

Bogotá D.C., junio de 2017

Señores
SUPERINTENDENTE DE INDUSTRIA Y COMERCIO
E.S.D.

Superintendencia de Industria y Comercio
Radicado: 14000419 Folios: 59
Dependencia: Patentes Fecha: 2017-06-13 15:37:20
Tipo aplicación: Documentos independientes

REF: Expediente actual: 14-000-419

TÍTULO SOLICITADO: FORMULACIONES FARMACÉUTICAS MUY
CONCENTRADAS QUE COMPRENDEN
ANTICUERPO ANTI-CD20

PUBLICACIÓN: GACETA 644 del 15 de mayo de 2012.

SOLICITANTE: F. HOFFMANN - LA ROCHE AG.

APODERADO: ALICIA LLOREDA RICAURTE.

TRÁMITE: REMISIÓN DE INFORMACIÓN – PRONUNCIAMIENTO
SOBRE EL NUEVO CAPITULO DE
REIVINDICACIONES CONTENIDO EN EL RECURSO
DE REPOSICIÓN PRESENTADO POR EL
SOLICITANTE

TITO NOÉ PARRA MURILLO, mayor de edad domiciliado y residenciado en la ciudad de Bogotá D.C., e identificado con cédula de ciudadanía No. 79.579.680 de Usaquén, abogado en ejercicio portador de la tarjeta profesional No. 71.168 del Consejo Superior de la Judicatura, obrando en calidad de representante legal de la sociedad **LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A.S.**, sociedad constituida de conformidad con las leyes colombianas e identificada con NIT 890.301.463-8, con domicilio principal en la ciudad de Cali, Valle del Cauca, de manera respetuosa y con el propósito de coadyuvar en la decisión que sobre la solicitud divisional **14-000-419** habrá de adoptarse próximamente, me permito presentar argumentos adicionales para demostrar que el nuevo capítulo reivindicatorio presentado por el solicitante persiste en incumplir los requisitos materiales de patentabilidad y debe por ello, ser negado al resolverse el trámite en cuestión.

Superintendencia de Industria y Comercio
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I. LEGÍTIMO INTERÉS

Asiste a **LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A.S.**, legítimo interés para presentar esta **REMISIÓN DE INFORMACIÓN**, pues la eventual concesión de la solicitud de patente comprendida en el Expediente 14-000-419 restringiría, en forma contraria al Régimen Común Andino de Propiedad Industrial, el empleo de ciertas sustancias y composiciones farmacéuticas, en detrimento de sus legítimos intereses comerciales y de la libre competencia, mencionando que mi representada ya se había pronunciado, mediante la presentación de OPOSICION a la solicitud de patente del expediente 14-000-419

II. COMPLEMENTO DE INFORMACIÓN

Debe señalarse que las modificaciones realizadas al capítulo reivindicatorio presentadas por el solicitante mediante recurso de Reposición y en subsidio Apelación, corresponden a un simple y básico cambio de forma en la cual esa medida no logra superar los argumentos planteados en un principio en la oposición presentada por el suscrito ni mucho menos los formulados por el examinador técnico y su despacho mediante resolución 11845 del 15 de marzo de 2017, en la cual se decidió **NEGAR la solicitud 14-000-419** de título "*COMPOSICIONES LIOFILIZADAS QUE COMPRENDEN ALTAS CONCENTRACIONES DE RITUXIMAB O OCRELIZUMAB (2H7) O OBINUTUZUMAB (GA101)*". Razón por la cual esta entidad considera debe ser tomado en cuenta el presente escrito para resolver, de manera negativa, el trámite que se encuentra en curso.

A) Incumplimiento de Requisitos de Patentabilidad.

la solicitud de patente correspondiente al Expediente No. **14-000-419** pretende proteger en Colombia, una formulación farmacéutica del anticuerpo anti-CD20 que comprende 120 mg/mL de **Rituximab**, **20 mM de L-histidina**, **210 mM de trehalosa dihidratada**, **10 mM de metionina**, **0.06% de polisorbato 80**, **2000 U/mL de rHuPH20** a pH 5.5. De igual manera una formulación farmacéutica del anticuerpo anti-CD20 que comprende 120 mg/mL de **Rituximab**, **20 mM de ácido acético**, **210**

mM de **trehalosa dihidratada**, 10 mM de metionina, 0.06% de **polisorbato 20**, 12.000 U/mL de **rHuPH20** a pH 5.5.

Además, menciona una formulación farmacéutica del anticuerpo anti-CD20 que comprende 120 mg/mL de **Rituximab**, **20 mM de L-histidina**, **120 mM de cloruro de sodio**, 10 mM de metionina, 0.02% de **polisorbato 80**, 12.000 U/mL de **rHuPH20** a pH 5.5.

También señala una formulación farmacéutica del anticuerpo anti-CD20 que comprende 120 mg/mL de **Rituximab**, 20 mM de ácido cítrico, **120 mM de cloruro de sodio**, **10 mM** de metionina, 0.02% de polisorbato 80, 12.000 U/mL de rHuPH20 a pH 6.5.

La solicitud reivindica una formulación farmacéutica del anticuerpo anti-CD20 que comprende 25 mg/mL de Obinutuzumab (GA101) (**huMAB<CD20>**), **20 mM de L-histidina**, 240 mM de **trehalosa dihidratada**, 0.02% de poloxámero 188, 2.000 U/mL de rHuPH20 a pH 6.0.

En consecuencia, es del caso reiterar que el objeto de la solicitud de patente del Expediente **No. 14-000-419** ha sido revelado previamente al 11 de agosto de 2009, fecha en la cual se señala la prioridad EP20090170110, en publicaciones disponibles en el estado de la técnica, las cuales se mencionan a continuación:

1. WO2009080541 del 02/07/2009.

"Antibody formulation".

Prioridad: EP20070150335 del **21/12/2007.**

Esta solicitud de patente, que es parte del estado de la técnica, pretende proteger una formulación que comprende: alrededor de **1 a cerca de 150 mg/mL de un anticuerpo tipo II anti-CD20**, opcionalmente **cerca de 0.001 a aproximadamente 1% p/v de al menos un tensoactivo**, y alrededor de **1 a cerca de 100 mM de un amortiguador**, a un **pH aproximadamente de 4,5 a cerca de 7.0.**

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Asimismo, señala que de acuerdo a la formulación anterior comprende: aproximadamente 10 a cerca de 30 mg/mL de un tipo II de anticuerpo anti-CD20, **20 mM L-histidina**, 240 mM trehalosa y **0,02% polisorbato 20** a un pH de alrededor de 6.

De la misma manera, menciona que dicha formulación además comprende uno o más **agentes isotonzantes** en una cantidad de aproximadamente **5 mM a alrededor de 350 mM** y donde los agentes isotonzantes son seleccionados del grupo que consiste en **cloruro de sodio (NaCl)**, cloruro de potasio, azúcares que comprende glucosa, glicerina, **aminoácidos** y combinaciones de estos.

También reivindica una formulación como la ya mencionada donde esta comprende: **25mg/mL HuMab<CD20>**, 0.02% polisorbato 20 p/v, **20 mM L-histidina**, y **240 mM trehalosa** a pH 6.0. Así como una formulación de **25mg/mL HuMab<CD20>**, 0.02% Polisorbato 80 p/v, **20 mM L-histidina**, y **240mM trehalosa**, a pH 6.0

2. US6991790 del 31/01/2006.

"Antibody formulation"

Prioridad: US19970053087P del **13/06/1997.**

Esta solicitud de patente, que es parte del estado de la técnica, pretende proteger un método para tratar un linfoma de células B en un mamífero, que comprende la administración de **una formulación farmacéutica** acuosa y estable que consiste en una cantidad terapéuticamente efectiva de un **anticuerpo que se une a CD20**, el anticuerpo no es sometido a liofilización previa, **un amortiguador acetato de aproximadamente un pH 4.8 a cerca de 5.5**, **un tensoactivo** y **un poliol**, donde la formulación carece de una cantidad de tonificante de cloruro de sodio.

También en la memoria descriptiva señala una formulación líquida multidosis del candidato **C2B8**, donde **el rhuMab CD20** fue formulado a 40 mg/mL en **acetato 25 mM**, **trehalosa 150 mM**, alcohol bencílico 0,9% y **polisorbato 20 al 0,02%** a pH 5,0.

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Adicional reivindica que en el método inicialmente mencionado el **poliol** es un azúcar no reductor y especifica que el **azúcar no reductor es trehalosa**. De igual manera en la memoria descriptiva destaca que la presencia **de trehalosa es beneficiosa para minimizar la agregación inducida por congelación y no es perjudicial para la estabilidad en el estado líquido**. La formulación líquida multidosis candidata antes descrita contiene preferiblemente al menos una proporción molar de trehalosa de 500: 1 respecto a C2B8.

3. WO2005044859 del 19/05/2005.

"Antigen binding molecules with increased Fc receptor binding affinity and effector function".

Prioridad: US20030517096P del **05/11/2003**.

Esta solicitud de patente, que es parte del estado de la técnica, pretende proteger un **polinucleótido** que comprende: (a) una secuencia selección del grupo que consiste de: SEQ ID NO.: 5, SEQ ID NO.: 6 y (b) una secuencia seleccionada a partir del grupo compuesto de: SEQ ID NO: 21, SEQ ID NO: 22 y SEQ ID NO: 23; y (c) secuencia ID NO: 24.

Asimismo, indica un **polinucleotido aislado** que comprende a) una secuencia que codifica un **polipéptido que tiene la región VH del anticuerpo B-Lyl murina**, o variantes del mismo; y b) una secuencia que codifica un polipéptido que tiene la secuencia de la región Fc del anticuerpo, o un fragmento del mismo, de una especie distinta del ratón. Además, **reivindica un polipéptido que comprende una secuencia derivada del anticuerpo B-Lyl murina y una secuencia derivada de un polipéptido heterólogo**. Una **molécula de unión a antígeno que comprende el polipéptido heterólogo, que se une a CD20 humano y que es un anticuerpo quimérico y más específicamente un anticuerpo humanizado**. También menciona que **la molécula de unión al antígeno es un anticuerpo, primatizado, humanizado**.

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En la memoria descriptiva señala que **el anticuerpo rituximab (RITUXANO)** es un dominio **quimérico** murino constante gamma 1 humano genéticamente modificado que **contiene anticuerpo monoclonal dirigido contra el antígeno D20 humano**.

También en la memoria descriptiva mencionan una **formulación líquida multidosis** que comprende 40 mg/mL **rituximab**, 25 mM **acetato**, 150 mM **trehalosa**, 0.9% alcohol bencílico, **0.02% polisorbato 20** a pH 5.0 que tiene una vida media mínima de dos años de almacenamiento a 2-8°C. Otra **formulación anti-CD20** de interés comprende 10 mg/mL **rituximab** en 9.0 mg/mL **cloruro de sodio**, 7.35 mg/mL citrato de sodio dihidratado, 0.7 mg/mL **polisorbato 80**, y agua estéril para inyección pH 6.5.

4. WO2006091871 del 31/08/2006.

"Soluble glycosaminoglycanases and methods of preparing and using soluble glycosaminoglycanases".

Prioridad: US20050065716 del 23/02/2005.

Esta solicitud de patente, que es parte del estado de la técnica, pretende proteger una **glicoproteína sustancialmente purificada**, que comprende un **polipéptido activo neutro y soluble de hialuronidasa** y al menos una porción de azúcar N-unido, donde la porción de azúcar N-unido está unido covalentemente a un residuo de asparagina del polipéptido. Donde el polipéptido consiste esencialmente del dominio hialuronidasa de **la glicoproteína hialuronidasa humana (sHASEGP)** o una porción catalíticamente activa de esta, y específicamente la porción hialuronidasa del polipéptido comprende el dominio hialuronidasa de sHASEGP o una porción catalíticamente activa de la misma.

De igual forma, menciona un kit que comprende: (a) un polipéptido **sHASEGP** a una dosis de entre 0,1 y 1500 unidades/mL en un vehículo aceptable; (b) al menos **una sustancia terapéutica u otra molécula o complejo macromolecular** (cada uno un agente) en un vehículo aceptable; y (c) opcionalmente, instrucciones para entregar sustancias terapéuticas u otros agentes.

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En la memoria descriptiva indican que en la presente invención se incluyen enzimas solubles de glicosaminoglicanasas, particularmente miembros de la **familia de glicoproteínas de hialuronidasa neutras solubles** (también denominadas aquí **sHASEGPs**), siendo los miembros **preferidos las glicoproteínas de hialuronidasa neutras humanas** solubles en humanos, particularmente las **glicoproteínas de hialuronidasa de PH-20 solubles en humanos (también denominadas rHuPH20)** y también menciona que el uso de rHuPH20 como un ejemplo de glicosaminoglucano (por ejemplo, **promueve la entrega de agentes farmacológicos y otros dentro de los tejidos del cuerpo de mamíferos**). De igual manera, reivindican una hialuronidasa PEGilada, donde la hialuronidasa comprende al menos tres porciones PEG por polipéptido, y en el que la hialuronidasa es una sHASEGP PEGilada y más específicamente es una rHuPH20 PEGilada.

Además, la memoria descriptiva indica que **sHASEGPs** son también **útiles para potenciar la entrega de biológicos como anticuerpos monoclonales, citoquinas, y otros fármacos a tumores que acumulan glicosaminoglucanos**. También señala a modo de ilustración que **hay biológicos que pueden ser mejoradas por combinación con uno o más sHASEGPs** (por ejemplo, por co-formulación o coadministración de sHASEGP antes, y coincidiendo después con la administración de un biológico), Incluyendo, por ejemplo: Abciximabs (por ejemplo, REOPRO™), Adalimumab (por ejemplo, HUMIRA™), Agalsidase betas (por ejemplo, FABRAZYME™), **Rituximab** (por ejemplo, RITUXAN™), entre otros.

5. WO2007005608 del 11/01/2007.

"IL-12/P40 Binding proteins".

Prioridad: US20050695679P del **30/06/2005.**

Esta solicitud de patente, que es parte del estado de la técnica, pretende proteger **una proteína de unión** que comprende **un antígeno con dominio de unión**, dicha proteína capaz de unirse a la subunidad p40 de IL-12, y cuyo antígeno con dominio de unión comprende al menos un CDR que abarca una secuencia de aminoácidos

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seleccionada del grupo que consiste de:
CDR-H1. X1-X2-X3-X4-X5-X6-X7 (SEQ ID NO: 55).

Asimismo, señala **un anticuerpo construido que comprende una proteína de unión** como la ya mencionada, y en donde dicho anticuerpo comprende además un polipéptido de unión o una inmunoglobulina de dominio constante. Además, especifica que dicha proteína de unión es seleccionada del grupo que consiste de: una molécula de inmunoglobulina, un Fv unido a un disulfuro, **un anticuerpo monoclonal**, un scFv, un anticuerpo quimérico, un anticuerpo con un solo dominio, un anticuerpo CDR-grafted, un daicuerpo, un anticuerpo humanizado, un Fab, un Fab', un F(ab')₂, un Fv, un anticuerpo multiespecífico, un anticuerpo dual específico y un anticuerpo biespecífico.

Adicional en la memoria descriptiva indica que **los anticuerpos de la invención**, o las **porciones de unión a antígeno** de estos, puede también ser combinados con agentes, como metotrexato, 6-MP, azatioprina, penicilina, AINEs, por ejemplo, ibuprofeno, corticosteroides como prednisolona, inhibidores de la fosfodiesterasa, agonistas de adenosina, **rituximab**, entre otros.

La solicitud también reivindica una **composición para la liberación de una proteína de unión** dicha composición comprende: (a) una formulación, donde dicha **formulación comprende una proteína de unión** cristalizada, conforme a cualquiera de las reivindicaciones 72-83, y **un ingrediente**; y (b) al menos un vehículo polimérico. Además, indica que en dicha composición **el ingrediente** es seleccionado del grupo de albumina, sacarosa, **trehalosa**, lactitol, gelatina, hidroxipropil-[beta]-ciclodextrina, metoxipolietilenglicol y polietilenglicol.

De la misma manera, reivindica una composición farmacéutica que comprende a **la proteína de unión** inicialmente mencionada y **un vehículo farmacéuticamente aceptable**. Donde más específicamente, **el vehículo farmacéuticamente aceptable** tiene funciones como adecuado **adyuvante** para incrementar la absorción, o dispersión de dicha proteína de unión y luego especifica que dicho **adyudante** es **hialuronidasa**.

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6. **BOOKBINDER L.H. et al.** "A recombinant human enzyme for enhanced interstitial transport of therapeutics", Journal of Controlled Release, 2006, vol. 114, p 230-241.

Esta publicación, que es parte del estado de la técnica, plantea la posibilidad de utilizar la **enzima hialuronidasa humana recombinante purificada (rHuPH20)** como un agente potenciador de liberación intersticial **capaz de incrementar la dispersión y biodisponibilidad de fármacos co-inyectados**. Dentro de los fármacos mencionados, se encuentran **anticuerpos monoclonales** como adalimumab e infliximab. Para el infliximab, el anticuerpo es administrado por vía intravenosa. Comparando el área bajo la curva del infliximab liberado intravascularmente y localmente muestra que los perfiles farmacocinéticos y la biodisponibilidad absoluta fueron dramáticamente mejorados cuando se co-administraba con rHuPH20. El Infliximab co-inyectada con rHuPH20 exhibió un perfil farmacocinético que fue muy similar a dosis intravenosas.

7. **US2007071675 del 29/03/2007.**

"Dual variable domain immunoglobulin and uses thereof".

Prioridad: US20050709911P del **19/08/2005.**

Esta solicitud de patente, que es parte del estado de la técnica, pretende proteger una proteína de unión que comprende **una cadena polipeptídica**, en la que dicha cadena polipeptídica comprende VD1- (X1) n-VD2-C- (X2) n, donde VD1 es un primer dominio variable, VD2 es un segundo dominio variable, C es un dominio constante, X1 representa un aminoácido o polipéptido, X2 representa una región Fc y n es 0 o 1. Además, en donde dicha proteína de unión es capaz de **unir uno o más blancos** y específicamente los blancos pueden ser seleccionados del grupo que consiste de ABCF1; ACVR1; ACVR1B; ACVR2; ACVR2B; ACVRL1; ADORA2A; AGR2; AICDA; AIF1; AIG1; AKAP1; AKAP2; AMH; AMHR2; ANGPT1; ANGPT2; ANGPTL3; ANGPTL4; ANPEP; APC; APOC1; AR; B7.1; B7.2; BAD; BAFF; BAG1; BAI1; BCL2; BCL6; BDNF; BLNK; BLR1 (MDR15); BlyS; BMP1; BMP2; BMP3B (GDF10); BMP4; BMP6; BMP8; BMPR1A; BMPR1B; BMPR2; ;

CD19; CD1C; **CD20**; CD200; CD-22; CD24; CD28; CD3; CD37; CD38; CD3E; CD3G; CD3Z; CD4; CD40; CD40L; CD44, entre otros.

De igual forma dentro de la memoria descriptiva señalan que **las proteínas de unión pueden también ser combinadas** con agentes como metotrexato, 6-MP, azatriopina, sulfasalazina, agentes adrenérgicos, **rituximab**, IL-1 TRAP, MRA, CTLA4-IG, IL-18 BP, anti-IL-18, Anti-IL15, B1RB-796, SCIO-469, VX-702, AMG-548, VX-740, entre otros.

B) Existencia de Resolución. Mediante resolución 11845 del 15 de marzo de 2017 esta entidad decidió **NEGAR** la solicitud 14-000-419 de título "*COMPOSICIONES LIOFILIZADAS QUE COMPRENDEN ALTAS CONCENTRACIONES DE RITUXIMAB O OCRELIZUMAB (2H7) O OBINUTUZUMAB (GA101)*". Razón por la cual, el solicitante al realizar e introducir cambios en el capítulo reivindicatorio mediante un recurso de reposición y en subsidio apelación, en realidad no está modificando la patente, ya que para esta ser concedida, debe realizar una modificación sustancial a la misma y en consecuencia comenzar un nuevo trámite de solicitud de patente, dado que los cambios realizados corresponden a cambios de forma simples y básicos, los cuales no logran superar los argumentos planteados en un principio en la oposición presentada por el suscrito ni mucho menos los formulados por el examinador técnico y su despacho mediante resolución 11845 del 15 de marzo de 2017, que NEGÓ la solicitud.

C) Conclusiones

Es del caso reiterar, como se demostró anteriormente, que el objeto de **la solicitud de patente con Expediente N° 14000419**, ha sido previamente revelada en diferentes publicaciones que se encuentran disponibles en el estado de la técnica y por lo tanto la invención que se pretende proteger **carece de nivel inventivo**, considerando que:

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- i. Las formulaciones farmacéuticas muy concentradas que comprenden el anticuerpo anti-CD20 pueden **ser desprendidas y deducidas, por un Experto Técnico en la materia**, del estado del arte previo.
 - ii. El objeto de la invención que se intenta proteger mediante la solicitud de patente con expediente No. 14-000-419 y los elementos que la componen, **son conocidos y forman parte del estado del arte previo.**

Por consiguiente, podemos afirmar que esta solicitud de patente junto con sus recursos de Reposición y en subsidio Apelación, **deben ser NEGADOS**, debido a que la invención que se intenta proteger **CARECE DE NIVEL INVENTIVO**, el cual, como es del conocimiento de su despacho, es un elemento *SINE QUA NON* para gozar los beneficios que otorga un privilegio de protección de Patentes de Invención en Colombia.

III. PRUEBAS

Ruego señores Superintendencia de Industria y Comercio se tengan como pruebas de este escrito, todos los documentos mencionados en el mismo y que relaciono a continuación, en el mismo orden en que fueron presentados en el acápite anterior:

1. WO2009080541 del 02/07/2009, "Antibody Formulation", Publicación internacional publicada bajo el Tratado de Cooperación de Patentes (PCT), páginas 1, 43 – 45.
 2. US6991790 del 31/01/2006 "Antibody Formulation" páginas 1, 51, 53 y 59.
 3. WO2005044859 del 19/05/2005, "Antigen binding molecules with increased Fc receptor binding affinity and effector function", Publicación internacional publicada bajo el Tratado de Cooperación de Patentes (PCT) páginas 1, 7, 79, 95, 97, 98.
 4. WO2006091871 del 31/08/2006, "Soluble glycosaminoglycanases and methods of preparing and using soluble glycosaminoglycanases" Publicación internacional publicada bajo el Tratado de Cooperación de Patentes (PCT). páginas 1, 6, 114, 163, 236, 241, 242, 247 y 260.
 5. WO2007005608 del 11/01/2007. "IL-12/P40 Binding proteins", Publicación internacional publicada bajo el Tratado de Cooperación de Patentes (PCT). páginas 1, 75, 76, 123, 139, 143 y 144.
 6. BOOKBINDER L.H. et al. "A recombinant human enzyme for enhanced interstitial of therapeutics", Journal of Controlled Release, 2006, Vol. 114, páginas 230-241
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7. US2007071675 del 29/03/2007 "Dual variable domain immunoglobulin and uses thereof" páginas 1, 37, 121 y 122.

Todos los anteriores son aportados por el suscrito y obrante en el expediente.

IV. 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.S.
2. Copia para correr traslado al solicitante.
3. Reivindicaciones realizadas por el Solicitante.
4. Caratula de la Solicitud internacional WO2011/029892 del 17 de marzo de 2011
5. Los documentos mencionados en el acápite de Pruebas.

V. NOTIFICACIONES Y/O COMUNICACIONES

Mi representada y el suscrito recibiremos cualquier notificación y/o comunicación en la Secretaría de su Despacho, o en la sede de LAFRANCOL S.A.S, ubicada en la Calle 108 No. 51-45 de la ciudad de Bogotá D.C.

Del Señor Superintendente,



TITO NOÉ PARRA MURILLO
C.C. 79.579.680 de Usaquén
T.P. 71.168 del C.S.J.

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REIVINDICACIONES

1. Una formulación farmacéutica del anticuerpo anti-CD20 que comprende 120 mg/mL de Rituximab, 20 mM de L-histidina, 210 mM de trehalosa dihidratada, 10 mM de metionina, 0.06% de polisorbato 80, 2000 U/mL de rHuPH20 a pH 5.5.
2. Una formulación farmacéutica del anticuerpo anti-CD20 que comprende 120 mg/mL de Rituximab, 20 mM de L-histidina, 210 mM de trehalosa dihidratada, 10 mM de metionina, 0.06% de polisorbato 80, 2000 U/mL de rHuPH20 a pH 6.1.
3. Una formulación farmacéutica estable, del anticuerpo anti-CD20 que comprende 120 mg/mL de Rituximab, 20 mM de L-histidina, 210 mM de trehalosa dihidratada, 10 mM de metionina, 0.06% de polisorbato 80, 12.000 U/mL de rHuPH20 a pH 5.5.
4. Una formulación farmacéutica del anticuerpo anti-CD20 que comprende 120 mg/mL de Rituximab, 20 mM de ácido acético, 210 mM de trehalosa dihidratada, 10 mM de metionina, 0.06% de polisorbato 20, 12.000 U/mL de rHuPH20 a pH 5.5.
5. Una formulación farmacéutica del anticuerpo anti-CD20 que comprende 120 mg/mL de Rituximab, 20 mM de L-histidina, 210 mM de trehalosa dihidratada, 10 mM de metionina, 0.06% de polisorbato 20, 12.000 U/mL de rHuPH20 a pH 5.5.
6. Una formulación farmacéutica del anticuerpo anti-CD20 que comprende 120 mg/mL de Rituximab, 20 mM de L-histidina, 120 mM de cloruro de sodio, 10 mM de metionina, 0.02% de polisorbato 80, 12.000 U/mL de rHuPH20 a pH 5.5.
7. Una formulación farmacéutica del anticuerpo anti-CD20 que comprende 120 mg/mL de Rituximab, 20 mM de ácido cítrico, 120 mM de cloruro de sodio, 10 mM de metionina, 0.02% de polisorbato 80, 12.000 U/mL de rHuPH20 a pH 6.5.

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8. Una formulación farmacéutica del anticuerpo anti-CD20 que comprende 120 mg/mL de Rituximab, 20 mM de ácido cítrico, 210 mM de trehalosa dihidratada, 10 mM de metionina, 0.06% de polisorbato 80, 12.000 U/mL de rHuPH20 a pH 6.5.
9. Una formulación farmacéutica del anticuerpo anti-CD20 que comprende 120 mg/mL de Rituximab, 20 mM de L-histidina, 120 mM de cloruro de sodio, 10 mM de metionina, 0.04% de polisorbato 80, 12.000 U/mL de rHuPH20 a pH 6.0.
10. Una formulación farmacéutica del anticuerpo anti-CD20 que comprende 25 mg/mL de Obinutuzumab (GA101) (huMAB<CD20>), 20 mM de L-histidina, 240 mM de trehalosa dihidratada, 0.02% de poloxámero 188, 2.000 U/mL de rHuPH20 a pH 6.0.
11. Una formulación farmacéutica del anticuerpo anti-CD20 que comprende 30 mg/mL de Ocrelizumab (2H7) humanizado, 30 mM de acetato de sodio, 8% de trehalosa dihidratada, 0.02% de polisorbato 20, 0 U/mL de rHuPH20 a pH 5.3.
12. Una formulación farmacéutica del anticuerpo anti-CD20 que comprende 30 mg/mL de Ocrelizumab (2H7) humanizado, 30 mM de acetato de sodio, 8% de trehalosa dihidratada, 0.02% de polisorbato 20, 1.500 U/mL de rHuPH20 a pH 5.3.
13. Una formulación farmacéutica del anticuerpo anti-CD20 que comprende 30 mg/mL de Ocrelizumab (2H7) humanizado, 30 mM de acetato de sodio, 8% de trehalosa dihidratada, 0.02% de polisorbato 20, 12.000 U/mL de rHuPH20 a pH 5.3.
14. Una formulación farmacéutica del anticuerpo anti-CD20 que comprende 50 mg/mL de Ocrelizumab (2H7) humanizado, 30 mM de acetato de sodio, 8% de trehalosa dihidratada, 0.02% de polisorbato 20, 0 U/mL de rHuPH20 a pH 5.3.
15. Una formulación farmacéutica del anticuerpo anti-CD20 que comprende 50 mg/mL de Ocrelizumab (2H7) humanizado, 30 mM de acetato de sodio, 8% de trehalosa dihidratada, 0.02% de polisorbato 20, 1.500 U/mL de rHuPH20 a pH 5.3.

16. Una formulación farmacéutica del anticuerpo anti-CD20 que comprende 50 mg/mL de Ocrelizumab (2H7) humanizado, 30 mM de acetato de sodio, 8% de trehalosa dihidratada, 0.02% de polisorbato 20, 12.000 U/mL de rHuPH20 a pH 5.3.
17. Una formulación farmacéutica del anticuerpo anti-CD20 que comprende 100 mg/mL de Ocrelizumab (2H7) humanizado, 30 mM de acetato de sodio, 8% de trehalosa dihidratada, 0.02% de polisorbato 20, 0 U/mL de rHuPH20 a pH 5.3.
18. Una formulación farmacéutica del anticuerpo anti-CD20 que comprende 100 mg/mL Ocrelizumab (2H7) humanizado, 30 mM de acetato de sodio, 8% de trehalosa dihidratada, 0.02% de polisorbato 20, 1.500 U/mL de rHuPH20 a pH 5.3.
19. Una formulación farmacéutica del anticuerpo anti-CD20 que comprende 100 mg/mL de Ocrelizumab (2H7) humanizado, 30 mM de acetato de sodio, 8% de trehalosa dihidratada, 0.02% de polisorbato 20, 12.000 U/mL de rHuPH20 a pH 5.3.

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

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
17 March 2011 (17.03.2011)

PCT

(10) International Publication Number
WO 2011/029892 A2

(51) International Patent Classification: Not classified

(21) International Application Number:
PCT/EP2010/063271

(22) International Filing Date:
10 September 2010 (10.09.2010)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09170110.2 11 September 2009 (11.09.2009) EP

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

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

Published:

— without international search report and to be republished upon receipt of that report (Rule 48.2(g))



WO 2011/029892 A2

(54) Title: HIGHLY CONCENTRATED PHARMACEUTICAL FORMULATIONS

(57) Abstract: The present invention relates to a highly concentrated, stable pharmaceutical formulation of a pharmaceutically active anti-CD20 antibody, such as e.g. Rituximab, Ocrelizumab or HuMab<CD20>, or a mixture of such antibody molecules for subcutaneous injection. In particular, the present invention relates to formulations comprising, in addition to a suitable amount of the anti-CD20 antibody, an effective amount of at least one hyaluronidase enzyme as a combined formulation or for use in form of a co-formulation. The said formulations comprise additionally at least one buffering agent, such as e.g. a histidine buffer, a stabilizer or a mixture of two or more stabilizers (e.g. a saccharide, such as e.g. α , α -trehalose dihydrate or sucrose, and optionally methionine as a second stabilizer), a nonionic surfactant and an effective amount of at least one hyaluronidase enzyme. Methods for preparing such formulations and their uses thereof are also provided.

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
2 July 2009 (02.07.2009)

PCT

(10) International Publication Number
WO 2009/080541 A1

(51) International Patent Classification:
A61K 9/08 (2006.01) A61K 47/18 (2006.01)

(21) International Application Number:
PCT/EP2008/067293

(22) International Filing Date:
11 December 2008 (11.12.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
07150335.3 21 December 2007 (21.12.2007) EP

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

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

Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments



WO 2009/080541 A1

(54) Title: ANTIBODY FORMULATION

(57) Abstract: The present invention relates to an anti-CD20 human monoclonal antibody formulation, a process for the preparation and uses thereof.

8. The formulation according to any one of claims 1 to 7, wherein the isotonicity agents are selected from the group consisting of sodium chloride (NaCl), potassium chloride, sugars comprising glucose, glycerin, amino acids, and combinations thereof.
9. The formulation according to any one of claims 1 to 8, which further comprises a sugar in
5 an amount of about 25 mM to about 500 mM.
10. The formulation according to any one of claims 1 to 9, wherein the sugars are selected from the group consisting trehalose, saccharose, lactose, glucose, mannose, maltose, galactose, fructose, sorbose, raffinose, glucosamine, N-Methylglucosamine ("Meglumine"), galactosamine, neuraminic acid.
- 10 11. The formulation according to any one of claims 1 to 10, which further comprises one or more ingredients selected from the group consisting of pharmaceutically acceptable: antioxidants, ascorbic acid, Glutathion, preservatives, in particular, m-cresol, phenol, benzylalcohol, methylparaben, propylparaben, chlorbutanol, thimersal, benzalkoniumchloride, polyethylenglycole, in particular PEG 3000, 3350, 4000 or 6000,
15 albumine, Humanes Serum Albumin (HSA), bovines serum albumin (BSA), polyhydric alcohol, glycerol, ethanol, mannitol, salts, acetate salts in particular sodium acetate, magnesiumchloride, calciumchloride, tromethamine, EDTA, in particular Na-EDTA.
12. The formulation according to any one of claims 1 to 11, wherein it comprises:
15mg/mL HuMab<CD20>,
20 20 mM L-histidine,
optionally 0.001 to 1% w/v of a surfactant,
at pH 6.0;
- or
- 10mg/mL HuMab<CD20>,
25 0.02% polysorbate 20 w/v,
20 mM L-histidine, and
240 mM trehalose,
at pH 6.0;
- or
- 25mg/mL HuMab<CD20>,
0.02% polysorbate 20 w/v,
20 mM L-histidine, and
240 mM trehalose,
35 at pH 6.0.

13. The formulation according to any one of claims 1 to 12, wherein it is in a lyophilized form and comprises:
- 10mg/mL HuMab<CD20>,
0.02% polysorbate 20 w/v,
5 20 mM L-histidine, and
240 mM trehalose,
at pH 6.0.
14. The formulation according to any one of claims 1 to 11, wherein it comprises:
- 10 25mg/mL HuMab<CD20>,
0.02% Poloxamer188TM w/v,
20 mM L-histidine, and
240mM trehalose,
at pH 6.0;
- 15 or
- 25mg/mL HuMab<CD20>,
0.01% Poloxamer188TM w/v,
20 mM L-histidine, and
240mM trehalose,
20 at pH 6.0;
- or
- 25mg/mL HuMab<CD20>,
25 0.1% Poloxamer188TM w/v,
20 mM L-histidine, and
240mM trehalose,
at pH 6.0;
- 30 or
- 25mg/mL HuMab<CD20>,
0.02% Polysorbate 80 w/v,
35 20 mM L-histidine, and

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240mM trehalose,
at pH 6.0;

or

5 25mg/mL HuMab<CD20>,
0.1% Polysorbate 80 w/v,
20 mM Acetate, and
240mM trehalose,
at pH 5.5;

or

10 25mg/mL HuMab<CD20>,
0.1% Polysorbate 80 w/v,
20 mM Acetate, and
140mM Sodium chloride,
at pH 5.5;

15 or

30mg/mL HuMab<CD20>,
0.01% Poloxamer188TM w/v,
20 mM L-histidine, and
20 200mM trehalose,
at pH 6.5;

15. The formulation according to claim 14, wherein it is in a liquid form and comprises:

25mg/mL HuMab<CD20>,
0.02% Poloxamer188TM w/v,
25 20 mM L-histidine, and
240mM trehalose,
at pH 6.0;

16. Use of a formulation according to any one of claims 1 to 15 for the preparation of a medicament useful for CD20-related treating diseases.

30 17. The use according to claim 16, wherein the disease is selected from the group consisting of breast cancer, colorectal cancer, non-small cell lung cancer (NSCLC) and prostate cancer.



US006991790B1

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(12) **United States Patent**
Lam et al.(10) **Patent No.:** **US 6,991,790 B1**
(45) **Date of Patent:** **Jan. 31, 2006**(54) **ANTIBODY FORMULATION**(75) **Inventors:** **Xanthe M. Lam**, San Francisco, CA (US); **James Q. Oeswein**, Moss Beach, CA (US); **Boonsri Ongpipattanakul**, Bangkok (TH); **Zehra Shahrokh**, San Francisco, CA (US); **Sharon X. Wang**, San Mateo, CA (US); **Robert P. Weissburg**, Greenville, DE (US); **Rita L. Wong**, San Mateo, CA (US)(73) **Assignee:** **Genentech, Inc.**, South San Francisco, CA (US)(*) **Notice:** Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 782 days.(21) **Appl. No.:** **09/724,868**(22) **Filed:** **Nov. 28, 2000****Related U.S. Application Data**

(63) Continuation of application No. 09/097,171, filed on Jun. 12, 1998, now Pat. No. 6,171,586.

(60) Provisional application No. 60/053,087, filed on Jun. 13, 1997.

(51) **Int. Cl.****A61K 39/395** (2006.01)**C07K 16/00** (2006.01)**C07K 16/28** (2006.01)(52) **U.S. Cl.** **424/130.1; 424/141.1; 424/152.1; 424/154.1; 424/173.1; 530/388.73**(58) **Field of Classification Search** **424/130.1, 424/152.1, 141.1, 154.1, 173.1; 530/388.73**
See application file for complete search history.(56) **References Cited****U.S. PATENT DOCUMENTS**

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Primary Examiner—Christina Chan**Assistant Examiner**—Marianne DiBrino(74) **Attorney, Agent, or Firm**—Lee K. Tan(57) **ABSTRACT**

A stable aqueous pharmaceutical formulation comprising a therapeutically effective amount of antibody not subjected to prior lyophilization, a buffer maintaining the pH in the range from about 4.5 to about 6.0, a surfactant and a polyol is described, along with uses for such a formulation.

20 Claims, 29 Drawing Sheets

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earlier eluting peaks on HIC and RP-HPLC, were all minimized at pH 5 (compared to pH 6), and in trehalose (compared to salt or mannitol). The smaller MW species may have been formed either by proteolytic cleavage and/or by disulfide scrambling, both of which may be enhanced at higher pH's. Reduction of the control and degraded samples led to only the light and heavy chains, consistent with either the cleavage to Fab, or mixed disulfide formation between heavy and light chains.

RhuMAb CD18 appeared to be least stable in salt formulations; at pH 5 a larger aggregate (equivalent to a trimer) appeared and at pH 6 precipitation was noted at 40° C. Without being bound to any one theory, one possible explanation is that NaCl does not provide hydrogen bonding, as sugars and mannitol do, which could potentially prevent self-association of this antibody at higher temperatures.

Based on the above preliminary data from the primary assays (IEX, SEC, and UV), the preferred aqueous formulation for rhuMAb CD18 is 10 mM sodium acetate, 8% trehalose w/v, 0.01% TWEEN 20™, pH 5.0. The shelf-life predicted from an Arrhenius fit to the first order rate constants (IEX data) is 1.7 to 4 years at 5° C. (95% confidence intervals). This formulation can be prepared by mixing 0.573 mL glacial acetic acid, 0.403 mL concentration NaOH, 80 g trehalose, 1 mL of a 10% TWEEN 20™ solution and making up to 1 L with MilliQ water (pH 5.0±0.1 at the 2 L scale). Conductivity of this formulation was found to be 502±10% microSiemens/cm using a Radiometer-Copenhagen CDM-83 with a CDC 314 probe, and density was 1.017 g/mL.

EXAMPLE 2

This example describes the production of a stable aqueous multidose formulation comprising a recombinant humanized anti-CD20 antibody, rhuMAb CD20. Acetate (pH 5) formulations stored at 40° C. for one month demonstrated greater stability than those samples formulated in histidine (pH 5 or 6). The histidine formulations after accelerated temperature storage became very opalescent and yellow in color. A buffering capacity of 10–30 mM acetate was sufficient to maintain the pH at 5.0. The effective amount of tonicity modifier needed to stabilize the antibody against freeze or thermal induced aggregation was compared using sodium chloride (NaCl) or trehalose. Trehalose was found to protect the formulation from freeze induced aggregation, particularly at levels ≥134 mM (500:1 molar ratio). The trehalose formulations (67–270 mM) were much more effective than NaCl in stabilizing formulations placed at 40° C. as evidenced by the clarity of the solution. These results led to the development of a stable prototype liquid multidose formulation comprising 40 mg/mL rhuMAb CD20, 25 mM acetate, 150 mM trehalose, 0.9% benzyl alcohol, 0.02% polysorbate 20 at pH 5.0 that has a minimum shelf life of two years storage at 2–8° C.

MATERIALS AND METHODS

RhuMAb CD20: The bulk material used in all studies was composed of 27 mg/mL rhuMAb CD20 in 50 mM Tris and 100 mM sodium chloride at pH 7.5 or 2.7 mg/mL rhuMAb CD20 in 25 mM Tris and 50 mM sodium chloride at pH 6.0. The bulk was stored aseptically at 2–8° C. in the absence of light.

Buffer exchange: The exchange of the bulk rhuMAb CD20 into different test buffers was done by dialysis at 2–8° C. using Spectra/Por® 7 membranes (MWCO=8 Kda) which were rinsed thoroughly with deionized water before

use. One volume of antibody was dialyzed against a minimum of 10 volumes of the appropriate test buffer. This process was repeated three to four times within a one day period. To obtain the final concentration of 40 mg/mL, the antibody was further concentrated using an Amicon UF/DF cell containing a YM30 membrane (MWCO=30 KDa). Due to the lower starting concentration of the 2.7 mg/mL material, this material was first concentrated to 40 mg/mL and then dialyzed against the appropriate test buffer. After the target concentration was reached and the buffer exchange completed, trehalose, benzyl alcohol and polysorbate 20 were added to the final concentrations described in Tables 15 to 17 below. The liquid multidose C2B8 candidate formulation was 40 mg/mL rhuMAb CD20 formulated in 25 mM acetate, 150 mM trehalose, 0.9% benzyl alcohol and 0.02% polysorbate 20 at pH 5.0. The formulation was then sterile filtered through a 0.2µm membrane and the concentration was determined by UV spectrophotometric scan. A 0.5 ml of each final formulation was then filled into sterile 3 cc glass vials, stoppered with teflon faced grey butyl rubber stoppers and then capped with crimp seals.

TABLE 15

pH and buffer species comparison study

Concentration of rhuMAb CD20 (mg/mL)	Concentration of buffer species (mM)	pH	Storage Temperature (° C.)
40	50 mM Acetate	5.0	2–8, 40, 50
40	50 mM Histidine	5.0	2–8, 40, 50
40	50 mM Histidine	6.0	2–8, 40, 50

All formulations contain 150 mM trehalose, 0.9% benzyl alcohol and 0.02% polysorbate 20.

TABLE 16

Acetate buffering capacity study

Concentration of rhuMAb CD20 (mg/mL)	mM Acetate	Storage temperature (° C.)
40	10	2–8, 40
40	15	2–8, 40
40	20	2–8, 40
40	25	2–8, 40
40	30	2–8, 40

All formulations contain 150 mM trehalose, 0.9% benzyl alcohol and 0.02% polysorbate 20 at pH 5.0.

TABLE 17

Effective ratio of tonicity modifier:rhuMAb CD20 study

Tonicity modifier	Molar ratio of sugar/salt:rhuMAb CD20	mM trehalose or sodium chloride
Trehalose	0	0
Trehalose	250:1	67
Trehalose	500:1	134
Trehalose	1000:1	267
Sodium Chloride	500:1	134
Sodium Chloride	1000:1	267

A). All formulations contained 40 mg/mL rhuMAb CD20, 20 mM acetate, 0.9% benzyl alcohol and 0.02% polysorbate 20 at pH 5.0.

B). A freeze-thaw comparison study was completed for the trehalose formulations. The stability of all formulations at 2–8° and 40° C. was conducted in parallel.

SEC HPLC: Samples were diluted to 10 mg/mL with formulation buffer before being assayed. The method uses a

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except in the case of gelation, and no changes were observed over the duration of the study.

TABLE 19

The effect of buffer species and pH on the percentage unoxidized Fe and percentage monomer of 40 mg/mL rhuMAb CD20 multidose formulations containing 50 mM acetate or histidine, 150 mM trehalose, 0.9% benzyl alcohol and 0.02% polysorbate 20 at pH 5 or 6.

Formulation	Temperature	% Unoxidized Fe (HIC HPLC)	% Monomer (SEC HPLC)
T = 0			
Acetate pH 5	2-8° C.	93.9	99.4
Histidine pH 5	2-8° C.	99.1	99.4
Histidine pH 6	2-8° C.	99.3	98.5
T = 8 weeks			
Acetate pH 5	40° C.	96.5	90.4
Histidine pH 5	40° C.	87.0	87.4
Histidine pH 6	40° C.	81.7	93.9

Since the acetate pH 5 formulation had only a slightly higher aggregation rate, did not turn yellow upon storage at high temperatures and had the greatest clarity under all conditions studied, it was chosen as the buffer species and pH of choice for all subsequent liquid C2B8 multidose formulation screens.

Amount of buffering species: The amount of acetate which maintained the pH of a 40 mg/mL C2B8 multidose formulation at 5.0 was determined. Summarized in Table 20 is the effect on pH as the acetate buffer concentration was increased from 10 to 30 mM. At two and four weeks there appeared to be a slight advantage in staying above 15 mM acetate, although this did not hold true upon long term storage. No change was seen in the pH in any of the test formulations studied after one years storage at 2-8° C. A range of 10 to 30 mM acetate is sufficient to maintain the pH at 5.0.

TABLE 20

The effect of acetate buffer concentration on maintaining the pH of liquid rhuMAb CD20 multidose formulations at 5.0. The formulations were composed of 40 mg/mL rhuMAb CD20, 10 to 30 mM acetate, 150 mM trehalose, 0.9% benzyl alcohol, 0.02% polysorbate 20 at pH 5.0.

mM	pH (weeks)			
Acetate	0	2	4	56
2-8° C.				
10	5.03	5.12	5.11	5.00
15	5.07	5.12	5.14	5.06
20	5.04	5.04	5.04	5.00
25	5.01	5.00	5.02	4.98
30	5.01	5.00	5.02	4.98
40° C.				
10		5.12	5.14	
15		5.10	5.17	
20		5.08	5.10	
25		5.05	5.05	
30		5.03	5.05	

Ratio of tonicity modifier:protein: The addition of trehalose was beneficial to the stability of rhuMAb CD20 multidose formulations after multiple freeze/thaw cycles (FIG. 25). A decrease in the formation of soluble aggregates was observed as the concentration of trehalose in the formulation was increased. At a ratio of 500 moles trehalose:1 mole

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C2B8 (134 mM trehalose), the percentage of aggregate formed was only ~0.5% after five freeze-thaw cycles while the control formulation containing no isotonicity modifier formed 4% aggregate. Having at least a 500 fold molar excess of trehalose in the liquid C2B8 formulation offered sufficient protection against freeze induced aggregation.

The ability of trehalose to stabilize rhuMAb CD20 during accelerated temperature storage was studied and compared to sodium chloride.

FIG. 26 describes the effect of excess molar ratios of trehalose or sodium chloride on the clarity of test formulations stored at 40° C. Sodium chloride is deleterious to the stability. After two weeks storage at 40° C., the 500:1 ratio sample had an O.D. of 0.44. At a 1000:1 molar ratio, the sodium chloride containing formulation separated into a two phase system composed of an opaque gel covered with an opalescent fluid on top. In contrast, there was little change in the solution clarity of the samples containing 0 to 1000:1 molar ratio of trehalose even after one months storage at 40° C. Although the 2-8° C. formulations were unchanged from the initial timepoint, the sodium chloride containing samples were more opalescent.

The effect of trehalose on minimizing the soluble aggregate formation was assessed by SEC HPLC (FIG. 27). No differences were observed between the trehalose formulations and the negative control which contained no tonicity modifier. The formulations appeared to degrade at the same rate to the same products upon storage at 40° C. The pH and concentration were also maintained over the duration of the study.

The presence of trehalose is beneficial in minimizing freeze-induced aggregation and is not deleterious to the stability in the liquid state. The candidate liquid multidose formulation preferably contains at least 500:1 molar ratio of trehalose to C2B8.

Stability of candidate liquid multidose formulation: Based on the aforementioned studies, a prototype liquid multidose formulation composed of 40 mg/mL rhuMAb CD20, 25 mM acetate, 150 mM trehalose, 0.9% benzyl alcohol and 0.02% polysorbate 20 at pH 5.0 was placed on stability at 2-8°, 30° C. and 40° C. The stability profile by SEC HPLC at each temperature studied is shown in FIG. 28. Although the rate of aggregation is slightly faster at 40° C. for the multidose formulation (40 mg/mL) compared to the reference control (10 mg/mL rhuMAb CD20, 25 mM citrate, 150 mM sodium chloride, 0.07% polysorbate 80 at pH 6.5), no decrease in percentage monomer was observed upon storage at 2-8° C. for two years. The bioactivity of the two year old 2-8° C. samples was 99.2% relative to the reference control as determined by the CDC assay.

CONCLUSIONS

The above screening studies indicated that a stable high concentration rhuMAb CD20 liquid multidose formulation was possible by buffering with acetate, maintaining the pH at 5 and including preferably at least about 500 moles of trehalose per mole of antibody. The preferred liquid multidose configuration is composed of 40 mg/mL rhuMAb CD20, 25 mM acetate, 150 mM trehalose, 0.9% benzyl alcohol and 0.02% polysorbate 20 at pH 5 and has a shelf life of two years at 2-8° C.

-continued

85	90	95
Arg Trp Arg Gly Leu Asn Tyr	Gly Phe Asp Val Arg Tyr	Phe Asp
100	105	110
Val Trp Gly Gln Gly Thr Leu	Val Thr Val Ser Ser Ala	Ser Thr
115	120	125
Lys Gly Pro Ser Val Phe Pro	Leu Ala Pro Ser Ser Lys	Ser Thr
130	135	140
Ser Gly Gly Thr Ala Ala Leu	Gly Cys Leu Val Lys Asp	Tyr Phe
145	150	155
Pro Glu Pro Val Thr Val Ser	Trp Asn Ser Gly Ala Leu	Thr Ser
160	165	170
Gly Val His Thr Phe Pro Ala	Val Leu Gln Ser Ser Gly	Leu Tyr
175	180	185
Ser Leu Ser Ser Val Val Thr	Val Pro Ser Ser Ser Leu	Gly Thr
190	195	200
Gln Thr Tyr Ile Cys Asn Val	Asn His Lys Pro Ser Asn	Thr Lys
205	210	215
Val Asp Lys Lys Val Glu Pro	Lys Ser Cys Asp Lys Thr	His Thr
220	225	230
Cys Pro Pro Cys Pro Ala Pro	Glu Leu Leu Gly Gly Arg	Met Lys
235	240	245
Gln Leu Glu Asp Lys Val Glu	Glu Leu Leu Ser Lys Asn	Tyr His
250	255	260
Leu Glu Asn Glu Val Ala Arg	Leu Lys Lys Leu Val Gly	Glu Arg
265	270	275 277

What is claimed is:

1. A method of treating a B cell lymphoma in a mammal, comprising administering a stable aqueous pharmaceutical formulation comprising a therapeutically effective amount of an antibody that binds CD20, the antibody not subjected to prior lyophilization, an acetate buffer from about pH 4.8 to about 5.5, a surfactant and a polyol, wherein the formulation lacks a tonicifying amount of sodium chloride.

2. The method of claim 1 wherein the formulation is isotonic.

3. The method of claim 1 wherein the formulation is stable at a temperature of about 2-8° C. for at least one year.

4. The method of claim 1 wherein the formulation is stable at a temperature of about 2-8° C. for at least two years.

5. The method of claim 1 wherein the formulation is stable at about 30° C. for at least one month.

6. The method of claim 1 wherein the formulation is stable following freezing and thawing of the formulation.

7. The method of claim 1 wherein the polyol is a nonreducing sugar.

8. The method of claim 7 wherein the nonreducing sugar is trehalose.

9. The method of claim 7 wherein the nonreducing sugar is sucrose.

10. The method of claim 1 wherein the antibody is an antibody fragment.

11. The method of claim 10 wherein the antibody fragment is a F(ab')₂.

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12. The method of claim 1 wherein the antibody concentration in the formulation is from about 0.1 to about 50 mg/mL.

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13. The method of claim 12 wherein the antibody is present in an amount of about 30-50 mg/mL.

14. The method of claim 1 wherein the surfactant is a polysorbate.

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15. The method of claim 1 wherein the acetate is present in an amount of about 5-30 mM.

16. The method of claim 15 wherein the acetate is present in an amount of 10-30 mM.

17. The method of claim 1 wherein the formulation further comprises a preservative.

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18. The method of claim 17 wherein the preservative is benzyl alcohol.

19. The method of claim 1 wherein the acetate buffer is at pH 5.0.

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20. The method of claim 1 wherein the buffer is 10-30 mM sodium acetate at pH 5, the polyol is trehalose in an amount of about 2-10% w/v, the surfactant is a polysorbate in an amount of about 0.01-0.1% v/v, wherein the formulation further comprises benzyl alcohol as a preservative and wherein the formulation is stable at a temperature of about 2-8° C. for at least two years.

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(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
19 May 2005 (19.05.2005)

PCT

(10) International Publication Number
WO 2005/044859 A2

(51) International Patent Classification⁷: **C07K 16/28**,
C12N 15/13, 15/63, 5/10, C07K 16/46, A61K 39/395

(21) International Application Number:
PCT/IB2004/003896

(22) International Filing Date:
5 November 2004 (05.11.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/517,096 5 November 2003 (05.11.2003) US

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

Published:

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

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: ANTIGEN BINDING MOLECULES WITH INCREASED FC RECEPTOR BINDING AFFINITY AND EFFECTOR
FUNCTION

(57) Abstract: The present invention relates to antigen binding molecules (ABMs). In particular embodiments, the present invention relates to recombinant monoclonal antibodies, including chimeric, primatized or humanized antibodies specific for human CD20. In addition, the present invention relates to nucleic acid molecules encoding such ABMs, and vectors and host cells comprising such nucleic acid molecules. The invention further relates to methods for producing the ABMs of the invention, and to methods of using these ABMs in treatment of disease. In addition, the present invention relates to ABMs with modified glycosylation having improved therapeutic properties, including antibodies with increased Fc receptor binding and increased effector function.

WO 2005/044859 A2

(1991); Jenkins *et al.*, *Nature Biotechnol.* 14:975-81 (1996)). Bacteria very rarely glycosylate proteins, and like other types of common hosts, such as yeasts, filamentous fungi, insect and plant cells, yield glycosylation patterns associated with rapid clearance from the blood stream, undesirable immune interactions, and in some specific cases, reduced biological activity. Among mammalian cells, Chinese hamster ovary (CHO) cells have been most commonly used during the last two decades. In addition to giving suitable glycosylation patterns, these cells allow consistent generation of genetically stable, highly productive clonal cell lines. They can be cultured to high densities in simple bioreactors using serum-free media, and permit the development of safe and reproducible bioprocesses. Other commonly used animal cells include baby hamster kidney (BHK) cells, NS0- and SP2/0-mouse myeloma cells. More recently, production from transgenic animals has also been tested. (Jenkins *et al.*, *Nature Biotechnol.* 14:975-81 (1996)).

[0015] All antibodies contain carbohydrate structures at conserved positions in the heavy chain constant regions, with each isotype possessing a distinct array of N-linked carbohydrate structures, which variably affect protein assembly, secretion or functional activity. (Wright, A., and Morrison, S. L., *Trends Biotech.* 15:26-32 (1997)). The structure of the attached N-linked carbohydrate varies considerably, depending on the degree of processing, and can include high-mannose, multiply-branched as well as biantennary complex oligosaccharides. (Wright, A., and Morrison, S. L., *Trends Biotech.* 15:26-32 (1997)). Typically, there is heterogeneous processing of the core oligosaccharide structures attached at a particular glycosylation site such that even monoclonal antibodies exist as multiple glycoforms. Likewise, it has been shown that major differences in antibody glycosylation occur between cell lines, and even minor differences are seen for a given cell line grown under different culture conditions. (Lifely, M. R. *et al.*, *Glycobiology* 5(8):813-22 (1995)).

[0016] One way to obtain large increases in potency, while maintaining a simple production process and potentially avoiding significant, undesirable side effects, is to enhance the natural, cell-mediated effector functions of monoclonal antibodies by engineering their oligosaccharide component as described in

particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

[0186] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antagonist, which matrices are in the form of shaped articles, e.g., films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOTTM (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid.

[0187] The formulations to be used for in vivo administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

[0188] The compositions of the invention may be in a variety of dosage forms which include, but are not limited to, liquid solutions or suspension, tablets, pills, powders, suppositories, polymeric microcapsules or microvesicles, liposomes, and injectable or infusible solutions. The preferred form depends upon the mode of administration and the therapeutic application.

[0189] The compositions of the invention also preferably include conventional pharmaceutically acceptable carriers and adjuvants known in the art such as human serum albumin, ion exchangers, alumina, lecithin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, and salts or electrolytes such as protamine sulfate.

[0190] The most effective mode of administration and dosage regimen for the pharmaceutical compositions of this invention depends upon the severity and course of the disease, the patient's health and response to treatment and the judgment of the treating physician. Accordingly, the dosages of the compositions should be titrated to the individual patient. Nevertheless, an effective dose of the compositions of this invention will generally be in the range of from about 0.01 to about 2000 mg/kg.

9. An isolated polynucleotide comprising
 - a. a sequence encoding a polypeptide having the sequence of SEQ ID No.:1; and
 - b. a sequence encoding a polypeptide having the sequence of an antibody Fc region, or a fragment thereof, from a species other than mouse.
10. An isolated polynucleotide comprising
 - a. a sequence encoding a polypeptide having the sequence of SEQ ID No.:2; and
 - b. a sequence encoding a polypeptide having the sequence of an antibody Fc region, or a fragment thereof, from a species other than mouse.
11. An isolated polynucleotide encoding a polypeptide having the sequence of SEQ ID No.:1.
12. An isolated polynucleotide encoding a polypeptide having the sequence of SEQ ID No.:2.
13. An isolated polynucleotide comprising SEQ ID NO.: 11.
14. An isolated polynucleotide comprising SEQ ID NO.: 12.
15. An isolated polynucleotide comprising a sequence having at least 80% identity to SEQ ID NO.:11 or to SEQ ID NO.:12.
16. An isolated polynucleotide comprising a sequence having at least 85% identity to SEQ ID NO.:11 or to SEQ ID NO.:12.
17. An isolated polynucleotide comprising a sequence having at least 90% identity to SEQ ID NO.:11 or to SEQ ID NO.:12.

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27. A host cell comprising an isolated polynucleotide according to any one of claims 1-23.
28. A polypeptide comprising a sequence derived from the murine B-Ly1 antibody and a sequence derived from a heterologous polypeptide.
29. An antigen-binding molecule comprising the polypeptide of claim 28.
30. An antigen-binding molecule according to claim 29, which binds human CD20.
31. An antigen-binding molecule according to claim 29, which is an antibody.
32. An antigen-binding molecule according to claim 31, which is a chimeric antibody.
33. An antigen-binding molecule according to claim 31, which is a humanized antibody.
34. An antigen-binding molecule according to claim 31, which is a primatized antibody.
35. A chimeric polypeptide comprising the sequence of SEQ ID NO:1 or a variant thereof.
36. A chimeric polypeptide comprising the sequence of SEQ ID NO:2 or a variant thereof.
37. A chimeric polypeptide comprising:
 - a. a polypeptide having a sequence selected from the group consisting of: SEQ ID NO:15, SEQ ID NO:16 and SEQ ID NO: 17; and

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b. a polypeptide having a sequence selected from the group consisting of: SEQ ID NO:25, SEQ ID NO:26 and SEQ ID NO: 27; and

c. SEQ ID NO:28.

38. A chimeric polypeptide according to claim 37, further comprising a heavy chain variable region framework, wherein said framework comprises an alanine residue at position 71, according to Kabat numbering.

39. A chimeric polypeptide according to claim 37, further comprising a heavy chain variable region framework, wherein said framework comprises an arginine residue at position 94, according to Kabat numbering.

40. A chimeric polypeptide comprising SEQ ID NO:18, SEQ ID NO:19 and SEQ ID NO: 20.

41. An isolated polypeptide comprising SEQ ID NO:13 or a variant thereof.

42. An isolated polypeptide comprising SEQ ID NO:14 or a variant thereof.

43. The isolated polypeptide according to any of claims 35-40, wherein said polypeptide is a fusion polypeptide.

44. An antigen binding molecule comprising the isolated polypeptide of claim 43.

45. The antigen binding molecule of claim 44, wherein said antigen binding molecule is an antibody.

46. The antibody of claim 45, wherein said antibody is a primatized antibody.

47. The antibody of claim 45, wherein said antibody is a humanized antibody.

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
31 August 2006 (31.08.2006)

PCT

(10) International Publication Number
WO 2006/091871 A1

(51) International Patent Classification:

C07K 16/00 (2006.01) C12N 5/00 (2006.01)
C07K 1/00 (2006.01) A01K 67/033 (2006.01)
C07H 21/02 (2006.01) A01N 37/18 (2006.01)
C12N 15/00 (2006.01) A01N 43/04 (2006.01)

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(21) International Application Number:

PCT/US2006/006700

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(22) International Filing Date:

23 February 2006 (23.02.2006)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

11/065,716 23 February 2005 (23.02.2005) US
11/238,171 27 September 2005 (27.09.2005) US

(81) Designated States (unless otherwise indicated, for every
kind of national protection available): 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, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV,
LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI,
NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG,
SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US
(patent), UZ, VC, VN, YU, ZA, ZM, ZW.

(63) Related by continuation (CON) or continuation-in-part
(CIP) to earlier application:

US 11/065,716 (CIP)
Filed on 27 September 2005 (27.09.2005)

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,
FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT,
RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA,
GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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Published:

— with international search report
— before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: SOLUBLE GLYCOSAMINOGLYCANASES AND METHODS OF PREPARING AND USING SOLUBLE GLY-
COSAMINOGLYCANASES

(57) Abstract: The invention relates to the discovery of novel soluble neutral active Hyaluronidase Glycoproteins (sHASEGPs), methods of manufacture, and their use to facilitate administration of other molecules or to alleviate glycosaminoglycan associated pathologies. Minimally active polypeptide domains of the soluble, neutral active sHASEGP domains are described that include asparagine-linked sugar moieties required for a functional neutral active hyaluronidase domain. Included are modified amino-terminal leader peptides that enhance secretion of sHASEGP. The invention further comprises sialated and pegylated forms of a recombinant sHASEGP to enhance stability and serum pharmacokinetics over naturally occurring slaughterhouse enzymes. Further described are suitable formulations of a substantially purified recombinant sHASEGP glycoprotein derived from a eukaryotic cell that generate the proper glycosylation required for its optimal activity.

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[0019] One aspect of the invention is based upon the discovery that a soluble, neutral-active hyaluronidase activity can be produced with high yield in a mammalian expression system by introducing nucleic acids that lack a narrow region encoding amino acids in the carboxy terminus of the human PH20 cDNA. Additional modifications of the sHASEGP to enhance secretion by use of non-native leader peptides are also provided. Further provided are methods to modify the sHASEGP to prolong its half life by way of masking the protein with polyethylene glycol (PEG) and/or post-translational modifications to native glycosylation. Previous attempts to generate soluble neutral-active human hyaluronidase glycoproteins were unsuccessful. It was concluded that truncations of the human hyaluronidase polypeptide resulted in both a loss of neutral enzymatic activity, and an inability of cells to secrete the recombinant protein in mammalian expression systems (Arming, et al Eur J Biochem 1997 Aug 1;247 (3):810-4). It is critical to generate neutral-acting secreted sHASEGP for commercial production and therapeutic utility as a hyaluronidase. The invention, disclosed herein, overcomes these and other challenges.

[0020] The invention further comprises a catalytically active human sHASEGP glycoprotein wherein the sHASEGP possesses at least one N-linked sugar moiety. The studies shown herein demonstrate that human PH20 requires N-linked glycans for catalytic activity, whereas bovine and bee venom hyaluronidases remain active without such N-linked glycans. A human hyaluronidase PH20 domain devoid of N-linked moieties is catalytically inactive. Thus classic recombinant DNA technology does not permit the production of a catalytically active human sHASEGP, unlike bee venom hyaluronidase, which can be produced in *E. coli*.

[0021] The invention includes methods and cells for generation of an N-linked sHASEGP glycoprotein polypeptide, by using of a cell capable of introducing said N-linked sugar moieties or by introduction of said N-linked moieties on a sHASEGP polypeptide. Methods of identifying properly glycosylated sHASEGPs are further disclosed.

[0022] Catalytically active PEGylated and/or super-Sialated sHASEGP glycoproteins are also provided. PEGylated and/or super-sialated sHASEGPs possess greater serum half-lives compared to naturally occurring non-sialated bovine and ovine testes sHASEGPs, and are thus preferable for both enzyme stability and use as drugs or adjuvants in circumstances in which prolonged half-lives are desirable (such as is

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DOXIL™); Dromostanolone propionates (e.g. DROMOSTANOLONE™ and MASTERONE™ Injection); Elliott's B Solutions (e.g. Elliott's B Solution™); Epirubicins (e.g. ELLENCE™); Epoetin alfas (e.g. EPOGEN™); Estramustines (e.g. EMCYT™); Etoposide phosphates (e.g. ETOPOPHOS™); Etoposide VP-16s (e.g. VEPESID™); Exemestanes (e.g. AROMASIN™); Filgrastims (e.g. NEUPOGEN™); Floxuridines (e.g. FUDR™); Fludarabines (e.g. FLUDARA™); Fluorouracils incl. 5-FUs (e.g. ADRUCIL™); Fulvestrants (e.g. FASLODEX™); Gemcitabines (e.g. GEMZAR™); Gemtuzumabs / Ozogamicins (e.g. MYLOTARG™); Goserelin acetates (e.g. ZOLADEX™); Hydroxyureas (e.g. HYDREA™); Ibritumomabs / Tiuxetans (e.g. ZEVALIN™); Idarubicins (e.g. IDAMYCIN™); Ifosfamides (e.g. IFEX™); Imatinib mesylates (e.g. GLEEVEC™); Interferon alfa-2as (e.g. ROFERON-A™); Interferon alfa-2bs (e.g. INTRON A™); Irinotecans (e.g. CAMPTOSAR™); Letrozoles (e.g. FEMARA™); Leucovorins (e.g. WELLCOVORIN™, LEUCOVORIN™); Levamisoles (e.g. ERGAMISOL™); Lomustines / CCNUs (e.g. CeeBU™); Mecllorethamines / Nitrogen mustards (e.g. MUSTARGEN™); Megestrol acetates (e.g. MEGACE™); Melphalans / L-PAMs (e.g. ALKERAN™); Mercaptopurine incl. 6-MPs (e.g. PURINETHOL™); Mesnas (e.g. MESNEX™); Methotrexates; Methoxsalens (e.g. UVADEX™); Mitomycin Cs (e.g. MUTAMYCIN™, MITOZYTREX™); Mitotanes (e.g. LYSODREN™); Mitoxantrones (e.g. NOVANTRONE™); Nandrolone Phenpropionates (e.g. DURABOLIN-50™); Nofetumomabs (e.g. VERLUMA™); Oprelvekins (e.g. NEUMEGA™); Oxaliplatins (e.g. ELOXATIN™); Paclitaxels (e.g. PAXENET™, TAXOL™); Pamidronates (e.g. AREDIA™); Pegademases (e.g. ADAGENT™); Pegaspargases (e.g. ONCASPAR™); Pegfilgrastims (e.g. NEULASTA™); Pentostatins (e.g. NIPENT™); Pipobromans (e.g. VERCYTE™); Plicamycin / Mithramycins (e.g. MITHRACIN™); Porfimer sodiums (e.g. PHOTOFRIN™); Procarbazines (e.g. MATULANE™); Quinacrine (e.g. ATABRINE™); Rasburicases (e.g. ELITEK™); Rituximabs (e.g. RITUXAN™); Sargramostims (e.g. PROKINET™); Streptozocins (e.g. ZANOSAR™); Talcs (e.g. SCLEROSOL™); Tamoxifens (e.g. NOLVADEX™); Temozolomides (e.g. TEMODAR™); Teniposides / VM-26s (e.g. VUMON™); Testolactones (e.g. TESLAC™); Thioguanines incl. 6-TG; Thiotepas (e.g. THIOPLEX™); Topotecans (e.g. HYCAMTIN™); Toremifenes (e.g. FARESTON™); Tositumomabs (e.g. BEXXAR™); Trastuzumabs (e.g. HERCEPTIN™); Tretinoin / ATRA (e.g. VESANOID™); Uracil

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[0556] VITREOUS HEMORRHAGE

[0557] In an effort to minimize the potential for causing further detachment or tearing of the retina during performance of vitrectomy, it has previously been proposed in U.S. Pat. No. 5,292,509 (Hageman), to inject certain protease-free glycosaminoglycanase enzymes into the vitreous body, to cause the vitreous body to become uncoupled or "disinserted" from the retina, prior to removal of the vitreous body. Such disinsertion or uncoupling of the vitreous body is purported to minimize the likelihood that further tearing or detachment of the retina will occur as the vitreous body is removed. Examples of specific protease-free glycosaminoglycanase enzymes which may be used to bring about this vitreal disinsertion purportedly include; chondroitinase ABC, chondroitinase AC, chondroitinase B, chondroitin 4-sulfatase, chondroitin 6-sulfatase, hyaluronidase and beta-glucuronidase.

[0558] Although hyaluronidase enzyme has been known to be usable for various ophthalmic applications, including the vitrectomy adjunct application described in U.S. Pat. No. 5,292,509 (Hageman), published studies have indicated that the hyaluronidase enzyme may itself be toxic to the retina and/or other anatomical structures of the eye. See, The Safety of Intravitreal Hyaluronidase; Gottlieb, J. L.; Antoszyk, A. N., Hatchell, D. L. and Soloupis, P., Invest Ophthalmol Vis Sci 31:11, 2345-52 (1990). Moreover, the use of impure slaughterhouse preparations of hyaluronidase can cause uveitis or inflammation of the eye. The use of human sHASEGP is thus preferable in both its increased potency, purity and lack of animal origin that can give rise to immunogenic reactions and antibody mediated neutralization following repeated administration. In another embodiment, a pegylated form of a sHASEGP can be injected into the eye. Such a pegylated sHASEGP is not cleared from the vitreous in such a rapid fashion and maintains its activity in the vitreous for a longer period of time.

[0559] The ophthalmic toxicity of some hyaluronidase preparations has been confirmed by other investigators, who have proposed that such hyaluronidase preparations be used as a toxic irritant for causing experimentally induced neovascularization of the eye, in animal toxicity models, (see An Experimental Model of Preretinal Neovascularization in the Rabbit; Antoszyk, A. N., Gottlieb, J. L., Casey, R. C., Hatchell, D. L. and Machemer, R., Invest Ophthalmol Vis Sci 32:1, 46-51 (1991).

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Table 25-1 Effects of sHASEGP on the Pharmacokinetic Parameters of REMICADE.

Time (Hours)	CPM REMICAD E (10 mg/kg) Intradermal	SD	CPM REMICADE (10 mg/kg) + sHASEGP 100 U Intradermal	SD	CPM REMICADE (10 mg/kg) Intravenous
0.5	27,451	2,221	94,888	31,318	1,657,849
1	37,213	10,775	146,793	42,014	1,645,571
2	66,001	6,078	412,047	61,240	1,576,145
4	120,704	21,744	691,626	89,002	1,260,646
8	256,987	32,459	833,826	133,887	1,157,268
24	493,004	75,694	853,423	65,777	790,357
48	515,439	53,431	728,302	74,848	684,599
72	454,717	54,388	640,303	81,492	558,307
96	375,993	39,744	598,413	59,533	496,658
120	336,067	66,964	518,861	53,643	434,008
AUC	53,350,555		118,424,609		82,718,550
C _{max}	515,439		853,423		1,657,849
T _{max}	48		24		0
Absolute Bioavailability	62%		121%		100%

[0875] As can be seen in Table 5-1, the bioavailability of the exemplary pharmacologic REMICADE (as assessed by AUC) was dramatically improved when the intradermal dosing was combined with a dose of sHASEGP. As was observed with enhanced delivery of PEG-INTRON, the overall levels of REMICADE bioavailability achieved with an intradermal dosing in combination with sHASEGP were statistically similar to those of the intravenous administration when all material was delivered directly into the bloodstream. Although the REMICADE T_{max} is necessarily longer following ID versus IV administration, within twenty four hours after ID dosing with sHASEGP, the concentrations of agent in the bloodstream exceeded that observed with IV dosing, and thereafter approached or exceeded the IV concentrations. Compared with ID dosing without sHASEGP, the observed T_{max} was reached about twice as quickly with sHASEGP (24 hours versus 48 hours); and the C_{max} and bioavailability (AUC) both substantially increased (by about 65% and 95%, respectively).

6. The polypeptide of claim 1, wherein said sugar moiety is of the complex type.
7. The polypeptide of claim 1, wherein said sugar moiety is a hybrid type.
8. The polypeptide of claim 1, wherein said sugar moiety further comprises at least one terminated with sialic acid.
9. The polypeptide of claim 1, wherein the polypeptide consists essentially of the hyaluronidase domain of human hyaluronidase glycoprotein (sHASEGP) or a catalytically active portion thereof.
10. The polypeptide of claim 1, wherein the hyaluronidase portion of the polypeptide comprises of the hyaluronidase domain of sHASEGP or a catalytically active portion thereof.
11. The polypeptide of claim 1, wherein the polypeptide is modified with a polymer.
12. The polypeptide of claim 11, wherein the polymer is PEG or dextran.
13. A polypeptide, consisting essentially of a hyaluronidase domain or a catalytically active portion thereof of a human hyaluronidase protein.
14. The polypeptide of claim 13, wherein the hyaluronidase is super-sialated.
15. The polypeptide of claim 13, wherein the hyaluronidase domain comprises the sequence of amino acids set forth as amino acids 1-450 of SEQ ID NO. 3.
16. The polypeptide of claim 13, wherein the hyaluronidase domain comprises the sequence of amino acids set forth as amino acids 1-438 of SEQ ID NO. 3.

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17. The polypeptide of claim 13, wherein the hyaluronidase domain comprises the sequence of amino acids set forth as amino acids 1-459 of SEQ ID NO. 3.
18. The polypeptide of claim 13, wherein the polypeptide is modified with a polymer.
19. The polypeptide of claim 18, wherein the polymer is PEG or dextran.
20. The polypeptide of claim 1, wherein the sHASEGP is a human polypeptide.
21. The polypeptide of claim 1, wherein the polypeptide is a soluble polypeptide as set forth in SEQ ID NO. 4.
22. The polypeptide of claim 1, wherein a signal sequence capable of directing the polypeptide outside the cell is operably attached to the sHASEGP polypeptide.
23. An isolated soluble human hyaluronidase glycoprotein (sHASEGP), wherein the potency of the sHASEGP is greater than 40,000 USP Units/mg protein.
24. The sHASEGP of claim 23, wherein the hyaluronidase is sialated.
25. The sHASEGP of claim 23, wherein the protein is modified with a polymer.
26. The sHASEGP of claim 25, wherein the polymer is PEG or dextran.
27. A nucleic acid molecule, encoding the polypeptide of claim 1.
28. A nucleic acid molecule, comprising a sequence selected from:
 - (a) a sequence as set forth in SEQ ID NO. 6;

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57. The method of claim 56, wherein the polypeptide is modified with a polymer.
58. The method of claim 57, wherein the polymer is PEG or dextran.
59. The method of claim 56, wherein the sHASEGP polypeptide is administered prior to, simultaneously with or following administration of the molecule.
60. A pharmaceutical composition, comprising, substantially purified soluble hyaluronidase glycoprotein (sHASEGP) polypeptide of claim 1 and a pharmaceutical carrier.
61. A pharmaceutical composition, comprising the substantially purified sHASEGP polypeptide of claim 1.
62. The pharmaceutical composition of claim 60, further comprising a pharmaceutically active or pharmacologic agent.
63. The pharmaceutical composition of claim 62, wherein the pharmaceutically active or pharmacologic agent is selected from the group of agents consisting of a chemotherapeutic agent, an analgesic agent, an anti-inflammatory agent, an antimicrobial agent, an amoebicidal agent, a trichomonocidal agent, an anti-parkinson agent, an anti-malarial agent, an anticonvulsant agent, an anti-depressant agent, an antiarthritics agent, an anti-fungal agent, an antihypertensive agent, an antipyretic agent, an anti-parasitic agent, an antihistamine agent, an alpha-adrenargic agonist agent, an alpha blocker agent, an anesthetic agent, a bronchial dilator agent, a biocide agent, a bactericide agent, a bacteriostatic agent, a beta adrenergic blocker agent, a calcium channel blocker agent, a cardiovascular drug agent, a contraceptive agent, a decongestant agent, a diuretic agent, a depressant agent, a diagnostic agent, an electrolyte agent, a hypnotic agent, a hormone agent, a hyperglycemic agent, a muscle relaxant agent, a muscle contractant agent, an ophthalmic agent, a parasympathomimetic agent, a psychic energizer agent, a sedative agent, a sleep inducer, a sympathomimetic agent, a tranquilizer agent, a urinary agent, a vaginal agent, a viricide agent, a vitamin agent, a non-steroidal

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biotin (5kDa biotinylated), PEG-p-nitrophenyl-carbonate (30kDa) and PEG-propionaldehyde (30kDa).

136. A PEGylated hyaluronidase produced according to the method of claim 134.

137. A PEGylated hyaluronidase produced according to the method of claim 135.

138. A method of delivering a molecule to a tissue containing excess amounts of glycosaminoglycan, comprising: administering a PEGylated hyaluronidase of claim 128 to a tissue in an amount sufficient to degrade glycosaminoglycans sufficiently to open channels less than about 500 nm in diameter; and administering a molecule to the tissue comprising the degraded glycosaminoglycans.

139. A pharmaceutical composition, comprising a PEGylated hyaluronidase of claim 128 and a pharmaceutical carrier.

140. A method for increasing the diffusion of a therapeutic substance or other molecule or macromolecular complex (each an Agent) smaller than about 0.5 microns in diameter in a subject, comprising:

administering to a subject a PEGylated hyaluronidase of claim 128 in an amount sufficient to open or to form channels smaller than about 0.5 microns in diameter and an Agent, whereby the diffusion of the Agent is increased.

141. The method of claim 140, wherein the Agent is selected from the group of agents consisting of a chemotherapeutic agent, an analgesic agent, an anti-inflammatory agent, an antimicrobial agent, an amoebicidal agent, a trichomonocidal agent, an anti-parkinson agent, an anti-malarial agent, an anticonvulsant agent, an anti-depressant agent, an antiarthritics agent, an anti-fungal agent, an antihypertensive agent, an antipyretic agent, an anti-parasitic agent, an antihistamine agent, an alpha-adrenargic agonist agent, an alpha blocker agent, an anesthetic agent, a bronchial dilator agent, a biocide agent, a bactericide agent, a bacteriostatic agent, a beta adrenergic blocker agent, a calcium

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(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
11 January 2007 (11.01.2007)

PCT

(10) International Publication Number
WO 2007/005608 A2

(51) International Patent Classification:
C07K 14/715 (2006.01)

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(21) International Application Number:
PCT/US2006/025584

(81) Designated States (unless otherwise indicated, for every kind of national protection available): 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, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(22) International Filing Date: 29 June 2006 (29.06.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/695,679 30 June 2005 (30.06.2005) US

(71) Applicant (for all designated States except US): ABBOTT LABORATORIES [US/US]; 100 Abbott Park Road, Abbott Park, IL 60064 (US).

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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Published:

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

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: IL-12/P40 BINDING PROTEINS

(57) Abstract: The present invention encompasses IL-12p40 binding proteins, particularly antibodies that bind human interleukin-12 (hIL-12) and/or human IL-23 (hIL-23). Specifically, the invention relates to antibodies that are chimeric, CDR grafted and humanized antibodies. Preferred antibodies have high affinity for hIL-12 and/or hIL-23 and neutralize hIL-12 and/or hIL-23 activity in vitro and in vivo. An antibody of the invention can be a full-length antibody or an antigen-binding portion thereof. Method of making and method of using the antibodies of the invention are also provided. The antibodies, or antibody portions, of the invention are useful for detecting hIL-12 and/or hIL-23 and for inhibiting hIL-12 and/or hIL-23 activity, e.g., in a human subject suffering from a disorder in which hIL-12 and/or hIL-23 activity is detrimental.

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bitartrate/apap, diclofenac sodium/misoprostol, fentanyl, anakinra, human recombinant, tramadol hcl, salsalate, sulindac, cyanocobalamin/fa/pyridoxine, acetaminophen, alendronate sodium, prednisolone, morphine sulfate, lidocaine hydrochloride, indomethacin, glucosamine sulf/chondroitin, amitriptyline hcl, sulfadiazine, oxycodone hcl/acetaminophen, olopatadine hcl, misoprostol, naproxen sodium, omeprazole, cyclophosphamide, rituximab, IL-1 TRAP, MRA, CTLA4-IG, IL-18 BP, anti-IL-18, Anti-IL15, BIRB-796, SCIO-469, VX-702, AMG-548, VX-740, Roflumilast, IC-485, CDC-801, and Mesopram. Preferred combinations include methotrexate or leflunomide and in moderate or severe rheumatoid arthritis cases, cyclosporine.

Nonlimiting additional agents which can also be used in combination with an IL-12 or IL-23 antibody, or antigen-binding portion thereof, to treat rheumatoid arthritis include, but are not limited to, the following: non-steroidal anti-inflammatory drug(s) (NSAIDs); cytokine suppressive anti-inflammatory drug(s) (CSAIDs); CDP-571/BAY-10-3356 (humanized anti-TNF α antibody; Celltech/Bayer); cA2/infliximab (chimeric anti-TNF α antibody; Centocor); 75 kDTNFR-IgG/etanercept (75 kD TNF receptor-IgG fusion protein; Immunex; see *e.g.*, *Arthritis & Rheumatism* (1994) Vol. 37, S295; *J. Invest. Med.* (1996) Vol. 44, 235A); 55 kDTNF-IgG (55 kD TNF receptor-IgG fusion protein; Hoffmann-LaRoche); IDEC-CE9.1/SB 210396 (non-depleting primatized anti-CD4 antibody; IDEC/SmithKline; see *e.g.*, *Arthritis & Rheumatism* (1995) Vol. 38, S185); DAB 486-IL-2 and/or DAB 389-IL-2 (IL-2 fusion proteins; Seragen; see *e.g.*, *Arthritis & Rheumatism* (1993) Vol. 36, 1223); Anti-Tac (humanized anti-IL-2R α ; Protein Design Labs/Roche); IL-4 (anti-inflammatory cytokine; DNAX/Schering); IL-10 (SCH 52000; recombinant IL-10, anti-inflammatory cytokine; DNAX/Schering); IL-4; IL-10 and/or IL-4 agonists (*e.g.*, agonist antibodies); IL-1RA (IL-1 receptor antagonist; Synergen/Amgen); anakinra (Kineret®/Amgen); TNF-bp/s-TNF (soluble TNF binding protein; see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S284; *Amer. J. Physiol. - Heart and Circulatory Physiology* (1995) Vol. 268, pp. 37-42); R973401 (phosphodiesterase Type IV inhibitor; see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S282); MK-966 (COX-2 Inhibitor; see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S81); Iloprost (see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S82); methotrexate; thalidomide (see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S282) and thalidomide-related drugs (*e.g.*, Celgen); leflunomide (anti-inflammatory and cytokine inhibitor; see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S131; *Inflammation Research* (1996) Vol. 45, pp. 103-107); tranexamic acid (inhibitor of plasminogen activation; see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S284); T-614 (cytokine inhibitor; see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S282); prostaglandin E1 (see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S282); Tenidap (non-steroidal anti-inflammatory drug; see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S280); Naproxen (non-steroidal anti-inflammatory drug; see *e.g.*, *Neuro Report* (1996) Vol. 7, pp. 1209-1213); Meloxicam (non-steroidal anti-inflammatory drug); Ibuprofen

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(non-steroidal anti-inflammatory drug); Piroxicam (non-steroidal anti-inflammatory drug); Diclofenac (non-steroidal anti-inflammatory drug); Indomethacin (non-steroidal anti-inflammatory drug); Sulfasalazine (see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S281); Azathioprine (see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S281); ICE inhibitor (inhibitor of the enzyme interleukin-1 β converting enzyme); zap-70 and/or lck inhibitor (inhibitor of the tyrosine kinase zap-70 or lck); VEGF inhibitor and/or VEGF-R inhibitor (inhibitors of vascular endothelial cell growth factor or vascular endothelial cell growth factor receptor; inhibitors of angiogenesis); corticosteroid anti-inflammatory drugs (*e.g.*, SB203580); TNF-converterase inhibitors; anti-IL-12 antibodies; anti-IL-18 antibodies; interleukin-11 (see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S296); interleukin-13 (see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S308); interleukin-17 inhibitors (see *e.g.*, *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S120); gold; penicillamine; chloroquine; chlorambucil; hydroxychloroquine; cyclosporine; cyclophosphamide; total lymphoid irradiation; anti-thymocyte globulin; anti-CD4 antibodies; CD5-toxins; orally-administered peptides and collagen; lobenzarit disodium; Cytokine Regulating Agents (CRAs) HP228 and HP466 (Houghten Pharmaceuticals, Inc.); ICAM-1 antisense phosphorothioate oligo-deoxynucleotides (ISIS 2302; Isis Pharmaceuticals, Inc.); soluble complement receptor 1 (TP10; T Cell Sciences, Inc.); prednisone; orgotein; glycosaminoglycan polysulphate; minocycline; anti-IL2R antibodies; marine and botanical lipids (fish and plant seed fatty acids; see *e.g.*, DeLuca *et al.* (1995) *Rheum. Dis. Clin. North Am.* 21:759-777); auranofin; phenylbutazone; meclofenamic acid; flufenamic acid; intravenous immune globulin; zileuton; azaribine; mycophenolic acid (RS-61443); tacrolimus (FK-506); sirolimus (rapamycin); amiprilose (therafectin); cladribine (2-chlorodeoxyadenosine); methotrexate; antivirals; and immune modulating agents.

In one embodiment, the IL-12 antibody, or antigen-binding portion thereof, is administered in combination with one of the following agents for the treatment of rheumatoid arthritis: small molecule inhibitor of KDR (ABT-123), small molecule inhibitor of Tie-2; methotrexate; prednisone; celecoxib; folic acid; hydroxychloroquine sulfate; rofecoxib; etanercept; infliximab; leflunomide; naproxen; valdecoxib; sulfasalazine; methylprednisolone; ibuprofen; meloxicam; methylprednisolone acetate; gold sodium thiomalate; aspirin; azathioprine; triamcinolone acetonide; propoxyphene napsylate/apap; folate; nabumetone; diclofenac; piroxicam; etodolac; diclofenac sodium; oxaprozin; oxycodone hcl; hydrocodone bitartrate/apap; diclofenac sodium/misoprostol; fentanyl; anakinra, human recombinant; tramadol hcl; salsalate; sulindac; cyanocobalamin/fa/pyridoxine; acetaminophen; alendronate sodium; prednisolone; morphine sulfate; lidocaine hydrochloride; indomethacin; glucosamine sulfate/chondroitin; cyclosporine; amitriptyline hcl; sulfadiazine; oxycodone hcl/acetaminophen; olopatadine hcl; misoprostol; naproxen sodium; omeprazole; mycophenolate mofetil; cyclophosphamide; rituximab; IL-1 TRAP; MRA; CTLA4-IG; IL-18 BP; ABT-874; ABT-325

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X_2 is G, T, R, P, or H;
 X_3 is I, R, F, Y, or Q;
 X_4 is R, V, Y, F, or A;
 X_5 is S, N, G, A, or R;
 X_6 is A, Y, L, F, or M;
 X_7 is M, A, D, L, or F;
 X_8 is D, M, Y, or W;
 X_9 is Y, D, or N;
 X_{10} is Y, A, or is not present;
 X_{11} is M, or is not present;
 X_{12} is D, or is not present; and
 X_{13} is Y, or is not present;

CDR-L1. X_1 - X_2 - X_3 - X_4 - X_5 - X_6 - X_7 - X_8 - X_9 - X_{10} - X_{11} - X_{12} - X_{13} - X_{14} - X_{15} (SEQ ID NO: 58), wherein;

X_1 is K, or R;
 X_2 is A;
 X_3 is S;
 X_4 is Q, or E;
 X_5 is S, or N;
 X_6 is V, or I;
 X_7 is S, G, or D;
 X_8 is N, T, or K;
 X_9 is D, N, or Y;
 X_{10} is V, G, or L;
 X_{11} is A, I, or H;
 X_{12} is S, or is not present;
 X_{13} is F, or is not present;
 X_{14} is M, or is not present; and
 X_{15} is N, or is not present;

CDR-L2. X_1 - X_2 - X_3 - X_4 - X_5 - X_6 - X_7 - X_8 (SEQ ID NO: 59), wherein;

X_1 is Y, or S;
 X_2 is A, or T;
 X_3 is S, or A;
 X_4 is N, H, S, or Q;
 X_5 is R, N, or S;
 X_6 is Y, Q, or I;
 X_7 is T, S, or G; and
 X_8 is S, or is not present;

and

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SEQ ID NO.:2

SEQ ID NO.:4

SEQ ID NO.:5.

SEQ ID NO.:3

and

66. An antibody conjugate comprising an antibody construct described in any one of claims 62-65, said antibody conjugate further comprising an agent selected from the group consisting of; an immunoadhesion molecule, an imaging agent, a therapeutic agent, and a cytotoxic agent.
67. The antibody conjugate according to claim 66, wherein said agent is an imaging agent selected from the group consisting of a radiolabel, an enzyme, a fluorescent label, a luminescent label, a bioluminescent label, a magnetic label, and biotin.
68. The antibody conjugate according to claim 66, wherein said imaging agent is a radiolabel selected from the group consisting of: ^3H , ^{14}C , ^{35}S , ^{90}Y , ^{99}Tc , ^{111}In , ^{125}I , ^{131}I , ^{177}Lu , ^{166}Ho , and ^{153}Sm .
69. The antibody conjugate according to claim 66, wherein said agent is a therapeutic or cytotoxic agent selected from the group consisting of; an anti-metabolite, an alkylating agent, an antibiotic, a growth factor, a cytokine, an anti-angiogenic agent, an anti-mitotic agent, an anthracycline, toxin, and an apoptotic agent.
70. The antibody construct according to claim 64, wherein said binding protein possesses a human glycosylation pattern.
71. The antibody conjugate according to claim 66, wherein said binding protein possesses a human glycosylation pattern.
72. The binding protein according to claim 3, wherein said binding protein exists as a crystal.
73. The antibody construct according to claim 62, wherein said antibody construct exists as a crystal.
74. The antibody conjugate according to claim 66, wherein said antibody construct exists as a crystal.

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108. The pharmaceutical composition of claim 107 wherein said adjuvant is hyaluronidase.
109. The pharmaceutical composition of claim 106 further comprising at least one additional therapeutic agent for treating a disorder in which IL-12 activity is detrimental.
110. The pharmaceutical composition of claim 109, wherein said additional agent is selected from the group consisting of: Therapeutic agent, imaging agent, cytotoxic agent, angiogenesis inhibitors; kinase inhibitors; co-stimulation molecule blockers; adhesion molecule blockers; anti-cytokine antibody or functional fragment thereof; methotrexate; cyclosporin; rapamycin; FK506; detectable label or reporter; a TNF antagonist; an antirheumatic; a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteroid, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, and a cytokine antagonist.
111. A method for reducing human IL-12 activity comprising contacting human IL-12 with the binding protein of any one of claims 1-61 such that human IL-12 activity is reduced.
112. A method for reducing human IL-12 activity in a human subject suffering from a disorder in which IL-12 activity is detrimental, comprising administering to the human subject the binding protein of any one of claims 1-61 such that human IL-12 activity in the human subject is reduced.
113. A method for treating a subject for a disease or a disorder in which IL-12 activity is detrimental by administering to the subject the binding protein of any one of claims 1-61 such that treatment is achieved.
114. The method of claim 113, wherein said disorder is selected from the group comprising rheumatoid arthritis, osteoarthritis, juvenile chronic arthritis, septic arthritis, Lyme arthritis, psoriatic arthritis, reactive arthritis, spondyloarthropathy, systemic lupus erythematosus, Crohn's

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disease, ulcerative colitis, inflammatory bowel disease, insulin dependent diabetes mellitus, thyroiditis, asthma, allergic diseases, psoriasis, dermatitis scleroderma, graft versus host disease, organ transplant rejection, acute or chronic immune disease associated with organ transplantation, sarcoidosis, atherosclerosis, disseminated intravascular coagulation, Kawasaki's disease, Grave's disease, nephrotic syndrome, chronic fatigue syndrome, Wegener's granulomatosis, Henoch-Schoenlein purpura, microscopic vasculitis of the kidneys, chronic active hepatitis, uveitis, septic shock, toxic shock syndrome, sepsis syndrome, cachexia, infectious diseases, parasitic diseases, acquired immunodeficiency syndrome, acute transverse myelitis, Huntington's chorea, Parkinson's disease, Alzheimer's disease, stroke, primary biliary cirrhosis, hemolytic anemia, malignancies, heart failure, myocardial infarction, Addison's disease, sporadic, polyglandular deficiency type I and polyglandular deficiency type II, Schmidt's syndrome, adult (acute) respiratory distress syndrome, alopecia, alopecia areata, seronegative arthropathy, arthropathy, Reiter's disease, psoriatic arthropathy, ulcerative colitic arthropathy, enteropathic synovitis, chlamydia, yersinia and salmonella associated arthropathy, spondyloarthropathy, atheromatous disease/arteriosclerosis, atopic allergy, autoimmune bullous disease, pemphigus vulgaris, pemphigus foliaceus, pemphigoid, linear IgA disease, autoimmune haemolytic anaemia, Coombs positive haemolytic anaemia, acquired pernicious anaemia, juvenile pernicious anaemia, myalgic encephalitis/Royal Free Disease, chronic mucocutaneous candidiasis, giant cell arteritis, primary sclerosing hepatitis, cryptogenic autoimmune hepatitis, Acquired Immunodeficiency Disease Syndrome, Acquired Immunodeficiency Related Diseases, Hepatitis B, Hepatitis C, common varied immunodeficiency (common variable hypogammaglobulinaemia), dilated cardiomyopathy, female infertility, ovarian failure, premature ovarian failure, fibrotic lung disease, cryptogenic fibrosing alveolitis, post-inflammatory interstitial lung disease, interstitial pneumonitis, connective tissue disease associated interstitial lung disease, mixed connective tissue disease associated lung disease, systemic sclerosis associated interstitial lung disease, rheumatoid arthritis associated interstitial lung disease, systemic lupus erythematosus associated lung disease, dermatomyositis/polymyositis associated lung disease, Sjögren's disease associated lung disease, ankylosing spondylitis associated lung disease, vasculitic diffuse lung disease, haemosiderosis associated lung disease, drug-induced interstitial lung disease, fibrosis, radiation fibrosis, bronchiolitis obliterans, chronic eosinophilic pneumonia, lymphocytic infiltrative lung disease, postinfectious interstitial lung disease, gouty arthritis, autoimmune hepatitis, type-1 autoimmune hepatitis (classical autoimmune or lupoid hepatitis), type-2 autoimmune hepatitis (anti-LKM antibody hepatitis), autoimmune mediated

A recombinant human enzyme for enhanced interstitial transport of therapeutics

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Received 16 March 2006; accepted 24 May 2006

Available online 7 June 2006

Abstract

Subcutaneously injected therapeutics must pass through the interstitial matrix of the skin in order to reach their intended targets. This complex, three-dimensional structure limits the type and quantity of drugs that can be administered by local injection. Here we found that depolymerization of the viscoelastic component of the interstitial matrix in animal models with a highly purified recombinant human hyaluronidase enzyme (rHuPH20) increased the dispersion of locally injected drugs, across a broad range of molecular weights without tissue distortion. rHuPH20 increased infusion rates and the pattern and extent of appearance of locally injected drugs in systemic blood. In particular, rHuPH20 changed the pharmacokinetic profiles and significantly augmented the absolute bioavailability of locally injected large protein therapeutics. Importantly, within 24 h of injection, the interstitial viscoelastic barriers were restored without histologic alterations or signs of inflammation. rHuPH20 may function as an interstitial delivery enhancing agent capable of increasing the dispersion and bioavailability of co-injected drugs that may enable subcutaneous administration of therapeutics and replace intravenous delivery.

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Keywords: Hyaluronan; Hyaluronidase; Subcutaneous drug delivery; Subcutaneous bioavailability; PH20; Monoclonal antibodies; Interferon; Adenovirus

1. Introduction

Following subcutaneous injection, most drugs must pass through the interstitial matrix, traverse capillaries or lymphatics, and reach the vascular compartment in order to reach the final site of pathology [1]. The rate by which drugs permeate the interstitium is regulated by both diffusion and convective fluid flow. The interstitial matrix is a complex three-dimensional dynamic structure that acts as a filter controlling the rate of drug flow. It is comprised of numerous structural macromolecules including collagens, elastin, and fibronectin, in which glycosaminoglycans and proteoglycans form a hydrated gel-like substance. Collagens are the predominant fibers that hold tissues

in place and maintain tissue integrity. Glycosaminoglycans such as hyaluronan contribute to the “ground substance” by creating a barrier to bulk fluid flow through the interstitial collagenous matrix by way of their viscosity and water of hydration [2]. Hyaluronan is a mega-dalton molecule consisting of repeating disaccharide units that allows the extracellular matrix to resist compressive forces. While hyaluronan is found at only 1% the concentration of collagen in the skin, it occupies a fluid exclusion volume 10-fold higher than that of collagen on a mL H₂O/mg basis [3]. In contrast to collagen, which has a half-life approaching 15 years [4], hyaluronan is rapidly turned over in the body with a half-life of 15–20 h in the skin [5,6].

“Spreading agents” derived from animal testes extracts containing interstitial matrix-degrading enzymes have been used clinically for over 50 years to facilitate the dispersion and absorption of other drugs [7]. The active agent in these testes

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extracts was later associated with hyaluronidase activity [8]. Hyaluronidases are a family of glycosaminoglycan-degrading enzymes that nature has utilized repeatedly as a delivery agent to facilitate the dispersion of venoms, toxins, bacteria and spermatozoa through interstitial spaces [9]. PH20 is the predominant hyaluronidase in mammalian testes. Among the six hyaluronidase-like genes in the human genome, only the PH20 gene product is a neutral pH-active hyaluronidase and degrades glycosaminoglycans under physiologic conditions. The human enzyme is a 509 amino acid glycoprotein anchored to the plasma membrane through a glycosyl-phosphatidylinositol (GPI) moiety that facilitates penetration of spermatozoa through the cumulus cells to enable fertilization [10].

While animal testes-derived hyaluronidase extracts have been used extensively in the clinic to disperse other injected drugs, they have generally been limited by both immunogenicity and impurity profiles, typically comprising less than 1% enzyme per mg total protein. Such preparations are frequently contaminated with proteases, immunoglobulin and factors that increase capillary permeability and can also give rise to IgE mediated allergic reactions upon repeat administration [11–13], generally precluding their repeated use.

To investigate whether a purified soluble recombinant human PH20 enzyme could mediate the same spreading properties described in the animal sourced hyaluronidase extracts, we generated a soluble form of human hyaluronidase (rHuPH20) lacking the GPI membrane attachment motif. We provide evidence that the enzyme enhanced the infusion rates and penetration of molecules up to 200 nm in diameter up to 20-fold without eliciting inflammation, vascular permeability, immunogenic or allergic reactions. In addition to increasing infusion rates and dispersion across a broad range of molecular weights, rHuPH20 was found to enhance the systemic bioavailability of locally-injected biotherapeutics such as alpha interferons and monoclonal antibodies with an absolute bioavailability comparable to the levels obtained by intravenous delivery. By increasing infusion rates, rHuPH20 also permitted injection of large volumes with minimal distortion and tearing of the subcutaneous fascia. Finally, we show that the effects of rHuPH20 were temporary and fully reversible within 24 h of injection.

2. Materials and methods

2.1. Engineering, purification, and characterization of recombinant neutral-active soluble human PH20 hyaluronidase

A construct encoding a soluble, neutral-active form of the human PH20 gene was identified from a full length human PH20 cDNA EST clone by screening progressive carboxy terminal truncations for soluble neutral active enzyme activity when transfected into CHO cells. Enzyme activity in culture supernatants was measured as described previously [14]. An expression system was constructed with a CMV-based bi-cistronic cassette driving both the soluble rHuPH20 domain (amino acids 1–447) and the murine DHFR gene separated by an internal ribosomal entry site. The selected clone was electroporated into DHFR^{-/-} CHO cells (DG44), previously adapted to grow in a chemically

defined, animal product-free medium (Invitrogen). Cells were cloned by limiting dilution and amplified in methotrexate.

Recombinant human PH20 was purified from conditioned media through a series of ion exchange, hydrophobic interaction, aminophenylboronate, and hydroxyapatite chromatography to greater than 90% purity based upon specific activity (>100,000 USP Units/mg). CHO host protein contaminants were found to be less than 1% by ELISA, and preparations had endotoxin content of less than 0.5 EU/mg protein. Enzyme potency in bulk preparations was determined by turbidimetric assay [15] with USP bovine hyaluronidase reference standard. Analysis of hyaluronan fragment sizes following digestion with rHuPH20 was performed as described previously [16].

2.2. Interstitial dispersion experiments

All animal studies were approved by IACUC. Unless specified, outbred ^{Nu/Nu} mice (Harlan) were anesthetized by intraperitoneal injection of Ketamine (150 mg/kg) and Xylazine (10 mg/kg) followed by injection of test article or carrier control (10 mM Hepes, 1 mM Calcium, 130 mM NaCl, 1 mg/mL Human Serum Albumin) in a 0.5 mL syringe with a 27 gauge needle in a final volume of 50 μ L. Test articles and controls were isotonic and ranged from pH 6.5 to 7.5. Following injection, dye areas were measured at the respective time points, under normal light (trypan blue) or black light (fluorescence) using microcalipers and measured in 2 dimensions, with areas calculated using the formula $\text{Area} = ((D_1 \times D_2) \times \pi / 4)$ (where D_1 and D_2 are the diameters of the dye front). A minimum of four injection sites per dose were tested and compared by single factor ANOVA with controls for each time point. For dermal barrier reconstitution experiments, rHuPH20, USP hyaluronidase reference standard or carrier control were injected and marked with a pen followed by injections of 50 μ L of trypan blue dye at 0.5, 1, 24, and 48 h.

2.3. Effects of rHuPH20 on IL-8 expression by microvascular endothelial cells

Human microvascular endothelial cells were grown as recommended by the supplier (Cascade Biologics). Endotoxin (purified fraction from *Escherichia coli* LPS) was purchased from Charles River Endosafe (lot EX32952), and Peptidoglycan from Staph A from Sigma. Cells were seeded into 96 well plates at 2000 viable cells/well for 16–24 h before adding test articles. Test articles were incubated with cells for 24 h followed by detection of IL-8 using a capture ELISA kit (Pharmingen).

2.4. Toxicity study of rHuPH20 administered by subcutaneous injection to non-human primates

Toxicology studies were performed at Charles River Laboratories, Inc. (Sierra Division, Sparks, NV). Forty-eight experimentally naive rhesus monkeys, 24 males, 2.6 to 8.1 years of age, weighing 3.6 to 8.6 kg and 24 females 3.2 to 7.1 years of age weighing 3.3 to 6.3 kg at the outset (Day 1) of the study were dosed with 0, 130, 3,800, and 38,000 Units of rHuPH20 in 1 mL of 10 mM Phosphate, pH 7.4 with 1 mM EDTA, 1 mM CaCl₂,

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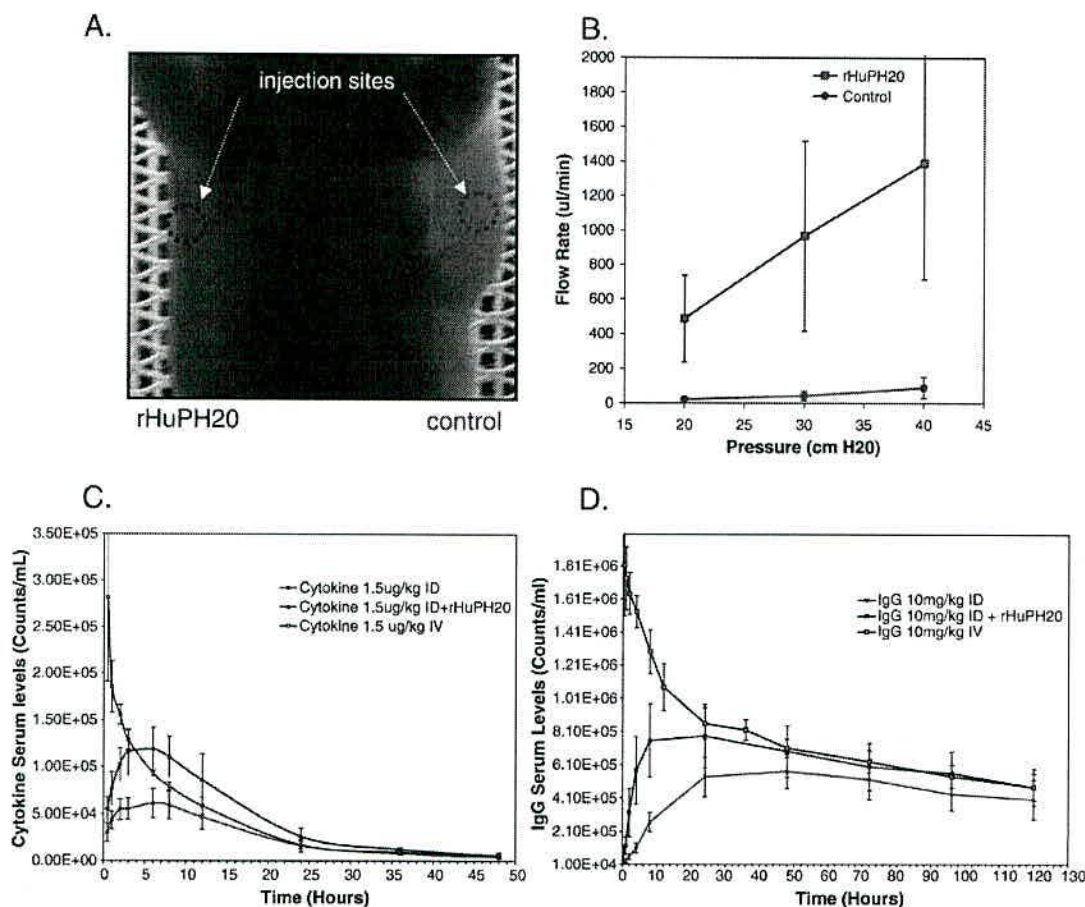


Fig. 6. rHuPH20 increases local infusion rates and speeds the appearance of locally injected drugs into the bloodstream. (A) Reduction of tissue distortion following injection of rHuPH20. Injection of 50 μ L rHuPH20 (5U) or carrier control followed by saline infusion at 300 μ L/min for 2 min in both sites by infusion pump demonstrates reduced tissue distortion with rHuPH20 compared to carrier control. (B) rHuPH20 increases infusion rates up to 20-fold in concentrated protein solutions ($P \leq 0.05$). Human serum albumin (50 mg/mL) was infused at pressures from 20–40 cm H₂O with and without hyaluronidase. Flow rates were measured at 0.1 mL intervals at each pressure ($n=3$ animals per group). (C) rHuPH20 increases C_{max} and bioavailability of a 125-I labeled cytokine injected locally. Pegylated cytokine (1.5 μ g/kg) was injected intravenously (IV) intradermally (ID) or intradermally with 100 Units rHuPH20 (ID+rHuPH20). Absolute bioavailability increased from 61% to 108% ($P < 0.001$) and C_{max} increased from 61,782 to 121,750 CPM/g ($P < 0.0003$). (D) rHuPH20 decreases T_{max} , increases C_{max} and bioavailability of a therapeutic monoclonal antibody. IgG1 (10 mg/kg) was injected intravenously (IV) intradermally (ID) or intradermally with 100 Units rHuPH20 (ID+rHuPH20). Absolute bioavailability increased from 59% to 94% ($P < 0.00001$), T_{max} decreased from 48 to 18.7 h ($P < 0.002$) and C_{max} increased from 531,866 CPM/g to 875,369 CPM/g ($P < 0.00002$).

the interstitial route and drives the pharmacokinetic profile towards an intravenous administration.

4. Discussion

While a spreading factor was first described in testes extracts over 70 years ago, its association with hyaluronidase activity in these preparations was based upon preparations containing less than 1% enzyme protein. Animal hyaluronidase containing extracts from cattle testes have been used for over 50 years to increase tissue permeability and promote the spread or dispersion of other co-injected drugs, most often in combination with local anesthetics in the setting of ophthalmic surgery. Despite their relative safety and potential for improving drug delivery, such preparations have had limited application so far, mostly due to the fact that they contain impurities in greater abundance than the animal hyaluronidase

enzyme, all of which are foreign antigens. The confounding activities of contaminants present in crude hyaluronidase preparations such as proteinases [28], anti-coagulants [29], growth factors [30], vasopermeability factors [9] immunoglobulins [31] and other unidentified components may in fact contribute to the various biological properties attributed to hyaluronidases, such as being pro-inflammatory or pro-angiogenic. To overcome the shortcomings of animal derived extracts, we generated a recombinant human form of PH20 in Chinese hamster Ovary cells. The recombinant enzyme is a soluble single chain polypeptide with N-linked glycosylation structures lacking the GPI-anchor that, like the bovine PH20, degrades hyaluronan by hydrolysis of the β 1,4 linkage between the C1 position of N-acetyl glucosamine and the C4 position of glucuronic acid. We found that rHuPH20 hyaluronidase is equally potent in its spreading activity on a Unit per Unit basis relative to the USP bovine hyaluronidase

reference standard that contains less than 1% hyaluronidase based upon specific activity.

The effects of rHuPH20 on the dispersion of other molecules in the interstitium are not anticipated from the relative abundance of glycosaminoglycans, compared to other molecules in the skin such as collagen. However, hyaluronan, through its volume of hydration and viscoelastic properties, can prevent bulk fluid flow through the collagenous interstitium [36]. By depolymerizing hyaluronan, rHuPH20 rapidly reduces its viscosity, changing the interstitial glycosaminoglycans from a gel-like phase to a fluid liquid phase, thereby making increased bulk fluid flow possible through the collagen fibers. The human body turns over nearly 1/3 of its hyaluronan (approximately 5 g) in the skin each day. As rHuPH20 has a very short half life, at least in the vascular compartment ($T_{1/2} < 1$ min), the reconstitution of hyaluronan in the interstitium within 15–20 h after injection of enzyme fits well with this model.

The clinical preparations used to date are non-human, unpurified, and can elicit immune reactions with repeat administration [12,13]. Some of the bovine preparations were reported to elicit allergic responses in as much as 10% of a naïve population [32]. In contrast to the side effects observed with testes-derived preparations, we established in cynomolgus monkeys, whose PH20 is 87% identical to the human enzyme, that rHuPH20 was well tolerated and did not elicit neutralizing antibodies. Repeated subcutaneous injections of rHuPH20 were neither inflammatory nor immunogenic based upon gross anatomy, histological and immunologic criteria, even when delivered at a dose over 500 fold above the therapeutic dose. As a result, the favorable safety profile of rHuPH20 opens the possibility to investigate co-formulation of the enzyme with therapeutic agents for the treatment and management of a range of diseases.

There is little published information regarding the spreading activity of even partially purified PH20 hyaluronidase from any species. Early studies on the mechanism of action of PH20 carried out with crude hyaluronidase enzyme preparations concluded that hyaluronidase acts principally by way of increasing hydraulic conductivity, or bulk fluid flow [33–35]. Hydrolysis of high molecular weight hyaluronan polymers by PH20 reduces their viscosity in the interstitium [36], thereby increasing the infusion rates in the interstitial matrix and facilitating bulk fluid flow. While increasing the bulk fluid flow appears to be the prevailing effect of rHuPH20 treatment, there are clearly sieving effect limitations, as shown by the results of injection of fluorescent particles of increasing size. We found that the size of the channels created in the interstitial matrix by the depletion of hyaluronan allowed the diffusion of particles up to 200 nm in diameter. Administration of PH20 does not affect the organization of interstitial matrix fibers as they are largely degraded by specific matrix metalloproteinases, including collagenases. One plausible explanation for the observed particle size limitations is that the degradation of interstitium hyaluronan by rHuPH20 results in the widening of the channels that typically exist between individual collagen fibers. The dimensions of these channels well exceed the diameter of high molecular weight therapeutic proteins (3–5 nm), monoclonal

antibodies (approximately 10 nm) and gene therapy vectors (35 nm), supporting a wide range of molecules from conventional small molecule drugs to large polypeptide therapeutics to be more consistently and predictably absorbed.

4.1. Implications for drug delivery

Many drugs must be injected intravenously due to limitations on drug solubility, bioavailability or tissue irritation. Tissue distortion, injection site pain and irritation negatively influence patient compliance for repeat subcutaneous injections. Historically, the use of crude hyaluronidase preparations was limited to acute use or emergency situations such as periorbital anesthesia, paravenous injections of toxic substances and emergency fluid hydration [37]. By increasing the infusion rates, co-injection with rHuPH20 reduced tissue distortion during injection and dispersed injected drugs into the interstitial matrix for systemic adsorption, potentially enabling subcutaneously injections of intravenous dosing volume.

Our studies also established that the pharmacokinetic profiles of macromolecule drugs injected subcutaneously could be significantly improved by co-administration with rHuPH20. We selected two biologics for their therapeutic relevance among the many therapeutic proteins that are presently marketed. In one example, we tested a long lasting peginterferon alfa-2b approved for the treatment of chronic HCV infection. Peginterferon alfa-2b is administered subcutaneously as a monotherapy or in combination with small molecular weight drugs once a week. In the second example, we tested a therapeutic antibody that blocks TNF- α . Infliximab is approved for the treatment of the inflammation of Crohn's disease, rheumatoid arthritis, and psoriatic arthritis. The antibody is administered by intravenous infusion. Comparison between the areas under the curve of either peginterferon or infliximab delivered intravascularly or locally shows that the pharmacokinetic profile and absolute bioavailability of both therapeutics were dramatically improved when co-administered with rHuPH20. As a result, peginterferon bioavailability increased from 60% to levels similar to those achieved by intravascular delivery. Similarly, Infliximab co-injected with rHuPH20 exhibited pharmacokinetic profiles that were more similar to intravenous dosing.

Importantly, the co-injection with rHuPH20 hyaluronidase permitted the administration of up to five times more volume than normally feasible for subcutaneously injected drugs, e.g., 5–10 mL vs. 1–2 mL, respectively. This may allow the conversion of intravenously-administered pharmaceuticals to more desirable local routes, and may make it possible for patients with chronic diseases to self-administer injectable medicines, thus reducing the need for hospital visits. It is also of interest to note that animal-derived hyaluronidase preparations have been historically used as an antidote to intravenous extravasation, promoting rapid resorption of necrotic drugs when intravenous lines have slipped. Similarly, rHuPH20 hyaluronidase might reduce injection site reactions for molecules that are locally toxic and improve patient compliance.

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US 20070071675A1

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(19) **United States**(12) **Patent Application Publication** (10) **Pub. No.: US 2007/0071675 A1**
Wu et al. (43) **Pub. Date: Mar. 29, 2007**(54) **DUAL VARIABLE DOMAIN
IMMUNOGLOBULIN AND USES THEREOF****Publication Classification**(76) **Inventors:** Chengbin Wu, Shrewsbury, MA (US);
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Grafton, MA (US)(51) **Int. Cl.**

<i>A61K</i>	<i>51/00</i>	(2006.01)
<i>C07H</i>	<i>21/04</i>	(2006.01)
<i>C12P</i>	<i>21/06</i>	(2006.01)
<i>C12N</i>	<i>1/21</i>	(2006.01)
<i>C12N</i>	<i>1/18</i>	(2006.01)
<i>C12N</i>	<i>5/06</i>	(2006.01)

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ABBOTT BIORESEARCH**100 RESEARCH DRIVE****WORCESTER, MA 01605-4314 (US)**(52) **U.S. Cl.** **424/1.49**; 530/388.8; 530/391.1;
530/388.22; 424/155.1; 424/178.1;
435/69.1; 435/326; 435/252.3;
435/254.21; 435/348; 536/23.53(21) **Appl. No.:** 11/507,050(22) **Filed:** Aug. 18, 2006(57) **ABSTRACT****Related U.S. Application Data**(60) Provisional application No. 60/709,911, filed on Aug.
19, 2005. Provisional application No. 60/732,892,
filed on Nov. 2, 2005.The present invention relates to engineered multivalent and
multispecific binding proteins, methods of making, and
specifically to their uses in the prevention and/or treatment
of acute and chronic inflammatory and other diseases.

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with the DVD Igs of this invention. Non-limiting examples of therapeutic agents for rheumatoid arthritis with which an antibody, or antibody portion, of the invention can be combined include the following: cytokine suppressive anti-inflammatory drug(s) (CSAIDs); antibodies to or antagonists of other human cytokines or growth factors, for example, TNF, LT, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-15, IL-16, IL-18, IL-21, IL-23, interferons, EMAP-II, GM-CSF, FGF, and PDGF. Binding proteins of the invention, or antigen binding portions thereof, can be combined with antibodies to cell surface molecules such as CD2, CD3, CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD80 (B7.1), CD86 (B7.2), CD90, CTLA or their ligands including CD154 (gp39 or CD40L).

[0200] Preferred combinations of therapeutic agents may interfere at different points in the autoimmune and subsequent inflammatory cascade; preferred examples include TNF antagonists like chimeric, humanized or human TNF antibodies, D2E7, (PCT Publication No. WO 97/29131), CA2 (Remicade™), CDP 571, and soluble p55 or p75 TNF receptors, derivatives, thereof, (p75TNFR1gG (Enbrel™) or p55TNFR1gG (Lenercept)), and also TNF α converting enzyme (TACE) inhibitors; similarly IL-1 inhibitors (Interleukin-1-converting enzyme inhibitors, IL-1RA etc.) may be effective for the same reason. Other preferred combinations include Interleukin 11. Yet another preferred combination include key players of the autoimmune response which may act parallel to, dependent on or in concert with IL-12 function; especially preferred are IL-18 antagonists including IL-18 antibodies or soluble IL-18 receptors, or IL-18 binding proteins. It has been shown that IL-12 and IL-18 have overlapping but distinct functions and a combination of antagonists to both may be most effective. Yet another preferred combination are non-depleting anti-CD4 inhibitors. Yet other preferred combinations include antagonists of the co-stimulatory pathway CD80 (B7.1) or CD86 (B7.2) including antibodies, soluble receptors or antagonistic ligands.

[0201] The binding proteins of the invention may also be combined with agents, such as methotrexate, 6-MP, azathioprine sulphasalazine, mesalazine, olsalazine chloroquine/hydroxychloroquine, pencillamine, aurothiomalate (intramuscular and oral), azathioprine, cochlincine, corticosteroids (oral, inhaled and local injection), beta-2 adrenoreceptor agonists (salbutamol, terbutaline, salmeterol), xanthines (theophylline, aminophylline), cromoglycate, nedocromil, ketotifen, ipratropium and oxitropium, cyclosporin, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, for example, ibuprofen, corticosteroids such as prednisolone, phosphodiesterase inhibitors, adenosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, agents which interfere with signalling by proinflammatory cytokines such as TNF α or IL-1 (e.g. IRAK, NIK, IKK, p38 or MAP kinase inhibitors), IL-1 β converting enzyme inhibitors, TNF α converting enzyme (TACE) inhibitors, T-cell signalling inhibitors such as kinase inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors and derivatives thereof (e.g. soluble p55 or p75 TNF receptors and the derivatives p75TNFR1gG (Enbrel™ and p55TNFR1gG (Lenercept)), sIL-1RI, sIL-1RII, sIL-6R), antiinflammatory cytokines (e.g. IL-4, IL-10, IL-11, IL-13 and TGF β), celecoxib, folic acid, hydroxychloroquine sulfate, rofecoxib, etanercept,

infliximab, naproxen, valdecoxib, sulfasalazine, methylprednisolone, meloxicam, methylprednisolone acetate, gold sodium thiomalate, aspirin, triamcinolone acetonide, propoxyphene napsylate/apap, folate, nabumetone, diclofenac, piroxicam, etodolac, diclofenac sodium, oxaprozin, oxycodone hcl, hydrocodone bitartrate/apap, diclofenac sodium/misoprostol, fentanyl, anakinra, human recombinant, tramadol hcl, salsalate, sulindac, cyanocobalamin/fa/pyridoxine, acetaminophen, alendronate sodium, prednisolone, morphine sulfate, lidocaine hydrochloride, indomethacin, glucosamine sulf/chondroitin, amitriptyline hcl, sulfadiazine, oxycodone hcl/acetaminophen, olopatadine hcl, misoprostol, naproxen sodium, omeprazole, cyclophosphamide, rituximab, IL-1 TRAP, MRA, CTLA4-IG, IL-18 BP, anti-IL-18, Anti-IL15, BIRB-796, SCIO-469, VX-702, AMG-548, VX-740, Roflumilast, IC-485, CDC-801, and Mesopram. Preferred combinations include methotrexate or leflunomide and in moderate or severe rheumatoid arthritis cases, cyclosporine.

[0202] Nonlimiting additional agents which can also be used in combination with a binding protein to treat rheumatoid arthritis include, but are not limited to, the following: non-steroidal anti-inflammatory drug(s) (NSAIDs); cytokine suppressive anti-inflammatory drug(s) (CSAIDs); CDP-571/BAY-10-3356 (humanized anti-TNF α antibody; Celltech/Bayer); cA2/infliximab (chimeric anti-TNF α antibody; Centocor); 75 kDTNFR-IgG/etanercept (75 kD TNF receptor-IgG fusion protein; Immunex; see e.g., *Arthritis & Rheumatism* (1994) Vol. 37, S295; *J. Invest. Med.* (1996) Vol. 44, 235A); 55 kDTNFR-IgG (55 kD TNF receptor-IgG fusion protein; Hoffmann-LaRoche); IDEC-CE9.1/SB 210396 (non-depleting primatized anti-CD4 antibody; IDEC/SmithKline; see e.g., *Arthritis & Rheumatism* (1995) Vol. 38, S185); DAB 486-IL-2 and/or DAB 389-IL-2 (IL-2 fusion proteins; Seragen; see e.g., *Arthritis & Rheumatism* (1993) Vol. 36, 1223); Anti-Tac (humanized anti-IL-2R α ; Protein Design Labs/Roche); IL-4 (anti-inflammatory cytokine; DNAX/Schering); IL-10 (SCH 52000; recombinant IL-10, anti-inflammatory cytokine; DNAX/Schering); IL-4; IL-10 and/or IL-4 agonists (e.g., agonist antibodies); IL-1RA (IL-1 receptor antagonist; Synergen/Amgen); anakinra (Kineret®/Amgen); TNF-bp/s-TNF (soluble TNF binding protein; see e.g., *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S284; *Amer. J. Physiol.—Heart and Circulatory Physiology* (1995) Vol. 268, pp. 37-42); R973401 (phosphodiesterase Type IV inhibitor; see e.g., *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S282); MK-966 (COX-2 Inhibitor; see e.g., *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S81); Iloprost (see e.g., *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S82); methotrexate; thalidomide (see e.g., *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S282) and thalidomide-related drugs (e.g., Celgen); leflunomide (anti-inflammatory and cytokine inhibitor; see e.g., *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S131; *Inflammation Research* (1996) Vol. 45, pp. 103-107); tranexamic acid (inhibitor of plasminogen activation; see e.g., *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S284); T-614 (cytokine inhibitor; see e.g., *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S282); prostaglandin E1 (see e.g., *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S282); Tenidap (non-steroidal anti-inflammatory drug; see e.g., *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S280);

-continued

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Asn Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Phe
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Ile Tyr Thr Ala Ser Thr Arg Ala Thr Asp Ile Pro Ala Arg Phe Ser
             165             170             175

Gly Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln
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Ser Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Asn Asn Trp Pro
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Ser Ile Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Arg
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<223> OTHER INFORMATION: Sequence of 1D4.1 VL region

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<400> SEQUENCE: 117

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             20             25             30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile
             35             40             45

Tyr Tyr Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg Phe Ser Gly
             50             55             60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Ala
             65             70             75             80

Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Asp Tyr Asn Ser Pro Trp
             85             90             95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
             100             105

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We claim:

1. A binding protein comprising a polypeptide chain, wherein said polypeptide chain comprises VD1-(X1)_n-VD2-C-(X2)_n, wherein VD1 is a first variable domain, VD2 is a second variable domain, C is a constant domain, X1 represents an amino acid or polypeptide, X2 represents an Fc region and n is 0 or 1.

2. The binding protein according to claim 1, wherein said VD1 and VD2 are heavy chain variable domains.

3. The binding protein according to claim 2, wherein said heavy chain variable domain is selected from the group consisting of a murine heavy chain variable domain, a human heavy chain variable domain, a CDR grafted heavy chain variable domain, and a humanized heavy chain variable domain.

4. The binding protein according to claim 2, wherein VD1 and VD2 are capable of binding the same antigen.

5. The binding protein according to claim 2, wherein VD1 and VD2 are capable of binding different antigens.

6. The binding protein according to claim 2, wherein C is a heavy chain constant domain.

7. The binding protein according to claim 6, wherein X1 is a linker with the proviso that X1 is not CH1.

8. The binding protein according to claim 7, wherein the linker is selected from the group consisting of AKTTP-KLEEGEFSEAR; AKTTPKLEEGEFSEARV; AKTTP-KLGG; SAKTTPKLGG; AKTTPKLEEGEFSEARV; SAKTTP; SAKTTPKLGG; RADAAP; RADAAPTVS; RADAAAAGGPGS; RADAAA(G₄S)₄; SAKTTP; SAKTTPKLGG; SAKTTPKLEEGEFSEARV; ADAAP; ADAAPTVSIFPP; TVAAP; TVAAPSVFIFPP; QPKAAP; QPKAAPSVTLFPP; AKTTPP; AKTTPPSVTPLAP; AKT-TAP; AKTTAPSVYPLAP; ASTKGP; ASTKGPSVFPLAP; GGGGGSGGGSGGGGS; GENKVEYAPALMALS; GPA-KELTPLKEAKVS; and GHEAAAVMQVQYPAS.

9. The binding protein according to claim 7, wherein X2 is an Fc region.

10. The binding protein according to claim 9, wherein said Fc region is a variant Fc region.

11. A binding protein comprising a polypeptide chain, wherein said polypeptide chain comprises VD1-(X1)_n-VD2-C-(X2)_n, wherein VD1 is a first heavy chain variable

domain, VD2 is a second heavy chain variable domain, C is a heavy chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 is an Fc region.

12. The binding protein according to claim 1, wherein said VD1 and VD2 are light chain variable domains.

13. The binding protein according to claim 12, wherein said light chain variable domain is selected from the group consisting of a murine light chain variable domain, a human light chain variable domain, a CDR grafted light chain variable domain, and a humanized light chain variable domain.

14. The binding protein according to claim 12, wherein VD1 and VD2 are capable of binding the same antigen.

15. The binding protein according to claim 12, wherein VD1 and VD2 are capable of binding different antigens.

16. The binding protein according to claim 12, wherein C is a light chain constant domain.

17. The binding protein according to claim 16, wherein X1 is a linker with the proviso that X1 is not CL1.

18. The binding protein according to claim 17, wherein the linker is selected from the group consisting of AKTTP-KLEEGEFSEAR; AKTTPKLEEGEFSEARV; AKTTP-KLGG; SAKTTPKLGG; AKTTPKLEEGEFSEARV; SAKTTP; SAKTTPKLGG; RADAAP; RADAAPTVS; RADAAAAGGPGS; RADAAAA(G₃S)₄; SAKTTP; SAKTTPKLGG; SAKTTPKLEEGEFSEARV; ADAAP; ADAAPTIVSIFPP; TVAAP; TVAAPSVIFPP; QPKAAP; QPKAAPSVTLFPP; AKTTPP; AKTTPPSVTPLAP; AKT-TAP; AKTTAPSVYPLAP; ASTKGP; ASTKGPSVEPLAP; GGGGSGGGGSGGGGS; GENKVEYAPALMALS; GPA-KELTPLKEAKVS; and GHEAAVMQVQYPAS.

19. The binding protein according to claim 17, wherein the binding protein does not comprise X2.

20. A binding protein comprising a polypeptide chain, wherein said polypeptide chain comprises VD1-(X1)_n-VD2-C-(X2)_n, wherein VD1 is a first light chain variable domain, VD2 is a second light chain variable domain, C is a light chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 does not comprise an Fc region.

21. A binding protein comprising first and second polypeptide chains, wherein said first polypeptide chain comprises VD1-(X1)_n-VD2-C-(X2)_n, wherein VD1 is a first heavy chain variable domain, VD2 is a second heavy chain variable domain, C is a heavy chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 is an Fc region; and said second polypeptide chain comprises VD1-(X1)_n-VD2-C-(X2)_n, wherein VD1 is a first light chain variable domain, VD2 is a second light chain variable domain, C is a light chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 does not comprise an Fc region.

22. A binding protein comprising four polypeptide chains, wherein two polypeptide chains comprise VD1-(X1)_n-VD2-C-(X2)_n, wherein VD1 is a first heavy chain variable domain, VD2 is a second heavy chain variable domain, C is a heavy chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 is an Fc region; and two polypeptide chains comprise VD1-(X1)_n-VD2-C-(X2)_n, wherein VD1 is a first light chain variable domain, VD2 is a second light chain variable domain, C is a light chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 does not comprise an Fc region.

23. The binding protein according to claim 1, wherein said binding protein is capable of binding one or more targets.

24. The binding protein according to claim 23, wherein the target is selected from the group consisting of ABCF1; ACVR1; ACVR1B; ACVR2; ACVR2B; ACVRL1; ADORA2A; Aggrecan; AGR2; AICDA; AIF1; AIG1; AKAP1; AKAP2; AMH; AMHR2; ANGPT1; ANGPT2; ANGPTL3; ANGPTL4; ANPEP; APC; APOC1; AR; AZGP1 (zinc-α-glycoprotein); B7.1; B7.2; BAD; BAFF; BAG1; BAI1; BCL2; BCL6; BDNF; BLNK; BLR1 (MDR15); BlyS; BMP1; BMP2; BMP3B (GDF10); BMP4; BMP6; BMP8; BMPR1A; BMPR1B; BMPR2; BPAG1 (plectin); BRCA1; C19orf10 (IL27w); C3; C4A; C5; C5R1; CANT1; CASP1; CASP4; CAV1; CCBP2 (D6/JAB61); CCL1 (I-309); CCL11 (eotaxin); CCL13 (MCP-4); CCL15 (MIP-1d); CCL16 (HCC-4); CCL17 (TARC); CCL18 (PARC); CCL19 (MIP-3b); CCL2 (MCP-1); MCAF; CCL20 (MIP-3a); CCL21 (MIP-2); SLC; exodus-2; CCL22 (MDC/STC-1); CCL23 (MPIF-1); CCL24 (MPIF-2/eotaxin-2); CCL25 (TECK); CCL26 (eotaxin-3); CCL27 (CTACK/ILC); CCL28; CCL3 (MIP-1a); CCL4 (MIP-1b); CCL5 (RANTES); CCL7 (MCP-3); CCL8 (mcp-2); CCNA1; CCNA2; CCND1; CCNE1; CCNE2; CCR1 (CKR1/HM145); CCR2 (mcp-1RB/RA); CCR3 (CKR3/CMKBR3); CCR4; CCR5 (CMKBR5/ChemR13); CCR6 (CMKBR6/CKR-L3/STRL22/DRY6); CCR7 (CKR7/EB11); CCR8 (CMKBR8/TER1/CKR-L1); CCR9 (GPR-9-6); CCRL1 (VSHK1); CCRL2 (L-CCR); CD164; CD19; CD1C; CD20; CD200; CD-22; CD24; CD28; CD3; CD37; CD38; CD3E; CD3G; CD3Z; CD4; CD40; CD40L; CD44; CD45RB; CD52; CD69; CD72; CD74; CD79A; CD79B; CD8; CD80; CD81; CD83; CD86; CDH1 (E-cadherin); CDH10; CDH12; CDH13; CDH18; CDH19; CDH20; CDH5; CDH7; CDH8; CDH9; CDK2; CDK3; CDK4; CDK5; CDK6; CDK7; CDK9; CDKN1A (p21Wap1/Cip1); CDKN1B (p27Kip1); CDKN1C; CDKN2A (p16INK4a); CDKN2B; CDKN2C; CDKN3; CEBPB; CER1; CHGA; CHGB; Chitinase; CHST10; CKLFSF2; CKLFSF3; CKLFSF4; CKLFSF5; CKLFSF6; CKLFSF7; CKLFSF8; CLDN3; CLDN7 (claudin-7); CLN3; CLU (clusterin); CMKLR1; CMKOR1 (RDC1); CNR1; COL18A1; COL1A1; COL4A3; COL6A1; CR2; CRP; CSF1 (M-CSF); CSF2 (GM-CSF); CSF3 (G-CSF); CTLA4; CTNND1 (b-catenin); CTSB (cathepsin B); CX3CL1 (SCYD1); CX3CR1 (V28); CXCL1 (GRO1); CXCL10 (IP-10); CXCL11 (I-TAC/IP-9); CXCL12 (SDF1); CXCL13; CXCL14; CXCL16; CXCL2 (GRO2); CXCL3 (GRO3); CXCL5 (ENA-78/LIX); CXCL6 (GCP-2); CXCL9 (MIG); CXCR3 (GPR9/CKR-L2); CXCR4; CXCR6 (TYMSTR/STRL33/Bonzo); CYB5; CYC1; CYSLTR1; DAB2IP; DES; DKFZp451J0118; DNCL1; DPP4; E2F1; ECGF1; EDG1; EFNA1; EFNA3; EFNB2; EGF; EGFR; ELAC2; ENG; ENO1; ENO2; ENO3; EPHB4; EPO; ERBB2 (Her-2); EREG; ERK8; ESR1; ESR2; F3 (TF); FADD; FasL; FASN; FCER1A; FCER2; FCGR3A; FGF; FGF1 (aFGF); FGF10; FGF11; FGF12; FGF12B; FGF13; FGF14; FGF16; FGF17; FGF18; FGF19; FGF2 (bFGF); FGF20; FGF21; FGF22; FGF23; FGF3 (int-2); FGF4 (iHST); FGF5; FGF6 (HST-2); FGF7 (KGF); FGF8; FGF9; FGF3R; FIGF (VEGF); FIL1 (EPSILON); FIL1 (ZETA); FLJ12584; FLJ25530; FLRT1 (fibronectin); FLT1; FOS; FOSL1 (FRA-1); FY (DARC); GABRP (GABA_A); GAGEB1; GAGEC1; GALNAC4S-6ST; GATA3; GDF5; GF11; GGT1; GM-CSF; GNAS1; GNRH1; GPR2 (CCR10); GPR31; GPR44; GPR81 (TKSG80); GRCC10 (C10); GRP; GSN (Gelsolin); GSTP1; HAVCR2; HDAC4; HDAC5; HDAC7A; HDAC9;

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**Cámara de
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NUMERO DE RADICACION: 20170270641-PRI

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PAGINAS: 1 - 9

REPUBLICA DE COLOMBIA

CERTIFICADO DE EXISTENCIA Y REPRESENTACION
EL SUSCRITO SECRETARIO DE LA CAMARA DE COMERCIO DE CALI

CERTIFICA

DENOMINACIÓN O RAZÓN SOCIAL: LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A.S.
SIGLA: LAFRANCOL S.A.S.
NIT. 890301463-8
DOMICILIO: CALI
AFILIADO.

CERTIFICA

DIRECCIÓN DEL DOMICILIO PRINCIPAL: CRA. 1 NRO. 46 84
MUNICIPIO: CALI-VALLE
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TELÉFONO COMERCIAL 2: 6877700
TELÉFONO COMERCIAL 3: 3155598201
FAX: 6877790
CORREO ELECTRÓNICO: lafrancolsas@lafrancol.com

DIRECCIÓN PARA NOTIFICACIÓN JUDICIAL: CRA. 1 NRO. 46 84
MUNICIPIO: CALI-VALLE
TELÉFONO PARA NOTIFICACIÓN 1: 6877700
TELÉFONO PARA NOTIFICACIÓN 2: 6877700
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FAX PARA NOTIFICACIÓN: 6877790
CORREO ELECTRÓNICO DE NOTIFICACIÓN: lafrancolsa@lafrancol.com

CERTIFICA

MATRÍCULA MERCANTIL: 5233-16
FECHA DE MATRÍCULA EN ESTA CAMARA : 02 DE OCTUBRE DE 1958
ÚLTIMO AÑO RENOVADO: 2017

CERTIFICA

ACTIVIDAD PRINCIPAL
C2100 FABRICACIÓN DE PRODUCTOS FARMACÉUTICOS, SUSTANCIAS QUÍMICAS MEDICINALES PRODUCTOS
BOTÁNICOS DE USO FARMACÉUTICO

ACTIVIDAD SECUNDARIA
C1089 ELABORACIÓN DE OTROS PRODUCTOS ALIMENTICIOS N.C.P.

OTRAS ACTIVIDADES
C1811 ACTIVIDADES DE IMPRESIÓN
N8299 OTRAS ACTIVIDADES DE SERVICIO DE APOYO A LAS EMPRESAS N.C.P.

CERTIFICA

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

CERTIFICA

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

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Cámara de Comercio de Cali

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NUMERO DE RADICACION: 20170270641-PRI

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CERTIFICA

QUE POR ACTA NRO. 124 DEL 30 DE MARZO DE 2012 ASAMBLEA GENERAL , INSCRITA EN LA CAMARA DE COMERCIO EL 31 DE MAYO DE 2012 BAJO EL NRO. 6645 DEL LIBRO IX , SE TRANSFORMO DE SOCIEDAD ANÓNIMA EN SOCIEDAD POR ACCIONES SIMPLIFICADA BAJO EL NOMBRE DE LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A.S. SIGLA: LAFRANCOL S.A.S. .

CERTIFICA

QUE POR ACTA NRO. 124 DEL 30 DE MARZO DE 2012 ASAMBLEA DE ACCIONISTAS , INSCRITA EN LA CAMARA DE COMERCIO EL 03 DE DICIEMBRE DE 2012 BAJO EL NRO. 14136 DEL LIBRO IX , SE APROBO LA ESCISION ENTRE (ESCIDENTE) AMERICAN GENERICS S.A.S. Y (BENEFICIARIA(S)) LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A.S. .

CERTIFICA

REFORMAS

DOCUMENTO	FECHA.DOC	ORIGEN	FECHA.INS	NRO.INS	LIBRO
E.P. 1104	30/06/1959	NOTARIA CUARTA DE CALI	07/07/1959	19467	
E.P. 2309	26/12/1959	NOTARIA CUARTA DE CALI	29/12/1959	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
E.P. 7222	14/12/1988	NOTARIA TERCERA DE CALI	19/12/1988	13847	IX
E.P. 4530	04/09/1989	NOTARIA TERCERA DE CALI	07/09/1989	21508	IX
E.P. 3865	02/08/1990	NOTARIA TERCERA DE CALI	13/08/1990	31708	IX
E.P. 8305	13/12/1995	NOTARIA TERCERA DE CALI	23/01/1996	557	IX
E.P. 4559	16/11/1999	NOTARIA TERCERA DE CALI	26/11/1999	7911	IX
E.P. 6.004	02/12/2004	NOTARIA TERCERA DE CALI	14/12/2004	13371	IX
E.P. 6241	27/12/2006	NOTARIA TERCERA DE CALI	19/01/2007	625	IX
E.P. 389	07/02/2008	NOTARIA TERCERA DE CALI	19/02/2008	1880	IX
E.P. 1907	11/05/2009	NOTARIA TERCERA DE CALI	26/05/2009	5950	IX
E.P. 3672	24/08/2009	NOTARIA TERCERA DE CALI	27/08/2009	9876	IX
ACT 116	04/03/2010	ASAMBLEA GRAL ORDINARIA	11/05/2010	5470	IX
E.P. 4421	21/10/2010	NOTARIA TERCERA DE CALI	26/10/2010	12622	IX
E.P. 1786	16/05/2011	NOTARIA TERCERA DE CALI	24/05/2011	6318	IX
ACT 124	30/03/2012	ASAMBLEA GENERAL	31/05/2012	6645	IX
ACT 125	27/07/2012	ASAMBLEA GENERAL DE ACCIONISTAS	23/08/2012	10177	IX
ACT 127	12/12/2012	ASAMBLEA GENERAL DE ACCIONISTAS	13/12/2012	14600	IX
ACT 131	17/06/2013	ASAMBLEA GENERAL DE ACCIONISTAS	24/06/2013	7217	IX
ACT 134	26/09/2014	ASAMBLEA DE ACCIONISTAS	17/10/2014	13923	IX
ACT 135	02/10/2014	ASAMBLEA DE ACCIONISTAS	17/10/2014	13928	IX

CERTIFICA

VIGENCIA: INDEFINIDA



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CERTIFICA

OBJETO SOCIAL. LA SOCIEDAD EN DESARROLLO DE SU OBJETO SOCIAL PODRÁ REALIZAR, ENTRE OTROS, LAS SIGUIENTES ACTIVIDADES COMERCIALES Y EMPRESARIALES: 2.1) FABRICACIÓN, DISTRIBUCIÓN Y VENTA EN EL TERRITORIO DE LA REPÚBLICA DE COLOMBIA O EN EL EXTRANJERO DE CUALESQUIERA TIPOS DE VACUNAS, PRODUCTOS QUÍMICOS, FARMACÉUTICOS, ALIMENTICIOS FUNCIONALES, COSMÉTICOS Y FISIOLÓGICOS, REACTIVOS, ANTIBIÓTICOS PARA USO HUMANO Y VETERINARIO, PRODUCTOS DIETÉTICOS, COLORANTES, DE PERFUMERÍA, DISPOSITIVOS MÉDICOS Y AFINES. 2.2) LA REPRESENTACIÓN DE CASAS FABRICANTES NACIONALES O EXTRANJERAS DE ESTOS MISMOS PRODUCTOS. 2.3) FABRICACIÓN, IMPORTACIÓN, DISTRIBUCIÓN Y VENTA DE CUALESQUIERA TIPO DE PRODUCTOS VETERINARIOS. 2.4) LA REALIZACIÓN DE TODOS LOS ACTOS, CONTRATOS, CONVENIOS, DIRECTAMENTE RELACIONADOS CON LOS FINES ANTES INDICADOS. 2.5) IMPORTAR CUALESQUIERA TIPOS DE VACUNAS, PRODUCTOS QUÍMICOS, FARMACÉUTICOS, ALIMENTICIOS FUNCIONALES, COSMÉTICOS Y FISIOLÓGICOS, REACTIVOS, ANTIBIÓTICOS PARA USO HUMANO Y VETERINARIO, PRODUCTOS DIETÉTICOS, COLORANTES, DE PERFUMERÍA, DISPOSITIVOS MÉDICOS Y AFINES Y/O PRODUCTOS INTERMEDIOS O A GRANEL. PARÁGRAFO UNO: 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 EN MUTUO DINERO Y CELEBRAR LOS CONTRATOS COMERCIALES DE CAMBIO EN TODAS SUS MANIFESTACIONES, FUNDAR EMPRESAS QUE TENGAN O NO LA MISMA FINALIDAD SOCIAL O ASOCIARSE CON ELLAS, O CUALESQUIERA OTRAS EMPRESAS DE OTROS OBJETOS SOCIALES. PARÁGRAFO DOS: ADEMÁS DE LAS ACTIVIDADES REFERIDAS EN ESTE ARTICULO, Y LAS ENUNCIADAS EN EL PARÁGRAFO UNO, COMO SOCIEDAD POR ACCIONES SIMPLIFICADA, PODRÁ REALIZAR CUALQUIER ACTIVIDAD LICITA DE COMERCIO EN EL PAÍS O EN EL EXTERIOR, POR EJEMPLO, Y DE MANERA ILUSTRATIVA MÁS NO LIMITATIVA, GESTIÓN DE LICENCIAS, REGISTROS DE EXPORTACIÓN O IMPORTACIÓN, CONVENIOS RELATIVOS A ESAS ACTIVIDADES Y LOS ACTOS RELACIONADOS CON EL MISMO. PARÁGRAFO TRES: POR RAZÓN DE SU OBJETO SOCIAL, LA SOCIEDAD NO PODRÁ CONSTITUIRSE GARANTE NI FIADORA DE OBLIGACIONES DISTINTAS DE LAS PROPIAS NI GARANTIZAR CON SUS BIENES OBLIGACIONES DE TERCEROS, A MENOS QUE LO AUTORICE EN CADA CASO LA ASAMBLEA GENERAL DE ACCIONISTAS CON EL VOTO DE LA TOTALIDAD DE LOS MIEMBROS PRESENTES EN LA REUNIÓN CORRESPONDIENTE.

CERTIFICA

DIRECCIÓN Y ADMINISTRACIÓN. LA DIRECCIÓN Y ADMINISTRACIÓN DE LA SOCIEDAD ESTARÁ A CARGO DE: A) LA ASAMBLEA GENERAL DE ACCIONISTAS; Y B) LOS REPRESENTANTES LEGALES COMO SE DESCRIBEN EN SUS CARGOS Y FUNCIONES EN LOS PRESENTES ESTATUTOS.

REPRESENTACIÓN LEGAL. ADMINISTRACIÓN. LA ADMINISTRACIÓN DE LA SOCIEDAD CORRESPONDE AL REPRESENTANTE LEGAL, QUE SE DENOMINARÁ GERENTE, QUIEN TENDRÁ HASTA CINCO SUPLENTE. EL GERENTE Y SUS SUPLENTE SERÁN LOS REPRESENTANTES LEGALES QUIENES SERÁN DESIGNADOS POR LA ASAMBLEA GENERAL DE ACCIONISTAS POR UN TÉRMINO INDEFINIDO Y SE MANTENDRÁN VIGENTES HASTA TANTO ESTOS NO SEAN REMOVIDOS DE SUS CARGOS POR LA ASAMBLEA GENERAL DE ACCIONISTAS. LOS SUPLENTE REEMPLAZARÁN AL GERENTE, DURANTE SUS AUSENCIAS TEMPORALES, ABSOLUTAS O ACCIDENTALES.

FUNCIONES DEL REPRESENTANTE LEGAL.: SON FUNCIONES DEL GERENTE: (A) REPRESENTAR A LA SOCIEDAD ANTE LOS ACCIONISTAS, ANTE TERCEROS Y ANTE AUTORIDADES DE LOS ÓRDENES ADMINISTRATIVO O JURISDICCIONAL, CON FACULTADES PARA TRANSIGIR, COMPROMETER Y DESISTIR Y PARA COMPARECER EN JUICIO; (B) SUPERVISAR EL COMPORTAMIENTO DE LOS EMPLEADOS DE LA SOCIEDAD PARA ASEGURAR EL CUMPLIMIENTO DE SUS OBLIGACIONES. (C) CUMPLIR Y HACER CUMPLIR LAS DETERMINACIONES DE LA ASAMBLEA GENERAL DE ACCIONISTAS. (D) PRESENTAR, EN LAS REUNIONES ORDINARIAS DE LA ASAMBLEA GENERAL DE ACCIONISTAS, EL BALANCE GENERAL DE LOS NEGOCIOS DEL RESPECTIVO EJERCICIO, JUNTO CON EL ESTADO DE RESULTADOS, UN PROYECTO SOBRE DISTRIBUCIÓN DE UTILIDADES O SOBRE CANCELACIÓN DE PÉRDIDAS, JUNTO CON UN INFORME SOBRE EL ESTADO DE LOS NEGOCIOS DE LA SOCIEDAD. (E) SUMINISTRAR A LA ASAMBLEA GENERAL DE ACCIONISTAS LOS INFORMES QUE ÉSTAS LE SOLICITEN. (F) OTORGAR LOS PODERES QUE SEAN NECESARIOS PARA LA DEFENSA O REPRESENTACIÓN DE LA SOCIEDAD. (G) SOMETER LAS DIFERENCIAS QUE PUEDAN SURGIR ENTRE LA SOCIEDAD Y TERCEROS A LA DECISIÓN DE ÁRBITROS Y CONCURRIR A



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LA DESIGNACIÓN DE DICHS ÁRBITROS Y DEL APODERADO QUE HAYA DE REPRESENTARLA ANTE EL RESPECTIVO TRIBUNAL. (H) EJERCER LIBREMENTE TODOS LOS ACTOS Y ACUERDOS APROPIADOS Y NECESARIOS PARA EL CUMPLIMIENTO DEL OBJETO SOCIAL, Y (I) EJERCER LAS DEMÁS ATRIBUCIONES QUE LE CONFIEREN LAS LEYES Y LOS ESTATUTOS SOCIALES Y LAS DEMÁS QUE LE CORRESPONDAN EN RAZÓN DE LA NATURALEZA DE SU CARGO.

EL GERENTE REQUIERE AUTORIZACIÓN PREVIA DE LA ASAMBLEA GENERAL DE ACCIONISTAS, PARA LLEVAR A CABO LOS SIGUIENTES ACTOS:

1. CELEBRAR TODA CLASE DE CONTRATOS CUYO OBJETO SEA LA APERTURA DE SUCURSALES, AGENCIAS O DEPENDENCIAS, O LA EMISIÓN DE BONOS DE LA SOCIEDAD.

2. APROBAR, CUALQUIERA SEA SU NATURALEZA, TODOS LOS ACTOS QUE IMPLIQUEN RENUNCIA A DERECHOS DE LA SOCIEDAD, TALES COMO TRANSACCIONES, DESISTIMIENTOS, CONCILIACIONES O ACTOS DE SIMILAR NATURALEZA, CUYA CUANTÍA ABSOLUTA SEA SUPERIOR AL EQUIVALENTE EN MONEDA LEGAL COLOMBIANA A LA SUMA DE QUINIENTOS MIL DÓLARES DE LOS ESTADOS UNIDOS DE AMÉRICA (USD \$500.000.00). EN LOS ASUNTOS DE NATURALEZA LABORAL ESTE REQUISITO SE EXIGIRÁ CUANDO LA REMUNERACIÓN ANUAL DEL RESPECTIVO TRABAJADOR SEA SUPERIOR A SEISCIENTOS CINCUENTA SALARIOS MÍNIMOS LEGALES MENSUALES VIGENTES (650 SMLMV) DE LA REPÚBLICA DE COLOMBIA.

3. INICIAR CUALQUIER ACCIÓN JUDICIAL O ADMINISTRATIVA CUYA CUANTÍA ABSOLUTA SEA SUPERIOR AL EQUIVALENTE EN MONEDA LEGAL COLOMBIANA A LA SUMA DE QUINIENTOS MIL DÓLARES DE LOS ESTADOS UNIDOS DE AMÉRICA (USD \$500.000.00).

4. SUSCRIBIR O REALIZAR CUALQUIER CONTRATO, ACUERDO, ACTO U OPERACIÓN PARA LA ADQUISICIÓN, VENTA, CESIÓN, TRASPASO O DISPOSICIÓN DE ACTIVOS FIJOS DE LA SOCIEDAD CUYO VALOR EN PESOS COLOMBIANOS, O EN CUALQUIER OTRA MONEDA EXCEDA DE UN MILLÓN DE DÓLARES DE LOS ESTADOS UNIDOS DE AMÉRICA (USD \$1.000.000).

5. GRAVAR O ASUMIR CUALQUIER GRAVAMEN SOBRE LOS ACTIVOS FIJOS DE LA SOCIEDAD, CUALQUIERA QUE SEA SU VALOR.

6. OTORGAR GARANTÍAS POR PARTE DE LA SOCIEDAD PARA GARANTIZAR OBLIGACIONES DE TERCEROS, SALVO POR/AS OBLIGACIONES DE COMPAÑÍAS FINALES DE LA SOCIEDAD.

7. LAS OPERACIONES FINANCIERAS Y BANCARIAS DEBEN REALIZARSE DE ACUERDO CON LAS POLÍTICAS ESTABLECIDAS POR LA ASAMBLEA GENERAL DE ACCIONISTAS, QUIEN CONFORMARÁ UN COMITÉ DE FINANZAS, PARA ESTE FIN.

PARA CALCULAR EL EQUIVALENTE DE LOS MONTOS ANTERIORES EN PESOS COLOMBIANOS, SERÁ USADA LA TASA REPRESENTATIVA DEL MERCADO (TRM) CERTIFICADA POR LA SUPERINTENDENCIA FINANCIERA O LA ENTIDAD COMPETENTE PARA EL DÍA EN QUE SE SUSCRIBA O REALICE LA TRANSACCIÓN.

CERTIFICA

DOCUMENTO: ACTA No. 134 DEL 26 DE SEPTIEMBRE DE 2014

ORIGEN: ASAMBLEA DE ACCIONISTAS

INSCRIPCION: 17 DE OCTUBRE DE 2014 No. 13926 DEL LIBRO IX

FUE(ON) NOMBRADO(S):

GERENTE

MANUEL CAMILO CAMACHO PEREZ

C.C.80504680

PRIMER SUPLENTE DEL GERENTE

MARIO FRANCISCO PEREZ ANZOLA



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C.C.79656956

SEGUNDO SUPLENTE DEL GERENTE
MARIA ISABEL RIVAS PARRA
C.C.52251884

CERTIFICA

DOCUMENTO: ACTA No. 135 DEL 02 DE OCTUBRE DE 2014
ORIGEN: ASAMBLEA DE ACCIONISTAS
INSCRIPCION: 17 DE OCTUBRE DE 2014 No. 13929 DEL LIBRO IX

FUE(ON) NOMBRADO(S):

TERCER SUPLENTE DEL GERENTE
FRANCISCO JAVIER PICART LAURENZ
C.E.399661

CUARTO SUPLENTE DEL GERENTE
TITO NOE PARRA MURILLO
C.C.79579680

CERTIFICA

DOCUMENTO: ACTA No. 139 DEL 23 DE NOVIEMBRE DE 2015
ORIGEN: ASAMBLEA DE ACCIONISTAS
INSCRIPCION: 07 DE DICIEMBRE DE 2015 No. 23664 DEL LIBRO IX

FUE(ON) NOMBRADO(S):

REVISOR FISCAL
ERNST & YOUNG AUDIT S.A.S.
NIT.860008890-5

CERTIFICA

DOCUMENTO: DOCUMENTO PRIVADO DEL 08 DE JUNIO DE 2016
ORIGEN: ERNST & YOUNG AUDIT S.A.S
INSCRIPCION: 17 DE JUNIO DE 2016 No. 9884 DEL LIBRO IX

FUE(ON) NOMBRADO(S):

REVISOR FISCAL PRINCIPAL
MANUEL ANTONIO RAMIREZ YANTEN
C.C.1117496898

REVISOR FISCAL SUPLENTE
CAROLINA MEJIA SALGADO
C.C.1130620779



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CERTIFICA

CAPITAL AUTORIZADO: \$40,000,000,224
NUMERO DE ACCIONES: 3,333,333,352
VALOR NOMINAL: \$12
CAPITAL SUSCRITO: \$24,140,480,940
NUMERO DE ACCIONES: 2,011,706,745
VALOR NOMINAL: \$12
CAPITAL PAGADO: \$24,140,480,940
NUMERO DE ACCIONES: 2,011,706,745
VALOR NOMINAL: \$12

CERTIFICA

CERTIFICA ESPECIAL POR SOLICITUD DEL USUARIO:

DOCUMENTO: DOCUMENTO PRIVADO DEL 22 ENERO DEL 2015
INSCRIPCIÓN: 11 DE FEBRERO DE 2015 NRO. 1943 DEL LIBRO IX

DOCUMENTO: DOCUMENTO PRIVADO DEL 07 DE FEBRERO DEL 2017
INSCRIPCIÓN: 17 DE FEBRERO DE 2017 NRO. 2423 DEL LIBRO IX

CONSTA EL GRUPO EMPRESARIAL :

CONTROLANTE: ABBOTT LABORATORIES
DOMICILIO:100 ABBOTT PARK ROAD, ABBOTT PARK ILLINOIS 60064
NACIONALIDAD: ESTADOS UNIDOS DE AMÉRICA
ACTIVIDAD: DESCUBRIMIENTO, DESARROLLO, FABRICACIÓN Y VENTA DE UN AMPLIO PORTAFOLIO DE PRODUCTOS PARA EL CUIDADO DE LA SALUD.

SUBORDINADA: ABBOTT LABORATORIES DE COLOMBIA S.A
NIT: 860002134-8
DOMICILIO: BOGOTA D.C.
NACIONALIDAD: COLOMBIANA.
ACTIVIDAD: IMPORTACION, EXPORTACION, DISTRIBUICION, COMPRA Y VENTA DE TODA CLASE DE MEDICAMENTOS Y PRODUCTOS QUIMICOS Y FARMACÉUTICOS Y ESPECIALIDADES MEDICINALES PARA USO HUMANO Y VETERINARIO.

SUBORDINADA: LABORATORIOS SYNTHESIS S.A.S
NIT; 860000760-1
DOMICILIO: BOGOTA D.C
NACIONALIDAD: COLOMBIANA.
ACTIVIDAD: IMPORTACION, EXPORTACION, FABRICACIÓN, COMERCIALIZACION, DISTRIBUCION Y VENTA DE PRODUCTOS FARMACÉUTICOS, HIGIENICOS, VETERINARIOS E IMPLEMENTOS MEDICOS.

SUBORDINADA: LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A.S.
NIT.890301463-8
DOMICILIO: CALI
NACIONALIDAD: COLOMBIANA
ACTIVIDAD: LA FABRICACIÓN, DISTRIBUCIÓN Y VENTA EN EL TERRITORIO DE LA REPÚBLICA DE COLOMBIA O EN EL EXTRANJERO, DE VACUNAS, PRODUCTOS QUÍMICOS, FARMACÉUTICOS, ALIMENTICIOS FUNCIONALES, COSMÉTICOS Y FISIOLÓGICOS, REACTIVOS, ANTIBIÓTICOS PARA USO HUMANO Y VETERINARIO

SUBORDINADA: LAFRANCOL INTERNACIONAL S.AS
NIT: 815003912-2
DOMICILIO: PALMIRA, VALLE.
NACIONALIDAD: COLOMBIANA



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ACTIVIDAD: ELABORACIÓN DE OTROS PRODUCTOS ALIMENTICIOS. FABRICACIÓN DE PRODUCTOS FARMACÉUTICOS, SUSTANCIAS QUÍMICAS MEDICINALES PRODUCTOS BOTÁNICOS DE USO FARMACÉUTICO.

SUBORDINADA: AMERICAN GENERICS S A S

NIT. 800223214-9

DOMICILIO: CALI

NACIONALIDAD: COLOMBIANA

ACTIVIDAD: FABRICAR, COMPRAR, VENDER, ADQUIRIR, MANTENER, IMPORTAR, EXPORTAR, DISTRIBUIR Y ENAJENAR A CUALQUIER TÍTULO PRODUCTOS, INSUMOS, ARTÍCULOS O MERCANCÍAS DE USO HUMANO Y/O VETERINARIO Y PRESTAR LOS SERVICIOS CORRESPONDIENTES CON DICHS INSUMOS, ARTÍCULOS, ETC.

SUBORDINADA: LABORATORIOS NATURMEDIK S.A.S.

NIT. 800226363-1

DOMICILIO: CALI

NACIONALIDAD: COLOMBIANA

ACTIVIDAD: FABRICAR, COMPRAR, VENDER, ADQUIRIR, MANTENER, IMPORTAR, EXPORTAR, DISTRIBUIR Y ENAJENAR A CUALQUIER TÍTULO PRODUCTOS, INSUMOS, ARTÍCULOS O MERCANCÍAS DE USO HUMANO Y/O VETERINARIO Y PRESTAR LOS SERVICIOS CORRESPONDIENTES CON DICHS INSUMOS, ARTÍCULOS, ETC.

SUBORDINADA: LABORATORIOS PAULY PHARMACEUTICAL S.A.S.

NIT: 800.097.402-6

DOMICILIO: CALI, VALLE

NACIONALIDAD: COLOMBIANA

ACTIVIDAD: FABRICAR, COMPRAR, VENDER, ADQUIRIR, MANTENER, IMPORTAR, EXPORTAR, DISTRIBUIR Y ENAJENAR A CUALQUIER TÍTULO PRODUCTOS, INSUMOS, ARTÍCULOS O MERCANCÍAS DE USO HUMANO Y/O VETERINARIO Y PRESTAR LOS SERVICIOS CORRESPONDIENTES CON DICHS INSUMOS, ARTÍCULOS ETC.

SUBORDINADA: DISTRIBUCIONES UQUIFA S.A.S.

NIT: 800182021-7

DOMICILIO: CALI, VALLE

NACIONALIDAD: COLOMBIANA

ACTIVIDAD: FABRICAR, COMPRAR, VENDER, ADQUIRIR, MANTENER, IMPORTAR, EXPORTAR, DISTRIBUIR Y ENAJENAR A CUALQUIER TÍTULO PRODUCTOS, INSUMOS, ARTÍCULOS O MERCANCÍAS DE USO HUMANO Y/O VETERINARIO Y PRESTAR LOS SERVICIOS CORRESPONDIENTES CON DICHS INSUMOS, ARTÍCULOS ETC.

SUBORDINADA: FOCUS PHARMACEUTICAL S.A.S

NIT: 900.104.555-8

DOMICILIO: CALI, VALLE

NACIONALIDAD: COLOMBIANA

ACTIVIDAD: FABRICAR, COMPRAR, VENDER, ADQUIRIR, MANTENER, IMPORTAR, EXPORTAR, DISTRIBUIR Y ENAJENAR A CUALQUIER TÍTULO PRODUCTOS, INSUMOS, ARTÍCULOS O MERCANCÍAS DE USO HUMANO Y/O VETERINARIO Y PRESTAR LOS SERVICIOS CORRESPONDIENTES CON DICHS INSUMOS, ARTÍCULOS ETC.

SUBORDINADA: LAFRANCOL PERU S.R.L.

DOMICILIO: LIMA

NACIONALIDAD: PERUANA

ACTIVIDAD: DISTRIBUCION Y COMERCIALIZACION DE PRODUCTOS FARMACEUTICOS DE USO HUMANO.

SUBORDINADA: LAFRANCOL DOMINICANA S.A.S.

DOMICILIO: SANTO DOMINGO

NACIONALIDAD: REPUBLICA DOMINICANA

ACTIVIDAD: FABRICACION DE PRODUCTOS FARMACEUTICOS.



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SUBORBINADA: LAFRANCOL GUATEMALA S.A.

DOMICILIO: GUATEMALA

NACIONALIDAD: GUATEMALTECA

ACTIVIDAD: PRODUCCION, FABRICACION, EXPORTACION Y DISTRIBUCION DE PRODUCTOS FARMACEUTICOS Y/O ALIMENTICIOS Y NATURALES DE CONSUMO HUMANO Y LA COMERCIALIZACION DE TODA CLASE DE MERCANCIAS POR CUENTA PROPIA O EN NOMBRE DE TERCEROS.

PRESUPUESTO DE CONTROL: ABBOTT INVESTMENTS LUXEMBOURG S.A. R.L ADQUIRIÓ EL 26 DE SEPTIEMBRE DE 2014 UNA PARTICIPACIÓN INDIRECTA DE MAS DEL 50% DEL CAPITAL DE LABORATORIO FRANCO COLOMBIANO LAFRANCOL SAS.

UNA PARTICIPACIÓN INDIRECTA EN EL CAPITAL DE LAS SOCIEDADES ACTUALMENTE REGISTRADAS COMO SUBORDINADAS DE LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A.S EN COLOMBIA Y EN EL EXTERIOR (LAS SOCIEDADES SUBORDINADAS A SABER: AMERICAN GENERICS S A S, LABORATORIOS NATURMEDIK S.A.S., LABORATORIOS PAULY PHARMACEUTICAL S.A.S., DISTRIBUCIONES UQUIFA S.A.S., FOCUS PHARMACEUTICAL S.A.S. LAFRANCOL PERU SRL, LAFRANCOL DOMINICANA S.A.S, LAFRANCOL GUATEMALA S.A.)

UNA PARTICIPACIÓN INDIRECTA DE MAS DEL 50% DEL CAPITAL DE LABORATORIOS SYNTHESIS S.A.S, CON DOMICILIO EN BOGOTA E IDENTIFICADA CON NIT: 860000760-1

ABBOTT INVESTMENTE LUXEMBOURG S.A.R.L. TIENE UNA PARTICIPACIÓN DIRECTA DEL MAS DEL 50% EN EL CAPITAL DE ABBOT LABORATORIES DE COLOMBIA S.A SITUACION DE CONTROL QUE FUE REGISTRADA MEDIANTE DOCUMENTO PRIVADO DEL 15 DE OCTUBRE DE 2013, INSCRITO EL 22 DE OCTUBRE DEL MISMO AÑO.

ADEMÁS DEL VÍNCULO DE SUBORDINACIÓN POR PARTICIPACIÓN QUE EXISTE ENTRE ABBOTT INVESTMENTS LUXEMBOURG S.A. R.L, (SOCIEDAD CONTROLANTE) Y LAS SOCIEDADES COLOMBIANAS ABBOTT LABORATORIES DE COLOMBIA S.A., LABORATORIOS SYNTHESIS S.A.S., LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A.S, Y LAS SOCIEDADES SUBORDINADAS, EN LOS TÉRMINOS DEL NUMERAL 1 DEL ARTÍCULO 261 DEL CÓDIGO DE COMERCIO; EXISTE ENTRE DICHAS SOCIEDADES UNIDAD DE PROPÓSITO Y DIRECCIÓN, SEGÚN LO DISPUESTO EN EL ARTÍCULO 28 DE LA LEY 222 DE 1995.

POR LO TANTO, SE CONFIGURA UN GRUPO EMPRESARIAL A PARTIR DEL 26 DE SEPTIEMBRE DE 2014.

SUBORBINADA: ST JUDE MEDICAL COLOMBIA LTDA.

NIT: 811.021.765-8

DOMICILIO: MEDELLIN

NACIONALIDAD: COLOMBIANA

ACTIVIDAD: IMPORTACIÓN, FABRICACIÓN, DISTRIBUCIÓN, COMERCIALIZACIÓN, VENTA Y EXPORTACIÓN DE PRODUCTOS MÉDICOS

PRESUPUESTO DE CONTROL: ABBOTT LABORATORIES EJERCE INFLUENCIA DOMINANTE EN LA TOMA DE LAS DECISIONES DE LOS ÓRGANOS DE ADMINISTRACIÓN DE LAS SOCIEDADES CONTROLADAS SEGÚN LO ESTABLECIDO EN EL ARTÍCULO 261 (3) DEL CÓDIGO DE COMERCIO; EN LA SOCIEDAD ST JUDE MEDICAL COLOMBIA LTDA. COMO SOCIEDAD CONTROLADA EN EL GRUPO EMPRESARIAL. LO ANTERIOR POR CUANTO ABBOTT LABORATORIES ADQUIRIÓ ST. JUDE MEDICAL, INC. EN EL EXTERIOR, COMPLETANDO LA FUSIÓN Y REORGANIZACIÓN EL DÍA 4 DE ENERO DE 2017.

CERTIFICA

QUE POR DOCUMENTO PRIVADO DEL 07 DE FEBRERO DE 2017, INSCRITO EN LA CÁMARA DE COMERCIO BAJO EL NRO. 2423 DEL 17 DE FEBRERO DE 2017, DEL LIBRO IX SE MODIFICÓ EL GRUPO EMPRESARIAL QUEDANDO COMO NUEVA CONTROLANTE LA SOCIEDAD ABBOTT LABORATORIES EN REEMPLAZO DE ABBOTT INVESTMET LUXEMBOURG S.A.R.L

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 2017



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QUE A NOMBRE DE LA SOCIEDAD FIGURA MATRICULADO EN LA CAMARA DE COMERCIO BAJO EL NRO.870576-2 ESTABLECIMIENTO DE COMERCIO: LABORATORIOS FRANCO COLOMBIANO LAFRANCOL SAS UBICADO EN: CL. 36 A NRO. 4 03 DE CALI
FECHA MATRICULA : 02 DE MAYO DE 2013
RENOVO : POR EL AÑO 2017

CERTIFICA

QUE LA SOCIEDAD EFECTUO LA RENOVACION DE SU MATRICULA MERCANTIL EL 27 DE MARZO DE 2017

CERTIFICA

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

LOS ACTOS ADMINISTRATIVOS DE REGISTRO QUEDAN EN FIRME DIEZ (10) DIAS HABILES DESPUES DE LA FECHA DE SU INSCRIPCION, SIEMPRE Y CUANDO DENTRO DE DICHO TERMINO NO SEAN OBJETO DE RECURSOS.

LA PERSONA O ENTIDAD A LA QUE USTED LE VA A ENTREGAR EL CERTIFICADO PUEDE VERIFICAR SU CONTENIDO INGRESANDO A <http://www.ccc.org.co/registraya/> Y DIGITANDO EL CODIGO DE VERIFICACION QUE SE ENCUENTRA EN EL ENCABEZADO DEL PRESENTE DOCUMENTO.

EL CERTIFICADO A VALIDAR CORRESPONDE A LA IMAGEN Y CONTENIDO DEL CERTIFICADO CREADO EN EL MOMENTO EN QUE SE GENERO EN LAS SEDES O A TRAVES DE LA PLATAFORMA VIRTUAL DE LA CAMARA.

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 15 DIAS DEL MES DE MAYO DEL AÑO 2017 HORA: 12:13:01 PM

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