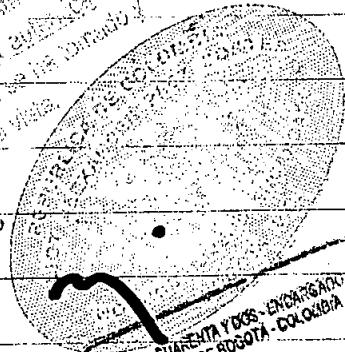
Villamizar Dec. 1772/79

Santafé de Bogotá D.C. 05 JUL. 2002

Qui Amun

Como NOTARIO NOVENO DE
BOGOTÁ D.C. después de la debida
calificación autenticada por el
Notario de la circunscripción del
Círculo de la villa.

6 ABR. 2008





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CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

FECHA: 2010/05/03

HORA: 10:49:35

OPERACION: 02CSI0503011

PAGINA: 1

EN JUNIO DE ESTE AÑO SE ELEGIRÁ JUNTA DIRECTIVA Y REVISOR FISCAL DE LA CAMARA DE COMERCIO. LAS INSCRIPCIONES DE CANDIDATOS DEBEN HACERSE EN LA PRIMERA QUINCENA DE MAYO. PARA INFORMACION DETALLADA DIRIGIRSE A LA SEDE PRINCIPAL O COMUNICARSE AL TELEFONO 8861321.

CERTIFICA:

NOMBRE: LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A.

SIGLA : LAFRANCOL S.A.

DOMICILIO: CALI VALLE

DIRECCION COMERCIAL : ---K 1 46 84

DIRECCION NOTIFICACION JUDICIAL: ---K 1 46 84

CIUDAD: CALI

MATRICULA MERCANTIL NRO. 5233-4 FECHA MATRICULA : 02 DE OCTOBER DE 1958

DIRECCION ELECTRONICA : lafrancolsa@lafrancol.co

DIRECCION WEB : www.lafrancol.com

AFILIADO

CERTIFICA:

NIT : 890301463-8

CERTIFICA:

QUE POR ESCRITURA NRO. 1434 DEL 29 DE SEPTEMBER DE 1958 NOTARIA CUARTA DE CALI , INSCRITA EN LA CAMARA DE COMERCIO EL 02 DE OCTOBER DE 1958 BAJO EL NRO. 18319 DEL LIBRO IX , SE CONSTITUYO LA SOCIEDAD DENOMINADA LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A.

CERTIFICA:

QUE POR ESCRITURA NRO. 3672 DEL 24 DE AUGUST DE 2009 NOTARIA TERCERA DE CALI , INSCRITA EN LA CAMARA DE COMERCIO EL 27 DE AUGUST DE 2009 BAJO EL NRO. 9876 DEL LIBRO IX , LA SOCIEDAD CAMBIO SU NOMBRE DE LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A. . POR EL DE LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A. . SIGLA: LAFRANCOL S.A.

CERTIFICA:

REFORMAS

DOCUMENTO	FECHA.DOC	ORIGEN	FECHA.INS	NRO.
INS LIBRO				
E.P. 1104	30/06/1959	NOTARIA CUARTA DE CALI	07/07/1959	
E.P. 2309	26/12/1959	NOTARIA CUARTA DE CALI	29/12/1959	

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RECIBIDO
CAMARA DE COMERCIO
DE BOGOTA
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CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

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PAGINA: 2

20089

E.P. 1725 24/07/1962 NOTARIA CUARTA DE CALI 26/07/1962

24528

E.P. 1824 31/07/1963 NOTARIA CUARTA DE CALI 14/08/1963

26360

E.P. 2422 25/10/1963 NOTARIA CUARTA DE CALI 28/10/1963

26717

E.P. 1720 16/09/1964 NOTARIA CUARTA DE CALI 22/09/1964

28406

E.P. 101 31/01/1966 NOTARIA CUARTA DE CALI 01/02/1966

31039

E.P. 552 21/03/1968 NOTARIA CUARTA DE CALI 01/04/1968

35711

E.P. 4964 12/12/1972 NOTARIA CUARTA DE CALI 16/01/1973

2975 IX

E.P. 1956 30/05/1973 NOTARIA CUARTA DE CALI 13/06/1973

4337 IX

E.P. 4964 12/12/1972 NOTARIA CUARTA DE CALI 28/11/1973

5994 IX

E.P. 5579 12/11/1973 NOTARIA CUARTA DE CALI 28/11/1973

5995 IX

E.P. 4406 30/09/1974 NOTARIA CUARTA DE CALI 28/11/1974

10887 IX

E.P. 192 03/02/1978 NOTARIA CUARTA DE CALI 14/02/1978

25355 IX

E.P. 3479 07/09/1979 NOTARIA CUARTA DE CALI 19/09/1979

34204 IX

E.P. 4274 12/12/1984 NOTARIA NOVENA DE CALI 19/12/1984

73088 IX

E.P. 1155 21/05/1985 NOTARIA SEPTIMA DE CALI 03/06/1985

76917 IX

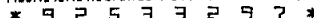
E.P. 1553 20/06/1986 NOTARIA SEPTIMA DE CALI 08/07/1986

85997 IX

E.P. 1458 09/06/1987 NOTARIA SEPTIMA DE CALI 24/12/1987

3508 IX

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PREMIOS
COLONIALES
AL CALIDAD
DE LA ESTACIÓN

CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

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PARA USO HUMANO Y VETERINARIO, PRODUCTOS DIETÉTICOS, COLORANTES, DE PERFUMERÍA, DISPOSITIVOS MÉDICOS Y AFINES Y/O PRODUCTOS INTERMEDIOS O A GRANEL. EN DESARROLLO DE SU OBJETO SOCIAL PODRÁ LA SOCIEDAD ADQUIRIR Y VENDER BIENES RAÍCES Y MUEBLES, DARLOS Y TOMARLOS EN ARRENDAMIENTO, GARANTIZAR CON HIPOTECA SOBRE LOS BIENES RAÍCES O CON PRENDA SOBRE LOS BIENES MUEBLES, OBLIGACIONES A CARGO DE LA SOCIEDAD; DAR Y RECIBIR A MUTUO COMERCIAL DINERO Y CELEBRAR LOS CONTRATOS COMERCIALES DE CAMBIO EN TODAS SUS MANIFESTACIONES, FUNDAR EMPRESAS QUE TENGAN LA MISMA FINALIDAD SOCIAL O ASOCIARSE CON ELLAS.

CERTIFICA:

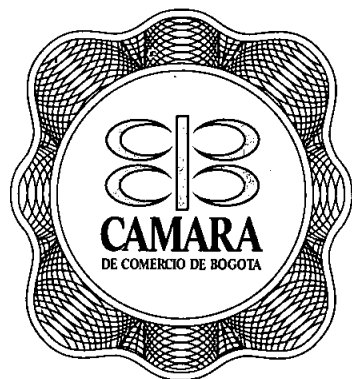
LA SOCIEDAD TIENE LOS SIGUIENTES ÓRGANOS PRINCIPALES 1) ASAMBLEA GENERAL DE ACCIONISTAS. 2) JUNTA DIRECTIVA. 3) REPRESENTANTES LEGALES.

FUNCIONES DE LA ASAMBLEA GENERAL, ENTRE OTRAS: A) DECLARAR ORDINARIA O EXTRAORDINARIAMENTE DISUELTA Y EN ESTADO DE LIQUIDACIÓN A LA SOCIEDAD; B) AUTORIZAR LA VENTA, TRANSFERENCIA, ACTOS DISPOSITIVOS, DE ADMINISTRACIÓN, O ARRIENDO, HIPOTECAR, GRAVAR, TOTAL O PARCIALMENTE LOS BIENES QUE COMPONEN SU PATRIMONIO; C) DECRETAR EL AUMENTO DE CAPITAL AUTORIZADO; D) DECRETAR LA PRÓRROGA DEL TÉRMINO DE DURACIÓN DE LA SOCIEDAD; E) REMOVER EN CUALQUIER MOMENTO AL PRESIDENTE EJECUTIVO, A LOS MIEMBROS DE LA JUNTA DIRECTIVA O AL REVISOR FISCAL, Y SUS RESPECTIVOS SUPLENTE; F) REALIZAR CUALQUIER MODIFICACIÓN ESTATUTARIA; G) DISPONER LA ESCISIÓN, FUSIÓN O TRANSFORMACIÓN DE LA SOCIEDAD; H) NOMBRAR A LOS MIEMBROS DE LA JUNTA DIRECTIVA Y EL REVISOR FISCAL DE LA SOCIEDAD Y SUS RESPECTIVOS SUPLENTE; N) DELEGAR EN LA JUNTA DIRECTIVA, CUANDO LO ESTIME OPORTUNO, ALGUNA O ALGUNAS DE SUS FUNCIONES CUYA DELEGACIÓN NO ESTÉ PROHIBIDA POR LA LEY O POR LOS ESTATUTOS, PERO, NO SON DELEGABLES LAS COMPRENDIDAS EN EL PRIMER LITERAL DE ESTE ARTÍCULO; O) LAS QUE NATURALMENTE LE CORRESPONDAN COMO SUPREMA ENTIDAD DIRECTIVA DE LA SOCIEDAD; S) NOMBRAR Y REMOVER EL LIQUIDADOR DE LA SOCIEDAD Y SU SUPLENTE, FIJAR SU REMUNERACIÓN Y CONSIDERAR, APROBAR O IMPROBAR SUS CUENTAS; T) ADOPTAR TODAS LAS MEDIDAS QUE RECLAMEN EL CUMPLIMIENTO DE LOS ESTATUTOS Y EL INTERÉS DE LOS ACCIONISTAS Y DE LA SOCIEDAD.

LA JUNTA DIRECTIVA SE COMPONE DE TRES (3) MIEMBROS QUE SERÁN ELEGIDOS POR LA ASAMBLEA GENERAL DE ACCIONISTAS. SUS SUPLENTE SERÁN PERSONALES.

FUNCIONES DE LA JUNTA DIRECTIVA, ENTRE OTRAS: B) CREAR LOS CARGOS QUE JUZGUE NECESARIOS PARA LA BUENA MARCHA DE LA EMPRESA, SEÑALAR SUS FUNCIONES, FIJAR SUS ASIGNACIONES O LA FORMA DE RETRIBUCIÓN, NOMBRAR Y REMOVER LIBREMENTE A LOS

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EMPLEADOS DE LA SOCIEDAD, EXCEPTO AQUELLOS CUYO NOMBRAMIENTO O REMOCIÓN CORRESPONDA A LA ASAMBLEA GENERAL DE ACCIONISTA A LAS PERSONAS QUE DEBEN CORRESPONDERLOS O LOS DESEMPEÑEN. C) NOMBRAR, REMOVER Y FIJAR ASIGNACIONES AL PRESIDENTE EJECUTIVO, VICEPRESIDENTES Y ADMINISTRADORES QUE REPORTEN DIRECTAMENTE A LA PRESIDENCIA EJECUTIVA H) AUTORIZAR POR VÍA GENERAL, LIBERALIDADES, BENEFICIOS O PRESTACIONES DE CARÁCTER EXTRALEGAL A FAVOR DEL PERSONAL DE LA SOCIEDAD. J) AUTORIZAR AL PRESIDENTE EJECUTIVO, PARA QUE SOLICITE, DE ACUERDO CON LA LEY VIGENTE, LLEGADO EL CASO, QUE SE ADMITA A LA COMPAÑÍA EN LA CELEBRACIÓN DE CONCORDATO PREVENTIVO CON SUS ACREEDORES, O REESTRUCTURACIÓN EMPRESARIAL. Q) CREAR LOS COMITÉS QUE ESTIME CONVENIENTES PARA ASESORAR AL PRESIDENTE EJECUTIVO Y REPRESENTANTES LEGALES DE LA SOCIEDAD EN DETERMINADOS ASUNTOS, FIJARLES SUS FUNCIONES Y SEÑALAR LA REMUNERACIÓN DE SUS MIEMBROS; R) AUTORIZAR AL TERCER REPRESENTANTE LEGAL QUE ES EL PRESIDENTE EJECUTIVO, AL CUARTO Y QUINTO REPRESENTANTES LEGALES PARA COMPROMETER A LA SOCIEDAD O A LOS NEGOCIOS SOCIALES EN CUANTÍA SUPERIOR A SEIS MIL SALARIOS MÍNIMOS MENSUALES LEGALES VIGENTES (6,000 SMMMLV) PARA CELEBRAR CONTRATOS COMERCIALES DE COMPRAVENTA CON LA NACIÓN (MINISTERIOS Y DEPARTAMENTOS ADMINISTRATIVOS), ENTIDADES DESCENTRALIZADAS (ESTABLECIMIENTOS PÚBLICOS, EMPRESAS INDUSTRIALES Y COMERCIALES DEL ESTADO, SOCIEDADES DE ECONOMÍA MIXTA), EMPRESAS SOCIALES CIVILES Y COMERCIALES, CUALQUIERA QUE SEA SU NATURALEZA CON FACULTADES PARA EXIGIR O SUSCRIBIR LOS DOCUMENTOS Y GARANTÍAS NECESARIOS HASTA LA CUANTÍA ANTES MENCIONADA Y REPRESENTAR A LA COMPAÑÍA EN CUALQUIER TRÁMITE O PROCESO JUDICIAL QUE SE ADELANTE ANTE LAS JURISDICCIONES CIVIL, LABORAL, COMERCIAL, PENAL Y CONTENCIOSO ADMINISTRATIVA EN CALIDAD DE DEMANDANTES O DEMANDADOS Y CUALQUIER OTRO TRÁMITE EN EL QUE SE REQUIERA REPRESENTAR A LA COMPAÑÍA ANTE ENTIDADES Y ORGANISMOS DEL ESTADO DE CUALQUIER ORDEN O ENTIDADES DE NATURALEZA PRIVADA. S) AUTORIZAR AL TERCER Y CUARTO REPRESENTANTE LEGAL PARA ADQUIRIR ACTIVOS FIJOS SUPERIOR A DOSCIENTOS UNO SALARIOS MÍNIMOS MENSUALES LEGALES VIGENTES (201 SMMMLV), CRÉDITOS CON ENTIDADES FINANCIERAS SUPERIORES A UN MILLÓN QUINIENTOS MIL DÓLARES AMERICANOS (US\$1.500.0000) Y DEFINIR LAS ATRIBUCIONES AL TERCER Y CUARTO REPRESENTANTE LEGAL CORRESPONDIENTE A OTRAS COMPRAS POR CONCEPTO DE ASESORÍAS Y GASTOS DIFERENTES A LOS ANTERIORES. T) AUTORIZAR AL TERCER Y CUARTO REPRESENTANTE LEGAL DE LA COMPAÑÍA PARA QUE CELEBREN CONTRATOS DE SOCIEDAD O PARTICIPEN EN OTRAS COMPAÑÍAS COMERCIALES. U) AUTORIZAR AL TERCER Y CUARTO REPRESENTANTE LEGAL

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PREMIO
CORPORATIVO
ALACID
DE LA GERENCIA

02/05/2010 10:50:35 02CSI0503011 005

CERTIFICADO NACIONAL

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PARA QUE SUSCRIBAN TODA CLASE DE DOCUMENTOS PÚBLICOS O PRIVADOS Y CON EL PROPÓSITO DE INSTRUMENTAR CUALQUIER TIPO DE ACTOS, NEGOCIOS JURÍDICOS, OPERACIONES DE CUALQUIER ÍNDOLE, Y SIEMPRE Y CUANDO SUPERE LA CUANTÍA PREVISTA PARA LA REALIZACIÓN DE ACTOS POR PARTE DEL REPRESENTANTE LEGAL DE LA COMPAÑÍA.

W) AUTORIZAR LA CONSTITUCIÓN O SUPRESIÓN DE SUCURSALES O AGENCIAS. BB) AUTORIZAR LA NOVACIÓN O CUALQUIER FORMA EXTINTIVA DE OBLIGACIONES. CC) AUTORIZAR LA ENAJENACIÓN A CUALQUIER TÍTULO RELACIONADA CON MAQUINARIA INDUSTRIAL, TERRENOS, EDIFICIOS E INTANGIBLES Y APROBAR DONACIONES. IGUALMENTE AUTORIZARÁ LA CONSTITUCIÓN DE GARANTÍAS HIPOTECARIAS, PRENDARIAS O DE CUALQUIER OTRA ÍNDOLE SOBRE DICHS ACTIVOS.

EL PRESIDENTE EJECUTIVO, TERCER REPRESENTANTE LEGAL DE LA COMPAÑÍA, PRESTARÁ SU ASESORÍA PERMANENTE A LA JUNTA DIRECTIVA CON LAS SIGUIENTES FUNCIONES: A) FIRMAR LAS CONVOCATORIAS DE LA ASAMBLEA GENERAL Y DE LA JUNTA DIRECTIVA. B) VIGILAR LA EJECUCIÓN DE LOS ACUERDOS ADOPTADOS, SIN PERJUICIO DEL CUMPLIMIENTO DE ESTA MISMA FUNCIÓN POR PARTE DEL PRESIDENTE O EL SECRETARIO DE LA JUNTA DIRECTIVA DE LA COMPAÑÍA. C) PROPONER LA CREACIÓN DE LOS ÓRGANOS ASESORES QUE ESTIME OPORTUNOS, INFORMANDO DE ELLO A LA JUNTA DIRECTIVA PARA SU APROBACIÓN POR ÉSTA. D) RENDIR ANUALMENTE INFORME DE SU ACTUACIÓN ANTE LA ASAMBLEA GENERAL Y SOMETER A APROBACIÓN LO CORRESPONDIENTE A LA JUNTA DIRECTIVA.

REPRESENTACIÓN LEGAL

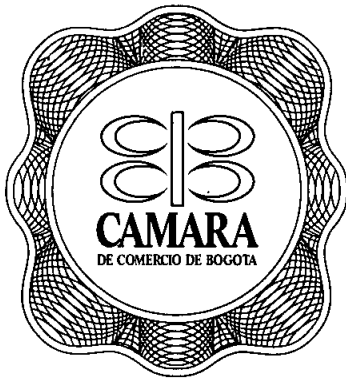
LA ADMINISTRACIÓN DE LA SOCIEDAD ESTA A CARGO DE LOS ACCIONISTAS, QUIENES LA DELEGAN EN EL PRESIDENTE EJECUTIVO, TERCER REPRESENTANTE LEGAL DE LA COMPAÑÍA, ASÍ COMO LA GESTIÓN Y EJECUCIÓN DE LOS NEGOCIOS Y ASUNTOS SOCIALES. EL PRESIDENTE EJECUTIVO DE LA SOCIEDAD, SERÁ DESIGNADO POR LA JUNTA DIRECTIVA PARA PERÍODOS DE UN (1) AÑO, SIN PERJUICIO DE SU LIBRE REMOCIÓN Y REELECCIÓN INDEFINIDA.

EL PRESIDENTE Y VICEPRESIDENTE DE LA JUNTA DIRECTIVA, SERÁN TAMBIÉN REPRESENTANTES LEGALES PRIMERO Y SEGUNDO DE LA SOCIEDAD. LAS ATRIBUCIONES INHERENTES A LA CALIDAD DE REPRESENTANTES LEGALES, Y EN ESPECIAL LAS DISPOSITIVAS, SERÁN EJERCIDAS SIN LIMITACIONES ESTABLECIDAS EN ÉSTOS ESTATUTOS.

EL CUARTO Y QUINTO REPRESENTANTE LEGAL, SERÁN REPRESENTANTES LEGALES DE LA SOCIEDAD QUIENES SON ELEGIDOS POR LA JUNTA DIRECTIVA CON SUS LIMITACIONES EN SUS FUNCIONES.

LAS ATRIBUCIONES INHERENTES A LA CALIDAD DEL CUARTO REPRESENTANTE LEGAL, Y EN

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ESPECIAL LAS DISPOSITIVAS, SERÁN EJERCIDAS CON SUJECCIÓN A LAS LIMITACIONES ESTABLECIDAS EN ÉSTOS ESTATUTOS PARA EL PRESIDENTE EJECUTIVO QUIEN ES EL TERCER REPRESENTANTE LEGAL.

EXISTIRÁ UN QUINTO REPRESENTANTE LEGAL DE LA SOCIEDAD QUE TENDRÁ LIMITACIÓN EN SUS FUNCIONES CUANDO SE TRATE DE COMPROMETER A LA SOCIEDAD O A LOS NEGOCIOS SOCIALES EN CUANTÍA NO SUPERIOR A SEIS MIL SALARIOS MÍNIMOS MENSUALES LEGALES VIGENTES (6,000 SMMLV) PARA CELEBRAR CONTRATOS COMERCIALES DE COMPRAVENTA CON LA NACIÓN (MINISTERIOS Y DEPARTAMENTOS ADMINISTRATIVOS), ENTIDADES DESCENTRALIZADAS (ESTABLECIMIENTOS PÚBLICOS, EMPRESAS INDUSTRIALES Y COMERCIALES DEL ESTADO, SOCIEDADES DE ECONOMÍA MIXTA), EMPRESAS SOCIALES CIVILES Y COMERCIALES, CUALQUIERA QUE SEA SU NATURALEZA CON FACULTADES PARA EXIGIR O SUSCRIBIR LOS DOCUMENTOS Y GARANTÍAS NECESARIOS HASTA LA CUANTÍA ANTES MENCIONADA Y REPRESENTAR A LA COMPAÑÍA EN CUALQUIER TRÁMITE O PROCESO JUDICIAL QUE SE ADELANTE ANTE LAS JURISDICCIONES CIVIL, LABORAL, COMERCIAL, PENAL Y CONTENCIOSO ADMINISTRATIVA EN CALIDAD DE DEMANDANTES O DEMANDADOS Y CUALQUIER OTRO TRÁMITE EN EL QUE SE REQUIERA REPRESENTAR A LA COMPAÑÍA ANTE ENTIDADES Y ORGANISMOS DEL ESTADO DE CUALQUIER ORDEN O ENTIDADES DE NATURALEZA PRIVADA.

FUNCIONES DEL PRESIDENTE EJECUTIVO: EL PRESIDENTE EJECUTIVO DE LA SOCIEDAD ES UN MANDATARIO CON REPRESENTACIÓN, INVESTIDO DE FACULTADES EJECUTIVAS Y ADMINISTRATIVAS, Y COMO TAL, TIENE A SU CARGO LA TERCERA REPRESENTACIÓN LEGAL, LA GESTIÓN COMERCIAL Y FINANCIERA, LA RESPONSABILIDAD DE LA ACCIÓN ADMINISTRATIVA, LA COORDINACIÓN Y SUPERVISIÓN GENERAL DE LA EMPRESA, LAS CUALES CUMPLIRÁ CON ARREGLO A LAS NORMAS DE ESTOS ESTATUTOS Y LAS DISPOSICIONES LEGALES, Y CON SUJECCIÓN A LAS ORDENES E INSTRUCCIONES DE LA JUNTA DIRECTIVA.

ADEMÁS DE LAS FUNCIONES ANTES INDICADAS, CORRESPONDE AL PRESIDENTE EJECUTIVO DE LA SOCIEDAD: A) COMPROMETER A LA SOCIEDAD O A LOS NEGOCIOS SOCIALES EN CUANTÍA NO SUPERIOR A SEIS MIL SALARIOS MÍNIMOS MENSUALES LEGALES VIGENTES (6,000 SMMLV) PARA CELEBRAR CONTRATOS COMERCIALES DE COMPRAVENTA CON LA NACIÓN (MINISTERIOS Y DEPARTAMENTOS ADMINISTRATIVOS), ENTIDADES DESCENTRALIZADAS (ESTABLECIMIENTOS PÚBLICOS, EMPRESAS INDUSTRIALES Y COMERCIALES DEL ESTADO, SOCIEDADES DE ECONOMÍA MIXTA), EMPRESAS SOCIALES CIVILES Y COMERCIALES, CUALQUIERA QUE SEA SU NATURALEZA CON FACULTADES PARA EXIGIR O SUSCRIBIR LOS DOCUMENTOS Y GARANTÍAS NECESARIOS HASTA LA CUANTÍA ANTES MENCIONADA Y

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COLLEGE
OF EDUCATION
OF CALIFORNIA
DE LA GESTA

CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

FECHA: 2010/05/03

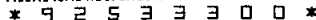
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OPERACION: 02CSI0503011

PAGINA: 8

REPRESENTAR A LA COMPAÑÍA EN CUALQUIER TRÁMITE O PROCESO JUDICIAL QUE SE ADELANTE ANTE LAS JURISDICCIONES CIVIL, LABORAL, COMERCIAL, PENAL Y CONTENCIOSO ADMINISTRATIVA EN CALIDAD DE DEMANDANTES O DEMANDADOS Y CUALQUIER OTRO TRÁMITE EN EL QUE SE REQUIERA REPRESENTAR A LA COMPAÑÍA ANTE ENTIDADES Y ORGANISMOS DEL ESTADO DE CUALQUIER ORDEN O ENTIDADES DE NATURALEZA PRIVADA; ADQUISICIÓN DE ACTIVOS FIJOS NO SUPERIOR A DOSCIENTOS UNO SALARIOS MÍNIMOS MENSUALES LEGALES VIGENTES (201 SMLLV); OBTENER CRÉDITOS CON ENTIDADES FINANCIERAS NO SUPERIORES A UN MILLÓN QUINIENTOS MIL DÓLARES AMERICANOS (US\$1.500.0000) Y CONTRATAR OTRAS COMPRAS POR CONCEPTO DE ASESORÍAS Y GASTOS DIFERENTES A LOS ANTERIORES PREVIA APROBACIÓN DE LA JUNTA DIRECTIVA. B) CUMPLIR Y HACER CUMPLIR LAS DECISIONES DE LA ASAMBLEA GENERAL DE ACCIONISTAS Y DE LA JUNTA DIRECTIVA Y HACER USO DE LA RAZÓN SOCIAL. C)... D)... E)... F)... G) COMPARECER EN LOS JUICIOS, TRANSIGIR Y COMPROMETER LOS NEGOCIOS SOCIALES DE CUALESQUIERA NATURALEZA QUE FUEREN, INTERPONER TODO GÉNERO DE RECURSOS Y CONSTITUIR APODERADOS QUE LO REPRESENTEN JUDICIAL O EXTRAJUDICIALMENTE. I) HACER DEPÓSITOS EN BANCOS Y AGENCIAS BANCARIAS, FIRMAR CHEQUES, LETRAS, PAGARÉS, GIROS, LIBRANZAS Y CUALQUIER OTRO DOCUMENTO, ASÍ COMO NEGOCIAR ESTOS INSTRUMENTOS, TENERLOS, COBRARLOS Y PAGARLOS, DESCARGARLOS, ETC. J)... K)... L) SUSCRIBIR TODA CLASE DE DOCUMENTOS PÚBLICOS O PRIVADOS, CUANDO LA JUNTA DIRECTIVA LO AUTORICE Y CON EL PROPÓSITO DE INSTRUMENTAR CUALQUIER TIPO DE ACTOS, NEGOCIOS JURÍDICOS, OPERACIONES DE CUALQUIER ÍNDOLE, Y SIEMPRE Y CUANDO NO SUPERE LA CUANTÍA PREVISTA EN ESTOS ESTATUTOS COMO LÍMITE DEL EJERCICIO DE SUS FUNCIONES, Y POR ENCIMA DE LA CUAL REQUIERE AUTORIZACIÓN DE LA JUNTA DIRECTIVA. M) PODRÁ EJERCER LA REPRESENTACIÓN LEGAL, SIN PERJUICIO DE LA MISMA FUNCIÓN ASIGNADA A LOS OTROS REPRESENTANTES LEGALES PARA OTORGAR PODERES A TERCEROS, NOMBRAR ABOGADOS Y PROCURADORES, INTERPONER RECLAMACIONES EN LA VÍA GUBERNATIVA, ANTE TRIBUNALES, JURISDICCIONES ORDINARIAS, LABORALES Y ESPECIALES, REPRESENTAR A LA SOCIEDAD ANTE ADMINISTRACIONES O HACIENDAS PÚBLICAS, PRIVADAS, NACIONALES, INTERNACIONALES Y AUTONÓMICAS, CONCURRIR A TODA CLASE DE SUBASTAS, CONCURSOS O SUMINISTROS OFICIALES O PARTICULARES, PUDIENDO POR TANTO REDACTAR, SUSCRIBIR, PRESENTAR Y, EN SU CASO, MEJORAR EN LICITACIÓN VERBAL O ESCRITA LAS OFERTAS O PROPOSICIONES PERTINENTES, ACEPTAR DONACIONES PARA LA SOCIEDAD Y ACEPTAR CON BENEFICIO DE INVENTARIO, LEGADOS, EJERCITAR ACCIONES O PONER EXCEPCIONES, PARA ACTUAR EN CALIDAD DE DEMANDANTE, DEMANDADO O TERCERO INTERVINIENTE. N) ORDENAR LOS PAGOS A REALIZAR POR LA

*** CONTINUA ***



**P R E M I O
COLOMBIANO
A LA CALIDAD
DE LA GESTIÓN**

CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

FECHA: 2010/05/03

HORA: 10:49:35

OPERACION: 02CSI0503011

PAGINA: 10

ESTHER ISABEL VENTURA DE RENDON

C.C.38979831

SEGUNDO RENGLON

JUAN MARIA RENDON GUTIERREZ

C.C.17125100

TERCER RENGLON

JUAN PABLO REYES VILLAMIZAR

C.C.80419709

SUPLENTES

PRIMER RENGLON

ADRIANA RENDON DE ANTORCHA

C.C.66901857

SEGUNDO RENGLON

JUAN MANUEL RENDON VENTURA

C.C.79939921

TERCER RENGLON

CARLOS ANTONIO ESPINOSA PEREZ

C.C.16684716

CERTIFICA:

DOCUMENTO: ACTA No. 99 DEL 14 DE MARCH DE 2002

ORIGEN: ASAMBLEA GENERAL DE ACCIONISTAS

INSCRIPCION: 09 DE SEPTEMBER DE 2002 No. 14675 DEL LIBRO IX

FUE(RON) NOMBRADO(S):

REVISOR FISCAL

ALFREDO LOPEZ & CIA. LTDA

NIT.800026893-5

CERTIFICA:

DOCUMENTO: DOCUMENTO PRIVADO DEL 08 DE MAY DE 2008

ORIGEN: ALFREDO LOPEZ & CIA LTDA

INSCRIPCION: 23 DE MAY DE 2008 No. 5771 DEL LIBRO IX

FUE(RON) NOMBRADO(S):

REVISOR FISCAL PRINCIPAL

ALFREDO LOPEZ CAMPO

C.C.8241912

REVISOR FISCAL SUPLENTE

*** CONTINUA ***



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CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

FECHA: 2010/05/03

HORA: 10:49:35

OPERACION: 02CSI0503011

PAGINA: 11

ANDERSON DAVID RODRIGUEZ REYES
C.C.16916373

CERTIFICA:

CAPITAL AUTORIZADO: \$20,000,000,000
NUMERO DE ACCIONES: 2,000,000,000
VALOR NOMINAL: \$10
CAPITAL SUSCRITO: \$10,000,000,000
NUMERO DE ACCIONES: 1,000,000,000
VALOR NOMINAL: \$10
CAPITAL PAGADO: \$10,000,000,000
NUMERO DE ACCIONES: 1,000,000,000
VALOR NOMINAL: \$10

CERTIFICA:

DOCUMENTO: DOCUMENTO PRIVADO DEL 03 DE DICIEMBRE DE 2009
INSCRIPCIÓN: 28 DE DICIEMBRE DE 2009 BAJO EL NRO. 14869 DEL LIBRO IX
CONSTA LA SITUACIÓN DE CONTROL:
CONTROLANTE: DORAL INVESTMENTS INTERNATIONAL INC
DOMICILIO: ISLAS VIRGENES BRITÁNICAS
ACTIVIDAD: OTRAS ACTIVIDADES EMPRESARIALES
CONTROLADA: LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A.
NIT: 890301463-8
DOMICILIO: CALI VALLE
NACIONALIDAD: COLOMBIANA

ACTIVIDAD: LA FABRICACIÓN, DISTRIBUCIÓN Y VENTA EN EL TERRITORIO DE LA
REPÚBLICA DE COLOMBIA O EN EL EXTRANJERO DE CUALESQUIERA TIPOS DE VACUNAS,
PRODUCTOS QUÍMICOS, FARMACÉUTICOS, ALIMENTOS FUNCIONALES, COSMÉTICOS Y
FISIOLÓGICOS, REACTIVOS, ANTIBIÓTICOS PARA USO HUMANO Y VETERINARIO, PRODUCTOS
DIETETICOS, COLORANTES DE PERFUMERÍA Y AFINES.

PRESUPUESTO DE CONTROL: POSEE PARTICIPACIÓN ACCIONARIA MAYOR AL 50% A PARTIR
DEL 10 DE NOVIEMBRE DE 2009.

CERTIFICA:

DOCUMENTO: DOCUMENTO PRIVADO DEL 30 DE ABRIL DE 2004
INSCRIPCIÓN: 03 DE MAYO DE 2004 No. 4859 DEL LIBRO IX
DOCUMENTO: DOCUMENTO PRIVADO DEL 29 DE ABRIL DE 2005
INSCRIPCIÓN: 06 DE MAYO DE 2005 No. 4983 DEL LIBRO IX

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PREMIO
COLOMBIANO
AL CUIDADO
DE LA GESTIÓN

14700000-0000-0000-0000-000000000000

CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

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DOCUMENTO: DOCUMENTO PRIVADO DEL 15 DE JUNIO DE 2006
INSCRIPCIÓN: 21 DE JUNIO DE 2006 No. 7526 DEL LIBRO IX
DOCUMENTO: DOCUMENTO PRIVADO DEL 01 DE NOVIEMBRE DE 2006
INSCRIPCIÓN: 28 DE NOVIEMBRE DE 2006 No. 13380 DEL LIBRO IX
DOCUMENTO: DOCUMENTO PRIVADO DEL 19 DE DICIEMBRE DE 2006
INSCRIPCIÓN: 21 DE DICIEMBRE DE 2006 No. 14301 DEL LIBRO IX
DOCUMENTO: DOCUMENTO PRIVADO DEL 19 DE DICIEMBRE DE 2007
INSCRIPCIÓN: 21 DE DICIEMBRE DE 2007 No. 13604 DEL LIBRO IX
DOCUMENTO: DOCUMENTO PRIVADO DEL 19 DE OCTUBRE DE 2009
INSCRIPCIÓN: 22 DE OCTUBRE DE 2009 No. 12216 DEL LIBRO IX
DOCUMENTO: DOCUMENTO PRIVADO DEL 23 DE FEBRERO DE 2010
INSCRIPCIÓN: 25 DE FEBRERO DE 2010 No. 2203 DEL LIBRO IX
DOCUMENTO: DOCUMENTO PRIVADO DEL 27 DE ABRIL DE 2010
INSCRIPCIÓN: 28 DE ABRIL DE 2010 NRO. 4911 DEL LIBRO IX
CONSTA EL SIGUIENTE GRUPO EMPRESARIAL:

CONTROLANTE

LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A.

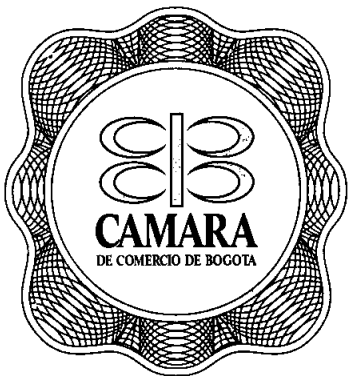
NIT. 890301463-8

DOMICILIO: CALI VALLE

NACIONALIDAD: COLOMBIA

ACTIVIDAD: A) LA FABRICACIÓN, DISTRIBUCIÓN Y VENTA EN EL TERRITORIO DE LA REPÚBLICA DE COLOMBIA O EN EL EXTRANJERO DE CUALESQUIERA TIPOS DE VACUNAS, PRODUCTOS QUÍMICOS, FARMACÉUTICOS, ALIMENTOS FUNCIONALES, COSMÉTICOS Y FISIOLÓGICOS, REACTIVOS, ANTIBIÓTICOS PARA USO HUMANO Y VETERINARIO, PRODUCTOS DIETÉTICOS, COLORANTES DE PERFUMERÍA Y AFINES. B) LA REPRESENTACIÓN DE CASAS FABRICANTES NACIONALES O EXTRANJERAS DE ESTOS MISMOS PRODUCTOS. C) FABRICACIÓN, IMPORTACIÓN, DISTRIBUCIÓN Y VENTA DE CUALESQUIERA TIPOS DE PRODUCTOS VETERINARIOS. D) LA REALIZACIÓN DE TODOS LOS ACTOS, CONTRATOS, CONVENIOS, DIRECTAMENTE RELACIONADOS CON LOS FINES ANTES INDICADOS. EN DESARROLLO DE SU OBJETO SOCIAL PUEDE LA SOCIEDAD ADQUIRIR Y VENDER BIENES RAÍCES Y MUEBLES, DARLOS Y TOMARLOS EN ARRENDAMIENTO, GARANTIZAR CON HIPOTECA SOBRE LOS BIENES RAÍCES O CON PRENDA SOBRE LOS BIENES MUEBLES, OBLIGACIONES A CARGO DE LA SOCIEDAD, DAR Y RECIBIR A MUTUO COMERCIAL DINERO Y CELEBRAR LOS CONTRATOS COMERCIALES DE CAMBIO EN TODAS SUS MANIFESTACIONES, FUNDAR EMPRESAS QUE TENGAN LA MISMA FINALIDAD SOCIAL O ASOCIARSE CON ELLAS.

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CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

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HORA: 10:49:35

OPERACION: 02CSI0503011

PAGINA: 13

SUBORDINADA

AMERICAN GENERICS S A S

NIT. 800223214-9

DOMICILIO: CALI VALLE

NACIONALIDAD: COLOMBIA

PRESUPUESTO DE CONTROL: NUMERAL 3, ARTÍCULO 27 DE LA LEY 222.

ACTIVIDAD: DISTRIBUCIÓN Y COMERCIALIZACIÓN DE PRODUCTOS FARMACÉUTICOS DE USO HUMANO.

SUBORDINADA

LAFRANCOL INTERNACIONAL S.A.

NIT. 815003912

DOMICILIO: PALMIRA VALLE

NACIONALIDAD: COLOMBIA

PRESUPUESTO DE CONTROL: LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A. POSEE EL 40% Y LABORATORIOS PAULY PHARMACEUTICAL S.A. POSEE EL 40% Y AMERICAN GENERICS S.A. POSEE EL 19.97%

ACTIVIDAD: REALIZACIÓN DE ACTIVIDADES DESDE LA ZONA FRANCA, LAS CUALES CONSISTEN EN LA PRESTACIÓN DE TODO TIPO DE SERVICIOS ASÍ COMO DISTRIBUIR, VENDER, COMPRAR, IMPORTAR, EXPORTAR Y ALMACENAR TODO TIPO DE MATERIAS PRIMAS, PRODUCTOS TERMINADOS Y SEMITERMINADOS RELACIONADOS CON LA INDUSTRIA DE ALIMENTOS, COSMÉTICA Y FARMACÉUTICA.

SUBORDINADA

LAFRANCOL PERÚ S.A

DOMICILIO: LIMA LIMA

NACIONALIDAD: PERU

PRESUPUESTO DE CONTROL: LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A. POSEE EL 93.0%

ACTIVIDAD: DISTRIBUCIÓN Y COMERCIALIZACIÓN DE PRODUCTOS FARMACÉUTICOS DE USO HUMANO.

SUBORDINADA

LABORATORIOS PAULY PHARMACEUTICAL S A S

NIT. 800097402-6

DOMICILIO: CALI VALLE

NACIONALIDAD: COLOMBIA

PRESUPUESTO DE CONTROL: NUMERAL 3, ARTÍCULO 27 DE LA LEY 222.

*** CONTINUA ***

RECIBIDO
BOGOTÁ
10/05/2010
10:50:00

IMPRESO POR: 888-001-1887 • TEL: 4 235 235 • 2010/05/03

CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

FECHA: 2010/05/03

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PAGINA: 14

ACTIVIDAD: DISTRIBUCIÓN Y COMERCIALIZACIÓN DE PRODUCTOS FARMACÉUTICOS DE USO HUMANO

SUBORDINADA

LAFRANCOL (USA) CORP

DOMICILIO:ESTADOS UNIDOS

NACIONALIDAD:ESTADOS UNIDOS

PRESUPUESTO DE CONTROL: LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A. POSEE EL 65.25%

ACTIVIDAD: DISTRIBUCIÓN Y COMERCIALIZACIÓN DE PRODUCTOS FARMACÉUTICOS DE USO HUMANO.

SUBORDINADA

GAMAR SOCIEDAD S A S

NIT. 890312050-7

DOMICILIO:CALI VALLE

NACIONALIDAD:COLOMBIA

PRESUPUESTO DE CONTROL:NUMERAL E, ARTICULO 27 DE LA LEY 222.

ACTIVIDAD:TODAS LAS ACTIVIDADES RELACIONADAS CON LAS ARTES GRÁFICAS

SUBORDINADA

LAFRANCOL GUATEMALA S.A.

NIT. 4088707

DOMICILIO:GUATEMALA

NACIONALIDAD:GUATEMALA

PRESUPUESTO DE CONTROL:NUMERAL 3, ARTÍCULO 27 DE LA LEY 222

ACTIVIDAD: PRODUCCIÓN, FABRICACIÓN, EXPORTACIÓN Y DISTRIBUCIÓN DE PRODUCTOS FARMACÉUTICOS Y/O ALIMENTICIOS Y NATURALES DE CONSUMO HUMANO Y LA COMERCIALIZACIÓN DE TODA CLASE DE MERCANCÍAS POR CUENTA PROPIA O EN NOMBRE DE TERCEROS.

SUBORDINADA

DISTRIBUCIONES UQUIFA S A S

NIT. 800182021-7

DOMICILIO:CALI VALLE

NACIONALIDAD:COLOMBIA

PRESUPUESTO DE CONTROL:NUMERAL 3, ARTÍCULO 27 DE LA LEY 222

ACTIVIDAD: DISTRIBUCIÓN Y COMERCIALIZACIÓN DE PRODUCTOS FARMACÉUTICOS Y ALIMENTOS FUNCIONALES DE CONSUMO HUMANO

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CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

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SUBORDINADA

LABORATORIOS NATURMEDIK S A S

NIT. 800226363-1

DOMICILIO: CALI VALLE

NACIONALIDAD: COLOMBIA

PRESUPUESTO DE CONTROL: NUMERAL 3, ARTÍCULO 27 DE LA LEY 222.

ACTIVIDAD: DISTRIBUCIÓN Y COMERCIALIZACIÓN DE PRODUCTOS FARMACÉUTICOS DE CONSUMO HUMANO.

SUBORDINADA

LAFRANCOL DOMINICANA S.A.

DOMICILIO: SANTO DOMINGO REPÚBLICA DOMINICANA

NACIONALIDAD: REPÚBLICA DOMINICANA

PRESUPUESTO DE CONTROL: NUMERAL 3; ARTÍCULO 27 DE LA LEY 222.

ACTIVIDAD: FABRICACIÓN DE PRODUCTOS FARMACÉUTICOS.

SUBORDINADA

LABORATORIO FRANCO-COLOMBIANO DEL ECUADOR S.A.

DOMICILIO: GUAYAQUIL, REPÚBLICA DEL ECUADOR

NACIONALIDAD: ECUATORIANA

PRESUPUESTO DE CONTROL: LA PARTICIPACIÓN QUE TIENE LAFRANCOL S.A. EN LABORATORIO FRANCO- COLOMBIANO DEL ECUADOR S.A., ES DE 96.8992%, LA FECHA DE LA PRIMERA TRANSACCIÓN COMERCIAL FUE EN JUNIO DE 2009 Y LA CAPITALIZACIÓN SE HIZO EN OCTUBRE DE 2008.

ACTIVIDAD: DEDICARSE A LA FABRICACIÓN, PRODUCCIÓN, EMPAQUE, COMERCIALIZACIÓN, DISTRIBUCIÓN, COMPRAVENTA, IMPORTACIÓN, EXPORTACIÓN Y/O REPRESENTACIÓN DE TODO TIPO DE PRODUCTOS FARMACÉUTICOS, HIGIÉNICOS, COSMÉTICOS, ALIMENTICIOS, DIETÉTICOS Y BAJOS EN CALORÍAS, VETERINARIOS, APARATOS E INSTRUMENTOS QUIRÚRGICOS, MÉDICOS, DENTALES Y VETERINARIOS. PODRÁ ADQUIRIR ACCIONES, PARTICIPACIONES O CUOTAS SOCIALES DE CUALQUIER CLASE DE COMPAÑÍA, SEA NACIONAL O EXTRANJERA, PUDIENDO INTERVENIR EN LA FUNDACIÓN O AUMENTO DE CAPITAL DE OTRAS SOCIEDADES QUE TENGAN OBJETOS SOCIALES RELACIONADOS CON EL SUYO. PODRÁ CONSTRUIR, ADQUIRIR EN PROPIEDAD, ARRENDAMIENTO O EN ASOCIACIÓN TODA CLASE DE PLANTAS INDUSTRIALES PARA PRODUCCIÓN, EMPAQUE, PROCESAMIENTO O CUALQUIER OTRA FORMA PARA LA COMERCIALIZACIÓN EN EL MERCADO INTERNO Y EXTERNO DE PRODUCTOS FARMACÉUTICOS YA SEA PARA EL CONSUMO HUMANO, VEGETAL O ANIMAL.

SUBORDINADA

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RECEIVED
COMMUNAL
ALCANTARA
DE LA FISCION

CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

FECHA: 2010/05/03

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OPERACION: 02CSI0503011

PAGINA: 16

FOCUS PHARMACEUTICAL S.A.

NIT: 900.104.555-8

DOMICILIO: BOGOTÁ D.C.

NACIONALIDAD: COLOMBIANA

PRESUPUESTO DE CONTROL: LA PARTICIPACIÓN QUE TIENE LAFRANCOL S.A. EN DICHA SOCIEDAD ES DE 39.9%.

ACTIVIDAD: EL OBJETO PRINCIPAL DE LA SOCIEDAD ES EL DESARROLLO, FABRICACIÓN O PRODUCCIÓN, DIRECTAMENTE O A TRAVÉS DE TERCEROS EN CUALQUIER MODALIDAD NEGOCIAL, LA COMERCIALIZACIÓN Y DISTRIBUCIÓN, NACIONAL O INTERNACIONAL, DE TODA CLASE DE ARTÍCULOS DE GENERO FARMACÉUTICO, ESPECÍFICAMENTE DE MEDICAMENTOS ESA ACTIVIDAD PRINCIPAL CONLLEVA LA HABILITACIÓN PARA DESARROLLAR TODA OTRA CONEXA O NECESARIA, INCLUYENDO DE MANERA ESPECIAL PERO NO LIMITATIVA, LA INVESTIGACIÓN FARMACÉUTICA, EL DESARROLLO DE PRODUCTOS MÉDICOS Y FARMACÉUTICOS, EQUIPOS MÉDICOS, TRATAMIENTOS ALTERNATIVOS Y EN GENERAL, TODO AQUELLO VINCULADO A LA PROTECCIÓN DE LA SALUD HUMANA.

PODRÁ EFECTUAR LA INVERSIÓN EN BIENES INMUEBLES, URBANOS O RURALES Y LA ADQUISICIÓN, ADMINISTRACIÓN, ARRENDAMIENTO, GRAVAMEN Y ENAJENACIÓN DE LOS MISMOS.

LA INVERSIÓN DE FONDOS PROPIOS, EN BIENES INMUEBLES, BONOS, VALORES BURSÁTILES Y PARTES DE INTERÉS EN SOCIEDADES COMERCIALES, ASÍ COMO LA NEGOCIACIÓN DE TODA CLASE DE DERECHOS DE CRÉDITO

CERTIFICA:

QUE A NOMBRE DE LA SOCIEDAD FIGURA MATRICULADO EN LA CAMARA DE COMERCIO BAJO EL NRO.5234- 2 ESTABLECIMIENTO DE COMERCIO: LABORATORIO FRANCO COLOMBIANO LAFRANCOL

UBICADO EN: ---K 1 46 84 DE CALI

RENOVO : POR EL AÑO 2010

CERTIFICA:

QUE A NOMBRE DE LA SOCIEDAD FIGURA MATRICULADO EN LA CAMARA DE COMERCIO BAJO EL NRO.369809- 2 ESTABLECIMIENTO DE COMERCIO: LABORATORIO FRANCO COLOMBIANO LAFRANCOL S A

UBICADO EN: --K 1 46A 70 DE CALI

FECHA MATRICULA : 29 DE ABRIL DE 1994

RENOVO : POR EL AÑO 2010

CERTIFICA:

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CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

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HORA: 10:49:35

OPERACION: 02CSI0503011

PAGINA: 17

QUE A NOMBRE DE LA SOCIEDAD FIGURA MATRICULADO EN LA CAMARA DE COMERCIO BAJO
EL NRO.369810- 2 ESTABLECIMIENTO DE COMERCIO: LABORATORIO FRANCO COLOMBIANO
LAFRANCOL S A

UBICADO EN: ---K 1A 46 A 32 DE CALI
FECHA MATRICULA : 29 DE APRIL DE 1994
RENOVO : POR EL AÑO 2010

CERTIFICA:

QUE A NOMBRE DE LA SOCIEDAD FIGURA MATRICULADO EN LA CAMARA DE COMERCIO BAJO
EL NRO.734908- 2 ESTABLECIMIENTO DE COMERCIO: LABORATORIO FRANCO COLOMBIANO
LAFRANCOL S.A

UBICADO EN: CRA. 1A NRO. 46A 28 DE CALI
FECHA MATRICULA : 28 DE MARCH DE 2008
RENOVO : POR EL AÑO 2010

CERTIFICA:

QUE A NOMBRE DE LA SOCIEDAD FIGURA MATRICULADO EN LA CAMARA DE COMERCIO BAJO
EL NRO.734911- 2 ESTABLECIMIENTO DE COMERCIO: LABORATORIO FRANCO COLOMBIANO
LAFRANCOL S.A

UBICADO EN: CRA. 1B NRO. 46A 121 DE CALI
FECHA MATRICULA : 28 DE MARCH DE 2008
RENOVO : POR EL AÑO 2010

CERTIFICA:

QUE A NOMBRE DE LA SOCIEDAD FIGURA MATRICULADO EN LA CAMARA DE COMERCIO BAJO
EL NRO.734913- 2 ESTABLECIMIENTO DE COMERCIO: LABORATORIO FRANCO COLOMBIANO
LAFRANCOL S.A

UBICADO EN: CRA. 3 NRO. 47 25 DE CALI
FECHA MATRICULA : 28 DE MARCH DE 2008
RENOVO : POR EL AÑO 2010

CERTIFICA:

QUE LA SOCIEDAD EFECTUO LA RENOVACION DE SU MATRICULA MERCANTIL EL 25 DE
MARCH DE 2010 .

CERTIFICA:

QUE NO FIGURAN OTRAS INSCRIPCIONES QUE MODIFIQUEN TOTAL O PARCIALMENTE EL
PRESENTE CERTIFICADO.

LOS ACTOS Y DOCUMENTOS REGISTRADOS QUEDAN EN FIRME CINCO (5) DIAS HABILES
DESPUES DE LA FECHA DE SU INSCRIPCION, SIEMPRE Y CUANDO DENTRO DE DICHO

*** CONTINUA ***

PREMIO
COLOMBIANO
ALA CALIDAD
DE LA GESTION

CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

FECHA: 2010/05/03

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TERMINO NO SEAN OBJETO DE RECURSOS EN LA VIA GUBERNATIVA.

DE CONFORMIDAD CON EL DECRETO 2150 DE 1.995 Y LA AUTORIZACION IMPARTIDA POR
LA SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO LA FIRMA MECANICA QUE APARECE A
CONTINUACION TIENE PLENA VALIDEZ PARA TODOS LOS EFECTOS LEGALES.

DADO EN CALI A LOS 03 DIAS DEL MES DE MAY DEL AÑO 2010 HORA: 10:49:52

[Firma manuscrita]



79

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 10 - 34,335
FECHA : MAYO 3 DE 2010

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 08-071244 - 00005-0000

Fecha: 2010-05-03 16:48:21 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 400 OPOSIOBSERVTER Folios: 79

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	Vr. PAGO
REYES Y REYES ABOGADOS	CONSIGNACION	BANCO DE BOGOTA	062754387	353266126	03/05/2010	295,000.00

***** CONCEPTO *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-04	PRESENTACION DE OBSERVACIONES ✓	
12		MARCAS Y LEMAS COMERCIALES	295,000.00 ✓
		TOTAL :	\$ 295,000.00

SON: DOSCIENTOS NOVENTA Y CINCO MIL PESOS

RESPONSABLE :

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____

2020
Bogotá, D.C.,

Doctor:
Emilio Ferrero
Apoderado

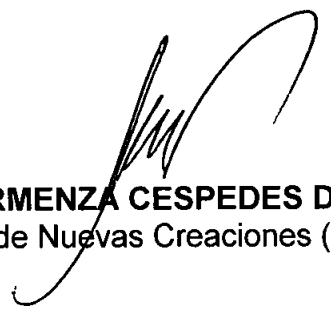
Oficio N°: 09204

ASUNTO: Radicación PCT N° 08-71244
Trámite 011
Evento 378
Actuación 440
Folios 068

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 el doctor José Luis Reyes Villamizar, en su calidad de apoderado de Lafrancol 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).

El anterior oficio fue notificado por fijación en lista de fecha, 23 JUL. 2010.

202021