Industria y Comercio
SUPERINTENDENCIA

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

No. 11-178794- -00004-0000

Fecha: 2012-07-16 10:12:41 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 379 FASENACIONALII
Act. 400 OPOSIOBSERVTER Folios: 31

DIRECCIÓN DE NUEVAS CREACIONES
PRESENTACIÓN DE OPOSICIÓN

1. Oposición a solicitud de:

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

Número de expediente: 11 178.794

Solicitante: F. HOFFMAN-LA ROCHE AG

Apoderado: ALICIA LLOREDA

Título de la Nueva Creación: FORMULACIÓN SUBCUTÁNEA DE ANTICUERPO ANTI-HER2

Gaceta: 643

2. Datos del Opositor

☐ Persona Natural ☐ Persona Jurídica

SYNTHESIS S.A.S.

Nombre o denominación /Razón social completa

LUIS PALACIOS ARAGON

Nombre del representante legal

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

Número 860000760-1_____

Dirección. CRA 44 20C 73_____Ciudad BOGOTA

Teléfono 3692222 Fax: _____ Correo electrónico_____

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

3. Datos del representante o apoderado:

MORENO BOHORQUEZ ELIANA ISAURA	51.975.664	83823
Apellidos y Nombre	**Documento de identificación**	**Tarjeta profesional**

Dirección del representante		
CRA 13 119-95 OFICINA 104		
Dirección electrónica negocios@optimum-co.com	No. Fax (571)6121979	Número telefónico (571)2131213

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

4. Fundamentos de la oposición

Causal: Art14, 16, 18,20 y 30 de la decisión 486 de la CAN no cumple con los requisitos De patentabilidad.

Justificación: La presente solicitud no cumple con los requisitos de patentabilidad dado que lo revelado, ya se encontraba en el estado de la técnica o es posible derivarlo de la misma tal y como se demostrará en el capitulo "fundamento de hecho".

Prórroga: **SI** ☐ **NO** ☐

5. Anexos

- ☑ Comprobante de pago de la tasa.
- ☑ Poder, si fuera el caso.
- ☑ Fundamentos de la oposición. Se anexan 40 folios (obligatorios indicar cuántos).
- ☑ Anexo de fundamentos de la oposición. Se anexan 9 folios (obligatorios indicar cuántos)
- ☑ Copias para traslado.
- ☐ Copia de la solicitud y sus anexos en formato magnético.

6. Firma	
Nombre del Firmante ELIANA ISAURA MORENO BOHORQUEZ	Firma
CC. 51.975.664	Tarjeta Profesional: 83823

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
NIT : 800.176.089-2
- / -

Industria y Comercio
SUPERINTENDENCIA

RECIBO DE CAJA

No. 12 - 0061406

Bogotá D.C., Junio 20 de 2012 - 15:54:46

RECIBIDO DE : LABORATORIOS SYNTHESIS S.A.S. NI 860.000.760

*** Soporte del Pago ***

TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	VR PAGO
CONSIGNACION	BANCO DE BOGOTA	062754387	408380781	19/06/2012	1.260.000.00

*** Conceptos Pagados ***

CANT.	RENTISTICO	CONCEPTO	Vr.UNDITARIO	Vr.CONCEPTO
1	50005-01-04 PRESENTACION DE OBSERVACIONES A SOLICITUDES	12 MARCAS Y LEMAS COMERCIALES	315.000.00	315.000.00
				$315.000.00

SON: **TRESCIENTOSQUINCE MIL PESOS MONEDA CORRIENTE***

Responsable: _____
-*******-

Recibo de Caja Aplicado al Expediente No. _____

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
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Derecho de la Competencia, Propiedad Industrial y Comercio Exterior

Señor Director,

DIRECCIÓN DE NUEVAS CREACIONES

Superintendencia de Industria y Comercio

E. S. D.

Referencia	: Expediente N° 11 178.794
Asunto	: Oposición a la solicitud de patente de Invención titulada *"FORMULACIÓN SUBCUTÁNEA DE ANTICUERPO ANTI-HER2"*
Solicitante	: F. HOFFMAN-LA ROCHE AG
Opositora	: LABORATORIOS SYNTHESIS S.A.S.

Publicada en la Gaceta de la Propiedad Industrial

N° 643 del 30 de abril de 2012

Yo, *ELIANA ISAURA MORENO BOHORQUEZ,* abogada en ejercicio, identificada como aparece al pie de su firma, con tarjeta profesional No. 83823 del Consejo Superior de la Judicatura, en mi condición de apoderada de la sociedad **LABORATORIOS SYNTHESIS S.A.S.** conformada bajo las leyes de la República de Colombia y domiciliada en la ciudad de Bogotá, Colombia, atentamente manifiesto que estando dentro del término de ley y por orden expresa de mi representada, presento oposición a la concesión del registro de la Patente de Invención titulada *"FORMULACIÓN SUBCUTÁNEA DE ANTICUERPO ANTI-HER2"*, cuyo titular es **F. HOFFMAN-LA ROCHE AG,** y pido a Usted declarar fundada esta oposición y en consecuencia negar el registro de la patente solicitada.

1. INTERÉS PARA ACTUAR

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

2. FUNDAMENTOS DE HECHOS

2.1. La solicitud objeto de la presente fue presentada el 26 de diciembre de 2011, reivindicando prioridad bajo la solicitud EP20090167025 del 2009-07-31; y fue publicada en Colombia, en la Gaceta de la Propiedad Industrial **N° 643 del 30 de abril de 2012**, cuya publicación internacional corresponde a la **WO2011012637** del **2011-02-03**.

2.2. La solicitud colombiana **11 178.794** objeto de la presente oposición, se refiere a una formulación farmacéutica altamente concentrada y estable de un anticuerpo anti-HER2 farmacéuticamente activo, como por ejemplo el Trastuzumab

(HERCEPIN™), Pertuzumab o T-DM1, o una mezcla de tales moléculas de anticuerpo para la inyección subcutánea. En particular, la presente invención está relacionada con formulaciones que com10 prenden, además de una cantidad adecuada del anticuerpo anti-HER2, una cantidad efectiva de al menos una enzima hialuronidasa como una formulación combinada o para su utilización en forma de una co-formulación. Dichas formulaciones comprenden adicionalmente al menos un agente tamponante, como por ejemplo un tampón de histidina, un estabilizante o una mezcla de dos o más estabilizantes (por ejemplo un sacárido, como por ejemplo la a,a-trehalosa dihidratada o la sacarosa, y opcionalmente metionina como segundo estabilizante), un tensioactivo no iónico y una cantidad efectiva de al menos una enzima hialuronidasa. También se proporcionan los métodos para preparar tales formulaciones y la utilización de las mismas.

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

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

- D1: US2006104968 publicada el 18 de mayo de 2006.

2.5.Ahora bien, el documento D1, particularmente en la descripción revela activos solubles neutros de Glicoproteínas hialuronidasa (sHASEGPs) y métodos de fabricación y su uso para facilitar la administración de otras moléculas o para aliviar glicosaminoglicano y patologías asociadas. Además se describen formulaciones adecuadas de una glicoproteína sHASEGP sustancialmente purificada recombinante derivado de una célula eucariota con la generación de la glicosilación apropiada requerida para su actividad óptima. Particularmente, en los ejemplos 18 a 21; párrafos (0433) y (558); ejemplos 25 y 26; y, reivindicación 52, se define en esta anterioridad D1, co-formulaciones de sHASEGP (= rHuPH20) con otras sustancias para formas inyectables para pequeños volúmenes o rápida administración subcutánea. En D1 se definen ejemplos como EpiPen ® con otros fluidos los cuales se pueden formular. Los métodos descritos en D1 incluyen la administración de las composiciones del polipéptido sHASEGP o compuestos farmacéuticos que contienen sHASEGP antes de, simultáneamente con o después de la administración de otras moléculas terapéuticas. De igual forma es sabido que

los anticuerpos humanizados se modifican para incluir secuencias de aminoácidos humanos de modo que la administración a un humano no provocaría una respuesta inmune; tales como un anticuerpo monoclonal donde se alteran por técnicas recombinantes ONA para expresar un anticuerpo en el que se basa la composición de aminoácidos de la región no variable en anticuerpos humanos. Como ejemplos de anticuerpos monoclonales en D1 se pueden escoger de Trastuzumabs (por ejemplo, Herceptin®) los cuales demuestran que han sido revelados con anterioridad. Por lo tanto, en cuanto a los requisitos de la actividad inventiva se refiere, una persona normalmente versada en la materia referida a esta técnica enfrenta el problema de buscar formulaciones farmacéuticas que comprenden el activo de un anticuerpo anti-HER2 en una formulación estable altamente concentrada siendo diseñado para la inyección subcutánea, y puede en principio capaz de deducir de una manera evidente a partir de D1, mostrando la posibilidad de una combinación con éxito en la forma del anticuerpo anti-HER2 y hialuronidasa, como los anticuerpos Trastuzumabs antiHER2 (por ejemplo HERCEPTIN®) como se describe en D1, combinada con hialuronidasa, siendo recomendado como un adyuvante posible, ya sea un recombinante purificado de sHASEGP o hialuronidasa testicular bovina, por lo que es obvio dicha combinación en una formulación subcutánea. Así las cosas, como lo evidencia dicho documento D1, la presente solicitud no comprende altura inventiva pues lo revelado se puede derivar del estado de la técnica.

2.6. Por lo tanto, este documento objeto de oposición a la luz del documento D1 carece evidentemente ALTURA INVENTIVA.

2.7. En consecuencia y de acuerdo a lo descrito anteriormente, se deriva que la solicitud colombiana **11 178.794** no puede pretender un monopolio por patente toda vez

que lo que pretende no cumple con dos de los tres requisitos necesarios para su protección.

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

2.8.1. CARENCIA DE ALTURA INVENTIVA. Según la Decisión 486 de la Comunidad Andina de Naciones, Artículo 18.- *"Se considerará que una invención tiene nivel inventivo, si para una persona del oficio normalmente versada en la materia técnica correspondiente, esa invención no hubiese resultado obvia ni se hubiese derivado de manera evidente del estado de la técnica."*. A la fecha de prioridad, 31 de julio de 2009, a partir del documento D1 en combinación con los conocimientos medios de la técnica, puede llegar de manera evidente a un una formulación farmacéutica altamente concentrada y estable de un anticuerpo anti-HER2 farmacéuticamente activo, como por ejemplo el Trastuzumab (HERCEPIN™), Pertuzumab o T-DM1, o una mezcla de tales moléculas de anticuerpo para la inyección subcutánea, sobre el cual busca protección en el capítulo reivindicatorio.

2.8.2. CARENCIA DE APLICACIÓN INDUSTRIAL La Decisión 486 de la CAN consagra en su artículo 14 lo siguiente: *"Los países miembros otorgarán patentes para las invenciones, sean de producto o procedimiento, en todos los campos de la tecnología, siempre que sean nuevas, tengan nivel inventivo y sean susceptibles de aplicación industrial"*. La presente solicitud carece de aplicación Industrial ya que como se demostró anteriormente, algunas cláusulas reclaman el método terapéutico y estos no son aplicables industrialmente porque primero no se

obtiene un producto o proceso; y segundo no es reproducible o repetible a escala industrial. Por lo tanto, el objeto de la presente invención referido a los usos y métodos terapéuticos, no cumplen con el requisito de la aplicación industrial.

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

2.10. Por todo lo anterior, atentamente solicito a usted que se sirva declarar fundada *"FORMULACIÓN SUBCUTÁNEA DE ANTICUERPO ANTI-HER2"*, cuyo titular es **F. HOFFMAN-LA ROCHE AG,** solicitud Colombiana que apareció publicada en la Gaceta de la Propiedad Industrial **N° 643 del 30 de abril de 2012.**

3. PRUEBAS

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

- D1: **US2006104968** publicada el 18 de mayo de 2006.

4. FUNDAMENTO DE DERECHO

Fundamento esta observación en las normas legales siguientes:

4.1. Numeral 1 del artículo 586 del Código de Comercio.

4.2. Artículo 42 de la Decisión 486 de la Comisión del Acuerdo de Cartagena.

4.3. En las demás normas concordantes.

5. ANEXOS

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

1. Poder para actuar en el presente.
2. Documento que acredita al representante legal de **LABORATORIOS SYNTHESIS S.A.S.**
3. Recibo de caja del respectivo pago de tasa.
4. Copias de los documentos:

- D1: **US2006104968** publicada el 18 de mayo de 2006.

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Derecho de la Competencia, Propiedad Industrial y Comercio Exterior

6. NOTIFICACIONES

Para efectos de notificaciones que por disposición del artículo 50 del Decreto 01 de 1984 debe hacer su Despacho, informo que mi representada y la suscrita recibiremos notificaciones en la Secretaría de su Despacho o en mis oficinas situadas en la Carrera 13 Nº 119-95, Of. 104 de esta ciudad de Bogotá.

Señor Superintendente,

ELIANA ISAURA MORENO BOHORQUEZ
C.C. 51'975.664 de Bogotá
T.P.A. 83823 del C.S. de la J.

ANEXO

US 20060104968A1

(19) United States

(12) Patent Application Publication (10) Pub. No.: US 2006/0104968 A1

Bookbinder et al. (43) Pub. Date: May 18, 2006

(54) SOLUBLE GLYCOSAMINOGLYCANASES AND METHODS OF PREPARING AND USING SOLUBLE GLYCOSAMINOGLYCANASES

(75) Inventors: **Louis D. Bookbinder**, San Diego, CA (US); **Anirban Kundu**, San Diego, CA (US); **Gregory L. Frost**, Del Mar, CA (US); **Michael F. Haller**, San Diego, CA (US); **Gilbert A. Keller**, Bernard, CA (US); **Tyler M. Dylan**, San Diego, CA (US)

Correspondence Address:
DLA PIPER RUDNICK GRAY CARY US, LLP
4365 EXECUTIVE DRIVE
SUITE 1100
SAN DIEGO, CA 92121-2133 (US)

(73) Assignee: **Halozyme, Inc.**, San Diego, CA

(21) Appl. No.: **11/238,171**

(22) Filed: **Sep. 27, 2005**

Related U.S. Application Data

(63) Continuation-in-part of application No. 11/065,716, filed on Feb. 23, 2005, which is a continuation-in-part of application No. 10/795,095, filed on Mar. 5, 2004.

(60) Provisional application No. 60/452,360, filed on Mar. 5, 2003.

Publication Classification

(51) **Int. Cl.**
A61K 38/47 (2006.01)
C12N 9/24 (2006.01)

(52) **U.S. Cl.** **424/94.61**; 435/200

(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.

agent can be administered in a high concentration without risk of the complications that can accompany systemic administration of a therapeutic agent.

[0432] As an exemplary class of pharmacologic agents that can be combined with sHASLGPs (or other glycosaminoglycanases) (e.g. combined by co-formulation with one or more sHASEGPs or by co-administration with one or more sHASEGPs (which may be administered prior to, coincident with or following administration of the agent), a number of agents having effectiveness or potential effectiveness in the treatment of various cancers have been described in the art, and novel anti-cancer agents are regularly being developed which may likewise be combined with one or more sHASEGPs (e.g. provided by co-formulation or by co-administration before, coincident with and/or following administration of the agent) to improve the pharmacokinetics, pharmacodynamics or other useful properties of the agent.

[0433] By way of illustration (and without limitation), a number of anti-cancer or chemotherapeutic agents have been approved for the treatment of various cancers which can be combined with sHASLGPs (or other glycosaminoglycanases) by co-formulation or co-administration (before, coincident with or after administration of the anti-cancer agent). Such agents include, for example, Aldesleukins (e.g. PROLEUKIN™); Alemtuzumabs (e.g. CAMPATH™); Alitretinoins (e.g. PANRETIN™); Allopurinols (e.g. ZYLOPRIM™); Altretamines (e.g. HEXALEN™); Amifostines (e.g. ETHYOL™); Anastrozoles (e.g. ARIMIDEX™); Arsenic Trioxides (e.g. TRISENOX™); Asparaginases (e.g. ELSPAR™); BCG Live (e.g. TICE™ BCG); Bexarotenes (e.g. TARGRETIN™); Bleomycins (e.g. BLENOXANE™); Busulfan intravenous (e.g. BUSULFEX™); Busulfan orals (e.g. MYLERAN™); Calusterones (e.g. METHOSARB™); Capecitabines (e.g. XELODA™); Carboplatins (e.g. PARAPLATIN™); Carmustines (e.g. BCNU™, BiCNU™); Carmustines with Polifeprosans (e.g. GLIADEL™ Wafer); Celecoxibs (e.g. CELEBREX™); Chlorambucils (e.g. LEUKERAN™); Cisplatins (e.g. PLATINOL™); Cladribines (e.g. LEUSTATIN™, 2-CdA™); Cyclophosphamides (e.g. CYTOXAN™, NEOSAR™); Cytarabines (e.g. CYTOSAR-U™, Cytarabine liposomals (e.g. DepoCyt™); Decarbazines (e.g. DTIC-Dome®); Dactinomycins (e.g. COSMEGEN™); Darbepoetin Altas (e.g. ARANESP™); Daunorubicin liposomals (e.g. DANUOXOME™); Daunorubicins/Doxorubicins (e.g. CERUBIDINE™); Denileukin Diftoxes (e.g. ONTAK™); Dexrazoxanes (e.g. ZINECARD™); Docetaxels (e.g. TAXOTERE™); Doxorubicins (e.g. ADRIAMYCIN™, RUBEX™); Doxorubicin liposomals (e.g. DOXIL™); Dromostanolone propionates (e.g. DROMOSTANOLONE™ and MASTERONE™ Injection); Elliott's B Solutions (e.g. Elliott's B Solution™); Epirubicins (e.g. ELLENCE™); Epoetin altas (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™); Mechlorethamines/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. PAXENE™, TAXOL™); Pamidronates (e.g. AREDIA™); Pegademases (e.g. ADAGEN™); Pegaspargases (e.g. ONCASPAR™); Pegfilgrasums (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™); Quinacrines (e.g. ATABRINE™); Rasburicases (e.g. ELITEK™); Rituximabs (e.g. RITUXAN™); Sargramostims (e.g. PROKINE™); Streptozocins (e.g. ZANOSAR™); Tales (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™); Tretinoins/ATRA (e.g. VESANOID™); Uracil Mustards; Valrubicins (e.g. VALSTAR™); Vinblastines (e.g. VELBAN™); Vincristines (e.g. ONCOVIN™); Vinorelbines (e.g. NAVELBINE™); Zoledronates (e.g. ZOMETA™); as well as other agents described herein and in the art.

[0434] As with other classes of pharmacologic agents described herein, a sHASEGP (or other glycosaminoglycanase) may be used in conjunction with the administration of (or co-formulated with) an individual pharmacologic agent (such as an antineoplastic for purposes of illustration, or with a member of another class of agents as described herein and/or known in the art). Alternatively, a sHASEGP (or other glycosaminoglycanase) may be used in conjunction with the administration of (or co-formulated with) more than one pharmacologic agent of a given class (such as multiple antineoplastic agents for purposes of illustration, or multiple members of other classes of agents as described herein and/or known in the art). In the case of combinations with two or more agents of a given class, it is often preferred that the two or more agents act by two or more different mechanisms of action. A sHASEGP (or other glycosaminoglycanase) may also be used in conjunction with the administration of (or co-formulated with) one pharmacologic agent of a first given class (such as an antineoplastic for purposes of illustration, or a member of one of the other classes of agents as described herein and/or known in the art), and one or more additional pharmacologic agents of one or more different classes that are useful for treating any of a variety of conditions. While certain principles are thus referred to here in the case of anticancer agents for purposes of illustration, these principles are also applicable to, for example, anti-infectives and the numerous members of other

formulation can include pharmaceutically acceptable vehicles (excipients, adjuvants, etc.). The substance leading to disorganization of the extracellular matrix and the nucleic acid of interest are preferably dissolved in a buffer which is suitable for pharmaceutical use and which can be hypertonic, hypotonic or isotonic. Various buffers can be envisaged. Those which may be mentioned by way of illustration are a physiological saline solution (0.9% NaCl), a nonphysiological saline solution (1.8% NaCl), a Hepes Ringer solution, a Lactate-Ringer solution, a buffer which is based on Tris-HCl (10 mM Tris-HCl, pH 7.5 to 8, 1 mM EDTA, 10 mM Tris-HCl, pH 7.5 to 8, 1 mM $MgCl_2$), a phosphate buffer (Krebs phosphate H2O buffer), a sugar (glucose, sucrose, trehalose, etc.) solution, or simply water.

[0550] Hypodermoclysis

[0551] Hypodermoclysis, the subcutaneous infusion of fluids, is a useful and easy hydration technique suitable for mildly to moderately dehydrated adult patients, especially the elderly. The method is considered safe and does not pose any serious complications. The most frequent adverse effect is mild subcutaneous edema that can be treated by local massage or systemic diuretics. Approximately 3 L can be given in a 24-hour period at two separate sites. Common infusion sites are the chest, abdomen, thighs and upper arms. The preferred solution is normal saline, but other solutions, such as half-normal saline, glucose with saline or 5 percent glucose, can also be used. Potassium chloride can be added to the solution bag if needed. Additionally, other drugs can be delivered through similar routes. Human sHASEGP can be added to enhance fluid absorption and increase the overall rate of administration. Human sHASEGP is preferable for repeated Hypodermoclysis over slaughter house-derived enzymes in that it not likely to be immunogenic as the bovine enzyme is known to be. It may be administered at home by family members or a nurse; the technique should be familiar to every family physician.

[0552] In ambulatory patients, hypodermoclysis sites include the abdomen, upper chest above the breast, over an intercostal space and the scapular area. In debilitated patients, preferred sites are the thighs, the abdomen and the outer aspect of the upper arm. After one to four days, the needle and tubing should be changed, although infusion sites have been left in place for much longer periods without complications. Administration of 500-mL boluses over one or two hours three times a day can also be given. About 150 U of sHASEGP given at the subcutaneous site before the first morning infusion

[0553] Facilitation of Therapeutic Injections

[0554] Many molecules injected percutaneously (e.g. by using a needle or other device that penetrates the skin and is used to deposit the molecules into a sub-dermal layer) reach circulation slowly or with very low efficiency. Several factors regulate the pharmacokinetics and pharmacodynamics of molecules injected subcutaneously (SC) or intramuscularly (IM). Generally, larger molecules reach circulation more slowly and less efficiently without active transport into circulation. Subcutaneous bioavailability is determined by calculating the ratio of area under the curves for SC verses intravenous administration ($AUC_{sc}/AUC_{intravenous}$). A second factor is charge and affinity for matrix molecules that may play a role in sequestration of molecules somewhat passively. If these materials are degraded locally they can never reach their desired targets and thus demonstrate a decreased overall systemic bioavailability to the target organs.

[0555] Large proteins are normally given intravenously so the medicament directly available in the blood stream. It would however be advantageous if a medicament could be given subcutaneously, intramuscularly or intradermally (ID) as these administration forms are much easier to handle for the patient. Especially, if the medicament must be taken regularly during the whole life and treatment is to start early, already when the patient is a child. However, a medicament with a very large and labile molecule, such as coagulation factor VIII of 170 to 300 kDa, have normally a very low bioavailability if given subcutaneously, intramuscularly or intradermally, since the uptake is not enough and degradation is severe.

[0556] In addition to the need to increase bioavailability of many subcutaneously administered biologics, more rapid pharmacokinetics is also critically important in instances of emergency medicine. The time required to reach intravenous access in many patients can prevent an otherwise rapid acting drug when administered systemically from being utilized. In some cases failure to reach intravenous access is then followed by subcutaneous injection, which leads to additional delay in reaching the target organs. Thus, the more rapid availability of subcutaneous drugs would be of benefit as a first line of treatment rather than to risk the time required to achieve intravenous access. Examples of molecules that can be delivered subcutaneously as well as intravenously include epinephrine, atropine, narcan, lignocaine, and dextrose.

[0557] An additional benefit of the invention lies in the ability to deliver equivalent or larger volumes of solutions I.D, SC or IM without the pain and morbidity associated with the pressure and volume of the solution at the site of injection.

[0558] By way of illustration (and without limitation), there are a number of biologics that can be enhanced by combination with one or more sHASEGPs (e.g. by co-formulation or co-administration of sHASEGP before, coincident with or after administration of the biologic), including for example: Abciximabs (e.g. REOPRO™), Adalimumabs (e.g. HUMIRA™), Agalsidase betas (e.g. FABRAZYME™), Aldesleukins (e.g. PROLEUKIN™), Alefacepts (e.g. AMEVIVE™), Alemtuzumabs (e.g. CAMPATH™), Alteplases (e.g. ACTIVASE™), Alteplases (e.g. CATHFLO ACTIVASE™), Anakinras (e.g. KINERET™), Arcitumomabs (e.g. CEA-Scan™), Asparaginases (e.g. ELSPAR™), Basiliximabs (e.g. SIMULECT™), Becaplermins (e.g. REGRANEX™), bevacizumabs (e.g. AVASTIN™), Botulinum Toxin Type A's (e.g. BOTOX™), Botulinum Toxin Type B's (e.g. MYOBLOC™), Capromab Pendetides (e.g. PROSTASCINT™), Cetuximabs (e.g. ERBITUX™), Daclizumabs (e.g. ZENAPAX™), Darbepoetin alfas (e.g. ARANESP™), Denileukin diftitoxs (e.g. ONTAK™), Dornase alfas (e.g. PULMOZYME™), Drotrecogin alfas (e.g. XIGRIS™), Efalizumabs (e.g. RAPTIVA™), Epoetin alfas (e.g. EPOGEN™), Etanercepts (e.g. ENBREL™), Filgrastims (e.g. NEUPOGEN™), Ibritumomabs/Tiuxetans (e.g. ZEVALIN™), Imciromab Pentetates (e.g. MYOSCINT™), Infliximabs (e.g. REMICADE™), Interferon alfa-2as (e.g. ROFERON-A™), Interferon alfacon-1s (e.g. INFERGEN™), Interferon alfa-n3s (e.g. ALFERON N™),

[0768] b. Results

2 UNITS	SALINE CONTROL
$t_{0.5}$ = 180	$t_{0.5}$ = 54
$t_{1.0}$ = 107	$t_{1.0}$ = 89
$t_{0.9}$ = 91	$t_{0.9}$ = 48

[0769] The results demonstrate that the dermal barrier reconstitutes within 24 hours of administration of 2 Units of enzyme.

EXAMPLE-18

Determination of the Size of Channels Opened by sHASEGP

[0770] It was shown that human sHASEGP opened channels in the interstitial space sufficient to permit the diffusion of a small molecule, i.e. trypan blue dye. However, it was unknown what the upper limits were on the size of particles that could diffuse in the presence of sHASEGP.

[0771] a. Protocol

[0772] Fluorescent molecules of varying sizes were used to determine the size of the channels opened by human sHASEGP. Fluoresceinated Dextrans of 4,400 and 2 million Da Average Molecular Weight (Sigma) as well as fluorescent labeled beads of defined diameters from 20 nanometers to 500 nanometers (Molecular Probes), were administered subcutaneously in a volume of 40 ul with following injection of sHASEGP or saline control in the same sites. Area of the dye front was then measured in two dimensions at 15 minutes post injection.

[0773] b. Results

Diffusion Agent	Diffusion Test Particle Size	Area at 15 min	Std. Dev.
sHASEGP	4400 Da	84.2	35.7
Control	4400 Da	38.6	3.9
sHASEGP	2 × 10E6 Da	14.2	4.3
Control	2 × 10E6 Da	8.1	8.1
sHASEGP	20 nm Diameter	92.3	21.2
Control	20 nm Diameter	51.6	8.9
sHASEGP	200 nm Diameter	50.1	8.7
Control	200 nm Diameter	40.2	7.1
sHASEGP	260 nm Diameter	35.3	2.9
Control	260 nm Diameter	37.6	8.1
sHASEGP	500 nm Diameter	44.8	13.2
Control	500 nm Diameter	41.2	9.8

[0774] The results demonstrated that molecules from approximately 1 kDa (Trypan Blue) to 50 nm in diameter (Latex Beads) showed enhanced diffusion following administration of sHASEGP. While bovine serum albumin (66 kDA) showed similar kinetics of diffusion to trypan blue, the 50 nm latex beads required significantly more time to diffuse. 500 nm beads showed no diffusion up to 480 minutes.

EXAMPLE-19

Serum Pharmacokinetics Profiles of Biotinylated Antibodies Following Subcutaneous Co-Injection of Human sHASEGP

[0775] a. Protocol

[0776] Female Balb/c mice were anesthetized with a mixture of ketamine/xylazine. The mice were then injected subcutaneously with 20 ul of 0.5 mg/ml solution of biotinylated mouse IgG mixed with 20 ul of either saline or 20 ul sHASEGP containing 4 Units of activity.

[0777] b. Results

TIME POST INJECTION	CONTROL	sHASEGP (4...)
Serum IgG t = 0 Hrs	0 ng/ml	0 ng/ml
Serum IgG t = 2 Hrs	0 ng/ml	360 ng/ml
Serum IgG t = 5 Hrs	4152 ng/ml	4176 ng/ml

[0778] The results demonstrate that sHASEGP increases the kinetics of serum distribution of large molecules in circulation. Where no biotinylated IgG could be detected in the control group at 2 hours, 360ng/ml was apparent by 2 hours in the sHASEGP group.

EXAMPLE-20

Spreading Activity of Subcutaneously Injected Molecules Following Intravenous Injection of Human sHASEGP

[0779] a. Protocol

[0780] Four sites for dye injection were utilized per dose of each Test Article and carrier control. Dye injection was 45 minutes after I.V. injection. Each dose of test or control article was injected I.V. into 2 animals. Measurement of the dye front area post 45 minute enzyme administration was calculated at 2.5, 5, 10 and 15 minutes for each dose or carrier control.

[0781] b. Results

[0782] Results demonstrated that highly purified sHASEGP was systemically available to distal tissues upon intravenous administration. The spreading activity of systemically administered sHASEGP was dose dependent, with a 16 unit injection being indistinguishable from carrier control.

Type	Dose IV	Time Minutes	Mean Area (mm...²	SD
PH2O	1600	2.5	86,417	2,834193
PH2O	1600	5	102,117	2,221148
PH2O	1600	10	124,55	6,364044
PH2O	1600	15	139,67	1,414214
PH2O	800	2.5	59,137	7,213615

-continued

Type	Dose IV	Time Min/Hr	Mean Area (mM*)	Std
PH20	300	5	73.638	2.51x07
PH20	300	10	87.092	8.38x08
PH20	300	15	92.537	1.06x09
PH20	100	2.5	56.308	6.xx x04
PH20	100	5	63.456	4.xx07
PH20	100	10	76.334	6.x6x09
PH20	100	15	77.462	1.xx20x
PH20	30	2.5	50.204	1.xx09x
PH20	30	5	59.493	5.x6x07
PH20	30	10	68.102	1.xx07
PH20	30	15	71.118	9.x64x03
PH20	10	2.5	36.6	3.x03x09
PH20	10	5	39.839	6.x08x09
PH20	10	10	45.645	2.xx536
PH20	10	15	48.07	6.x6x03
Control	0	2.5	24.963	3.xx07
Control	0	5	26.279	2.xx05
Control	0	10	44.68	1.x03x03
Control	0	15	52.002	2.81x09

EXAMPLE 21

Illustrative Use of Pegylation to Generate Modified sHASEGPs Exhibiting Improved Pharmacokinetics

[0783] As an illustrative example of the use of pegylation to generate modified forms of sHASEGPs having improved pharmacokinetics, such as increased serum half life, we attached either linear or branched polyethylene glycol (PEG) moieties to an exemplary sHASEGP, recombinant human PH20 (rHuPH20) as described above.

[0784] A number of different functionally activated groups can be used for attaching PEG molecules to sHASEGPs such as rHuPH20 (including, by way of illustration, aldehydes, succinimides, maleimides and others). A number of different pegylation reagents are commercially available from various sources including Nektar Therapeutics (www.nektar.com) as well as a number of other suppliers (www.prnia.dow.com, www.nof.co.jp, www.sunbio.com, www.celares.com). For purposes of illustration, rHuPH20 has been effectively pegylated as described herein using a number of illustrative PEG reagents to generate a number of different PEGylated rHuPH20 enzymes. Analogous methodologies have also been applied to other hyaluronidase enzymes, including an illustrative animal-derived hyaluronidase (using a preparation of ovine testicular hyaluronidase available as Vitrase® from ISTA Pharmaceuticals), an illustrative bacterial derived hyaluronidase (using a preparation of bacterial hyaluronidase available as Hyaluronate Lyase from Sigma) and an illustrative bacterial-derived chondroitinase having hyaluronidase activity (using a preparation of bacterial chondroitinase ABC available as Chondroitinase ABC from Associates of Cape Cod, Inc./Seikagaku Corporation).

[0785] In addition to varying the functional group, PEGs of a variety of different lengths and structures are available which can be incorporated using a particular functional group. For purposes of illustration, PEG lengths ranging from 5 to 60 KDa (e.g. 5, 10, 20, 30, 40 and 60 KDa) have been conjugated to rHuPH20. Again a variety of different PEGs having different lengths and structures are readily available from sources including Nektar, the Dow Chemical Corporation (Dowpharma), NOF Corporation, Sunbio, Celares and others. A large number of references are known in the art related to PEG reagents and methods of PEGylating proteins, including a number of reviews and articles cited herein; see, e.g., Roberts, M J et al. Advanced Drug Delivery Review 2002, 54: 459-476; Harris J M and Zalipsky, S (eds) of "Poly(ethylene glycol), Chemistry and Biological Applications" ACS Symposium Series 680 (1997), Mehvar, R et al. J. Pharm. Pharmaceut. Sci., 3(1): 125-136 (2000); Harris, J M, Nature Reviews 2: 215 et seq. (2003); Tsubery, H., J Biol. Chem 279(37): 38118-24 (2004); and are further described in a number of references describing PEGylation and various reagents and methodologies (see, e.g., Moulardini, C et al, Bioconjugate Chem. 6: 62-69 (1995); Veronese F M et al., J. Bioactive Compatible Polymers 12: 197-207 (1997); and a variety of U.S. and other published patents and applications including U.S. Pat. No. 5,672,662; U.S. Pat. No. 5,932,462; U.S. Pat. No. 6,495,659; and U.S. Pat. No. 6,737,505; as well as U.S. Pat. Nos. 4,002,531; 4,179,337; 5,122,614; 5,183,550; 5,324,844; 5,446,090; 5,612,460; 5,643,575; 5,766,581; 5,795,569; 5,808,096; 5,900,461; 5,919,455; 5,985,263; 5,990,237; 6,113,906; 6,214,966; 6,258,351; 6,340,742; 6,413,507; 6,420,339; 6,437,025; 6,448,369; 6,461,802; 6,828,401; 6,858,736; US Appl Publ 20010021763; 20010044526; 20010046481; 20020052430; 20020072573; 20020156047; 20030114647; 20030143596; 20030158333; 20030220447; 20040013637; 20050000360; 20050114037; 20050171328 and 20050209416; and European publications EP01064951; and EP0822199; and PCT publications WO 00176640; WO 00020017; WO 0249673; WO 9428024; and WO 0187925 (each of which publications is incorporated herein by reference).

EXAMPLE 21-A

Illustrative Pegylated Versions of Human Recombinant Hyaluronidases

[0786] As an exemplary illustration of the pegylation of an illustrative sHASEGP, PEG aldehydes, succinimides and carbonates have each been applied to conjugate PEG moieties to rHuPH20. Of these three chemistries, succinimidyl PEGs have typically been the most convenient to use in the case of rHuPH20. For example, rHuPH20 has been conjugated with exemplary succinimidyl monoPEG (mPEG) reagents including mPEG-Succinimdyl Propionates (mPEG-SPA), mPEG-Succinimidyl Butanoates (mPEG-SBA), and (for attaching "branched" PEGs) mPEG2-N-Hydroxylsuccinimide. These pegylated succinimidyl esters contain different length carbon backbones between the PEG group and the activated cross linker, and either a single or branched PEG group. These differences can be used, for example, to provide for different reaction kinetics and to potentially restrict sites available for PEG attachment to rHuPH20 during the conjugation process.

[0787] Succinimidyl PEGs (as above) comprising either linear or branched PEGs have been conjugated to rHuPH20. Using compositions and techniques as described in the art, succinimidyl PEGs have been used to generate rHuPH20s reproducibly comprising a combination of molecules having between about three to six PEG molecules per sHASEGP. Such pegylated rHuPH20 compositions were readily purified to yield compositions having specific activities of

approximately 25,000 Units/mg protein hyaluronidase activity, and being substantially free of non-PEGylated rHuPH20 (less than 5% non-PEGylated). As is known in the art and described for example in PEGylation references including those cited above, a variety of reagents and techniques are also available for achieving mono-disperse as opposed to poly-disperse PEG products (see, e.g., the reviews and references cited in the detailed description above related to site-specific monoPEGylation and site-directed PEGylation).

[0788] Improvements in both pharmacokinetics and pharmacodynamics have been observed in animal models following administration of a PEGylated sHASEGP as compared to the unmodified version of the sHASEGP. For example, following intravenous administration to mice of either 1,000 Units of an unmodified rHuPH20 or 1,000 Units of rHuPH20 conjugated to PEGs having a molecular weight of approximately 40 kDa (rHuPH20-PEG40), it was observed that the serum half-life of neutral active hyaluronidase activity (PH20) in serum of mice treated with rHuPH20-PEG40 was significantly longer (15-20 fold) than that of mice treated with unmodified rHuPH20. In addition, the effectiveness of rHuPH20-PEG40 has been compared to non-PEGylated rHuPH20 in a rat stroke model. Initial results from these studies demonstrate greater effectiveness of rHuPH20-PEG40 (greater than 75-85% survival, versus less than 50% in controls) in rat survival 7 days post stroke-inducement.

[0789] Using various PEG reagents as available from Nektar, we have prepared exemplary versions of human recombinant hyaluronidases (particularly preferred enzymes based on rHuPH20) using mPEG-SBA (30 kD), mPEG-SMB (30 kD), and branched versions based on mPEG2-NHS (40 kD), mPEG2-NHS (60 kD). These reagents and numerous others are available in various molecular weights (see, e.g., the 2005-06 Nektar Product Catalog which is available on-line at http://www.nektar.com or http://www.nektar.com/pdf/nektar_catalog.pdf, references therein, which catalog and references are incorporated herein by reference). A number of other PEG reagents and methodologies are available from other suppliers and/or are described in the art. By way of illustration, PEGylated versions of rHuPH20 have been generated using NHS chemistries, as well as carbonates, and aldehydes, using each of the following reagents available from Nektar: mPEG2-NHS-40K branched (Nektar #2z3y00r1); mPEG-NHS-10K branched (Nektar #2z3x0Lc1-10); mPEG-NHS-20K branched (Nektar #2z3x0Lc1-20); mPEG-NHS-40K branched (Nektar #2z3x0Lc1-40); mPEG2-NHS-60K branched (Nektar #2z3y0r01); mPEG-SBA-5K (Nektar #2m450h01); mPEG-SBA-20K (Nektar #2M450p01); mPEG-SBA-30K (Nektar #2M450r01); mPEG-SMB-20K (Nektar #2m4k0p01); mPEG-SMB-30K (Nektar #2m4k0r01); mPEG-butyraldehyde-30K (Nektar #2072M0R01); mPEG-SPA-20K (Nektar #2m4m0p01); mPEG-SPA-30K (Nektar #2m4m0r01); and PEG-NHS-5K-biotin (Nektar #0114M01102).

[0790] We have also generated PEGylated versions of hyaluronidases (e.g. rHuPH20) using PEG reagents available from Dowpharma, a division of Dow Chemical Corporation, including hyaluronidases PEGylated with Dowpharma's p-nitrophenyl-carbonate PEG (30 kDa) and with propionaldehyde PEG (30 kDa). Nektar and Dowpharma reagents and their resulting products were used and tested essentially as described below to generate a large variety of PEGylated hyaluronidase compositions. Other reagents, and other hyaluronidases, can be similarly reacted using analogous techniques.

[0791] For generating PEGylated versions of rHuPH20, purified enzyme (at a concentration of between about 1 to 14 mg/ml) was mixed with a molar excess of each PEG reagent (generally at about a 10:1 molar excess of PEG:enzyme), at pH and other conditions as recommended for each of the PEG reagent or chemistries (generally aldehydes reactions were performed at about pH4.5, NHS reactions at neutral pH and carbonate reactions at about pH9.5). The reaction components were reacted with mixing for approximately 16 hours at 4 degrees C.

[0792] SDS-PAGE was used to initially examine reaction products. Generally unmodified rHuPH20 was observed to migrate as a single band of approximately 63 kDa molecular weight. The various PEGylated versions of rHuPH20 generally comprised a band in the range of about 150-300 kDa (typically about 180 kDa) as well as several bands of greater than 200 kDa.

[0793] We then performed gel filtration of the modified enzyme products followed by enzyme activity of fractionated proteins. Briefly, following techniques as known in the art, separations of PEG-modified hyaluronidases from their respective native unmodified forms, were performed by gel filtration chromatography, using a Superose 6 column (GE/Pharmacia). Phosphate buffered saline was used as mobile phase for the separations, with a flow rate of 0.2 mL/minute. The column was calibrated using Gel Filtration Calibration Standards from GE/Pharmacia. Fractions containing separated hyaluronan degrading enzymes were assayed for relative hyaluronidase activity by a modified USP Turbidity assay, performed in a microtiter format. Peak retention volume (time) for rHuPH20 hyaluronidase enzyme activity fractionating through a Superose 6 gel filtration column, shifted from about 16 mL (approximately 60 to 80 KD apparent molecular weight) for the unmodified hyaluronidase, to about 10 mL (greater than 100,000 KD to approximately 670,000 KD apparent molecular weight) for the Peg-modified hyaluronidase. Peg modification can apparently, completely shift all measurable enzyme activity to the higher molecular weight modified forms, when molar excess peg is reacted with the enzyme.

[0794] We then examined enzymatic activity of various native and modified enzymes using zymography (analyses of glycosaminoglycan-degrading activity by substrate gel electrophoresis). Briefly, hyaluronan-containing SDS-PAGE zymogram gels were cast and analyses performed essentially in accordance with the procedure previously described by Miura K O, et al. Analysis of glycosaminoglycan-degrading enzymes by substrate gel electrophoresis (zymography). Anal Biochem. 1995 Mar 1;225(2):333-40.

[0795] Native rHuPH20 hyaluronidase demonstrated hyaluronan-degrading activity in zymogram gels as a single band with an apparent molecular weight of approximately 60 kDa. Peg modifications of rHuPH20 hyaluronidase resulted in a shift in hyaluronan degrading activity in zymogram gels with enzymatically active signals at approximately 180 kDa, and with at least 2 additional bands having enzyme activity with molecular masses greater than 200 kDa.

[0796] These results and others described herein demonstrate that glycosaminoglycanases can be effectively modified by PEGylation (which can be used to alter their pharmacokinetic and/or other profiles) using any of a wide variety of PEG reagents, and that the resulting enzymes can retain substantial enzymatic activity making them useful in any of variety of different applications—particularly in vivo or other applications in which modifying the enzyme to extend its half life can be useful.

EXAMPLE 21.B

Illustrative Pegylated Versions of Non-Human Hyaluronidases

[0797] Although human recombinant hyaluronidases are particularly preferred for applications in which the enzyme is being used in association with human cells, and even more particularly when it is being applied to or administered to the human body, the techniques illustrated herein can also be employed to generate pharmacokinetically-improved versions of other hyaluronidase enzymes that may have suitable application in certain contexts or environments. Thus, by applying methodologies as illustrated herein and described in the art to other hyaluronidase enzymes, we have generated a number of modified enzymes that are PEGylated (which can be used to improve their pharmacokinetics as illustrated herein) and yet retain substantial enzymatic activity. For purposes of illustration, methodologies as described and illustrated herein have been applied to a series of different exemplary "non-human" hyaluronidase enzymes, including an illustrative animal-derived hyaluronidase, an illustrative bacterial-derived hyaluronidase, and an illustrative bacterial derived chondroitinase having hyaluronidase activity. PEGylated versions of such non-human hyaluronidases, as described herein, can be used to effectively provide extended half lives (such as following intravenous or other in vivo administration).

[0798] As an illustrative example of a non-human animal-derived hyaluronidase, we have generated PEGylated versions of ovine testicular hyaluronidase (available as Vitrase™ from ISTA Pharmaceuticals. Vitrase (hyaluronidase for injection, Lyophilized, Ovine N.D. 67125-3-401, 2,000 USP Units/vial), purchased from ISTA pharmaceuticals, was a gift from an ophthalmologist. Vitrase solutions were prepared by reconstituting the lyophilized Vitrase with 0.9% Sodium Chloride Injection USP (Baxter).

[0799] As an illustrative example of a non-human bacterial-derived hyaluronidase, we have generated PEGylated versions of bacterial hyaluronidase available as Hyaluronate Lyase from Sigma. Hyaluronate Lyase from *Streptomyces hyalurolyticus* or other species, CAS Number 9067-54-7, EC Number 4.2.2.1) (674 units /vial) was purchased from Sigma. Hyaluronate lyase was reconstituted with sterile filtered phosphate buffered saline. See, e.g. Chien, J., and Kaneko, Y. Biochim. Biophys. Acta 198, 607, (1970).

[0800] As an illustrative example of a non-human chondroitinase having hyaluronidase activity, we have generated PEGylated versions of bacterial chondroitinase ABC (available as Chondroitinase ABC from Associates of Cape Cod, Inc., Seikagaku Corporation). Chondroitinase ABC from *Proteus vulgaris* (Chondroitin ABC lyase, Chondroitin ABC eliminase, EC Number: 4.2.2.4, CAS Number: 9024-13-0, cat. # 100330 or 10033) was purchased from Associates of Cape Cod Inc. Chondroitinase ABC solutions were prepared in 0.1% BSA solution. References include: (1) Yamagata, T., et al., J. Biol. Chem., 243, 1523 (1968). (2) Saito, H., et al., J. Biol. Chem., 243, 1536 (1968); (3) Suzuki, S., et al., J. Biol. Chem., 243, 1543 (1968); and (4) Oike, Y., et al., J. Biol. Chem., 257, 9751 (1982).

[0801] As an illustrative PEG reagent, we used an mPEG-Succinimidyl Butanoate (available as mPEG-SBA (30 kD) from Nektar). While mPeg-SBA-30K (N-hydroxysuccinimidyl ester of methoxy poly(ethylene glycol) butanoic acid, average M.W. 30,000, Cat # 2M450B01), was used for this example, other N-hydroxysuccinimidyl ester of methoxy poly(ethylene glycol) of any molecular weight or N-hydroxysuccinimidyl esters of poly(ethylene glycol) of any molecular weight, or peg-aldehydes of any size, or peg-carbonates of any size, or other Nektar PEG reagents, or other pegylation reagents can also be used. See, e.g., Zalipsky, S and C. Lee, "Poly(ethylene glycol) Chemistry: Biotechnical and Biomedical Applications," J. M. Harris, ed., Plenum, N.Y., 1992, cf. chapter 21.

[0802] For the generation of exemplary PEGylated versions of animal-derived hyaluronidases, a vial of ovine testicular hyaluronidase (Vitrase) was reconstituted with 0.62 mL of 0.9% saline (10 units/microliter), and 0.3 mL of the reconstituted enzyme solution was transferred to a sterile tube. The exemplary PEG reagent, mPeg-SBA-30K (9.9 mg), was added to the tube containing the Vitrase solution. The tube was mixed until all the mPeg appeared to enter into solution, and then the mixture was reacted for approximately 16 hours at 4 degrees C., with mixing.

[0803] For the generation of exemplary PEGylated versions of bacterial-derived hyaluronidases, one ampoule of Sigma hyaluronate lyase was reconstituted with 0.7 mL sterile filtered phosphate buffered saline (~1 unit/microliter), and 0.3 mL of the reconstituted enzyme solution was transferred to a sterile tube. The exemplary PEG reagent, mPeg-SBA-30K (9.8 mg), was added to the tube containing the hyaluronate lyase solution, the tube was then mixed until all the mPeg appeared to be in solution, and the mixture was reacted for approximately 16 hours at 4 degrees C., with mixing.

[0804] For the generation of exemplary PEGylated versions of bacterial-derived chondroitinase having hyaluronidase activity, one vial of Chondroitinase ABC (10 Units) was reconstituted with 0.2 mL of 0.9% saline, and the exemplary PEG reagent, mPeg-SBA-30K (12.4 mg), was added to the vial containing Chondroitinase ABC solution, and mixed until all mPeg appeared to be in solution, and the mixture was reacted for approximately 11 hours at 4 degrees C., with mixing.

[0805] Initial analyses included the application of SDS-PAGE to examine the modified enzymes compared to unmodified controls. Briefly, total protein was visualized with either Coomassie Blue or silver staining after separation through 3% to 8% NuPage Tris/Acetate gels (Invitrogen). Molecular weights of stained bands were determined by comparison to standard proteins of known mass run on the same gel.

[0806] Results with Vitrase were as follows: (A) Vitrase (unmodified), 5 bands of approximately the following

TABLE 24-1

Effects of sHASEGP on the pharmacokinetic profile of interferon

Time (Hours)	PEG INTRON (1.5 ug/kg) Intradermal	SD	PEG INTRON (1.5 ug/kg) + sHASEGP 100 U Intradermal	SD	PEG INTRON (1.5 ug/kg) Intravenous
0.5	30,289	9,089	54,808	12,955	281,730
1	43,137	9,134	76,673	17,570	185,630
2	54,655	11,123	102,159	19,526	155,521
3	55,261	11,093	113,349	24,970	128,656
6	61,214	13,528	113,354	23,817	93,055
8	59,297	14,734	110,843	27,133	79,275
12	46,696	12,752	85,710	28,078	58,457
24	15,233	6,595	26,047	6,026	16,966
36	7,854	1,777	12,498	2,762	9,277
48	3,867	459	5,517	1,650	5,847
AUC	1,223,682		2,213,583		1,910,725
Cmax	61,214		113,354		—
Tmax	6		6		—
Absolute Bioavailability	64%		116%		100%

[0837] As can be seen in Table 24-1, the bioavailability of the exemplary pharmacologic PEG-INTRON (as assessed by AUC) was dramatically improved when the intradermal dosing was combined with a dose of sHASEGP. In fact, the absolute bioavailability achieved with an intradermal dosing in combination with sHASEGP were statistically indistinguishable from the direct intravenous administration, where all of the material is delivered directly into the bloodstream. Thus, the use of sHASEGP in combination with a pharmacologic that is introduced by non-IV parenteral administration can provide levels of delivery comparable to administration directly into the bloodstream. Although the T_{max} is necessarily longer following ID administration (since some amount of time is necessary for the agent to reach the bloodstream), within several hours after ID dosing with sHASEGP, the concentrations of agent in the bloodstream essentially reached those achieved by IV dosing, and thereafter apparently exceeded them.

EXAMPLE 25

Illustrative Use of a sHASEGP to Facilitate Intradermal Delivery of a Macromolecule Normally Delivered by Intravenous Injection

[0838] As discussed above, sHASEGPs can be used to facilitate the delivery of known or newly developed pharmacologic and other agents by different routes of administration that may provide for greater convenience, safety, reduced costs and/or other benefits. As an illustrative example of such a reformulation, we used a sHASEGP to facilitate intradermal delivery of a macromolecular drug that is normally administered by intravenous injection. For this particular example, we combined recombinant human PH20 (rHuPH20) with a chimeric human-murine anti-TNF alpha monoclonal antibody (Infliximab, available as REMICADE™ from Centocor). REMICADE is a macromolecule of approximately 150 kD that is typically administered by intravenous injection for the treatment of arthritis or Crohn's disease. Administration of REMICADE thus generally requires the involvement of intravenous access and concomitant professional care and risks, and also requires on the order of two hours for each dosing.

[0839] REMICADE (20 mg/ml) was labeled with ^{125}I to a specific activity of 10 μCi/ug. Eighteen rats were divided into three groups of six animals each and were administered a mixture of radioactive and cold REMICADE (combined 10 mg/kg) in a final volume of 100 μl (i) by intravenous administration, (ii) by intradermal administration or (iii) or by intradermal administration in combination with 100 Unit sHASEGP (from a 100,000 U/ml stock); and then blood and tissues processed by gamma counting at various points after dosing, essentially as described for the preceding example. The results are summarized in Table:

TABLE 25-1

Effects of sHASEGP on the Pharmacokinetic Parameters of REMICADE

Time (Hours)	CPM REMICADE (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	2,222	95,889	31,018	2,557,849
1	37,213	10,775	146,793	42,014	1,625,571
2	66,901	6,078	412,642	61,540	1,576,145
4	120,704	21,744	591,625	89,662	2,260,645

TABLE 25-2

Effects of sHASEGP on the Pharmacokinetic Parameters of REMICADE

Time (Hours)	CPM REMICADE (10 mg/kg) Intradermal	SD	CPM REMICADE (10 mg/kg) + sHASEGP (300 U/kg) Intradermal	SD	CPM REMICADE (10 mg/kg) Intravenous
8	356,087	32,436	[illegible]	33,887	1,157,268
24	495,864	75,654	[illegible]	97,777	720,557
48	513,439	63,437	[illegible]	74,848	586,559
72	454,717	54,388	[illegible]	61,402	558,307
96	376,903	39,648	[illegible]	59,618	496,658
120	356,067	66,964	[illegible]	53,643	434,308
AUC	53,550,555		[illegible]		83,178,550
C_{max}	513,439		[illegible]		1,657,869
T_{max}	48		[illegible]		0
Absolute bioavailability	62%		[illegible]		100%

[0840] As can be seen in Table 5-1, the bioavailability of the exemplary pharmacologic REMICADE, as assessed by AUC) was dramatically improved when [illegible] intradermal dosing was combined with a dose of sHASEGP. As was observed with enhanced delivery of 8% 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 [illegible] 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 [illegible] observed with IV dosing, and thereafter approximated 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 Cmax and bioavailability (AUC) both substantially increased (by about 65% and 95%, respectively).

EXAMPLE 26

Illustrative Use of a sHASEGP to Facilitate Intradermal Delivery of a Larger Macromolecular Complex Normally Delivered by Non-IV Parenteral Injection

[0841] As another illustrative example of the use of sHASEGPs to enhance the pharmacokinetics of a macromolecular drug that is normally administered by non-intravenous parenteral injection, we combined recombinant human PH20 (rHuPH20) with recombinant human Factor VIII (RECOMBINATE™ available from Baxter). Factor VIII is a large glycoprotein complex comprising multiple polypeptides, and has a combined molecular of approximately 280 kD. Factor VIII is typically only administered by intravenous injection for the treatment of hemophilia. Like many macromolecules administered subcutaneously, Factor VIII is relatively slowly absorbed and a substantial portion of the agent never makes it to the bloodstream, with bioavailability on the order of 0.5-1%.

[0842] Eighteen rats were divided into three groups of six animals each and were administered 125-I labeled Factor VIII (i) by intravenous administration, (ii) by intradermal administration or (iii) by intradermal administration in combination with sHASEGP, and then blood and tissues processed at various points after dosing to measure counts per minute (CPM) per gram plasma, essentially as described for the preceding example.

TABLE 26-1

Effects of sHuPH20 on the pharmacokinetics of Factor VIII

Time (Hours)	CPM FACTOR VIII (50 IU/kg) Intradermal	SD	CPM FACTOR VIII (50 IU/kg) + sHASEGP (300 U/kg) Intradermal	SD	CPM FACTOR VIII (50 IU/kg) Intravenous
0.5	29,718	12,062	46,576	5,513	671,753
1	43,767	9,415	70,246	14,614	379,558
2	49,503	14,582	72,736	14,985	247,397
3	56,087	17,621	76,631	14,454	203,502
4	55,494	12,721	84,366	8,646	177,956
5	55,277	14,181	79,735	9,263	165,914
6	50,195	13,970	72,176	6,848	151,198
8	52,298	13,017	66,419	10,495	114,579

TABLE 26-1-continued

Effects of rHuPH20 on the pharmacokinetics of Factor VIII

Time (Hours)	CPM FACTOR VIII (50 IU/kg) Intradermal	SD	CPM FACTOR VIII (50 IU/kg) + sHASEGP (20 U) Intradermal	SD	CPM FACTOR VIII (50 IU/kg) Intravenous
12	48,336	10,765	58,[illegible]7	[illegible]	75,9[illegible]2
24	21,[illegible]	4,[illegible]	14,[illegible]	4,426	29,6[illegible]
AUC	1,014,521		1,[illegible]		2,554,597
C_{max}	55,[illegible]		84,[illegible]		—
T_{max}	4		4		—
Absolute Bioavailability	39.7%		5[illegible]%		100%

[0843] As shown in Table 26-1, co-administration of Factor VIII with rHuPH20 significantly increased the systemic bioavailability relative to Factor VIII alone (P<0.01). The use of TCA precipitation was necessary to eliminate the contribution of completely degraded I-125 labeled Factor VIII protein entering the blood stream.

EXAMPLE 27

Illustrative Use of a sHASEGP to Improve Survival and/or Behavioral Outcomes Following Stroke

[0844] In a first set of studies, sHASEGPs were evaluated in a rat model of stroke. Briefly, stroke was surgically induced in Sprague-Dawley rats by middle cerebral artery occlusion, followed at various times by intravenous administration of either a control solution (saline) or a test solution (saline comprising a sHASEGP). Animals were allowed to recover and were followed over a period of time to assess rates of survival in control versus test groups. In addition, survivors were assessed over a range of timepoints using multiple analyses of behavioral conditions typically associated with stroke. Only rats that performed normally in an initial qualifying forelimb flexion test were included in the study, and were then randomly assigned to a control or test group of animals.

[0845] Aseptic surgical procedures were performed by Zivic Laboratories essentially as follows. Rats were anesthetized in a chamber supplied with 2.5% halothane. A ventral midline incision was made and the muscle tissue was separated to expose the right external carotid artery which was then isolated and double ligated. An untied but constrictive 5/0 polyamide ligature was then placed around the external carotid stump. A small hole was cut into the lumen of the stump and an 18-20 mm length of 4/0 monofilament suture material was inserted into the lumen and advanced into the internal carotid artery. When completely advanced, the internalized tip of the monofilament should block flow into the middle cerebral artery. The constrictive polyamide ligature was then tied to secure the monofilament. Retraction was removed and the skin incision closed with a wound clip.

[0846] Intravenous injection procedures were performed essentially as follows. Rats were anesthetized in a chamber supplied with 2.5% halothane. A ventral skin incision was made to expose the jugular vein. A sterile 1 ml disposable syringe was fitted with a 30-gauge needle and loaded with injection material. Using the clavicle for support, the syringe was inserted into the jugular vein (using slight negative pressure on the syringe to draw bloodflow into the syringe and thereby confirm proper placement for IV injection) and then the injection material was slowly injected into the jugular vein. After injection, the needle was slowly withdrawn (and any bleeding controlled with a sterile cotton applicator), and then the skin incision was closed with a wound clip.

[0847] Survival of animals in the various groups (approximately 40 animals in each group) was monitored following induction of stroke, and the number of survivors at each day were then plotted against days following stroke to generate a Kaplan-Meier stroke curve. Substantial differences between the groups were apparent by the 3rd day following stroke. By one week following the induction of stroke, more than half of the control animals had died, whereas nearly three quarters of the sHASEGP-treated animals had survived, which was a statistically significant difference ($p=0.03$).

[0848] In addition to measuring rates of survival following stroke, survivors were also tested (at 1, 2, 3 and 4 weeks following stoke) using other analyses designed to assess the incidence of certain behavioral problems associated with stroke. Behavioral conditions assessed included forelimb flexion, torso twist, hind limb, gait disturbances and wall walk, which were used to generate a PNEGS behavorial score (higher scores reflecting an increase in behavioral problems associated with the stroke). By the second week following induction of stroke, the sHASEGP-treated animals exhibited significantly higher behavioral scores ($p=0.02$), and the relative differences between the treated group and the control were even greater over subsequent weeks ($p=0.007$ and $p=0.008$ for weeks three and four respectively).

[0849] Although the invention has been described with reference to the above examples, it will be understood that modifications and variations are encompassed within the spirit and scope of the invention. Accordingly, the invention is limited only by the following claims.

-continued

gaatggagac ccacttgggc aagaaactgg aaacctaaag atgtttacaa gaataggtct	900
attgaattgg ttcagcaaca aaatgtacaa cttagtctca cagaggccac tgagaaagca	960
aaacaagaat ttgaaaaggc agggaaggat ttcctggtag agactataaa attgggaaaa	1020
ttacttcggc caaatcactt gtggggttat tatcttttac cggattgtta caaccatcac	1080
tataagaaac ccggttacaa tggaagttgc ttcaatgtag aaataaaaag aaatgatgat	1140
ctcagctggt tgtggaatga aagcactgct ctttacccat ccatttattt gaacactcag	1200
cagtctcctg tagctgctac actctatgtg cgcaatcgag ttcgggaagc catcagagtt	1260
tccaaaatac ctgatgcaaa aagtccactt ccggtttttg catatacccg catagttttt	1320
actgatcaag ttttgaaatt cctttctcaa gatgaacttg tgtatacatt tggcgaaact	1380
gttgctctgg gtgcttctgg aattgtaata tgggggaccc tcagtataat gcgaagtatg	1440
aaatcttgct tgctcctaga caattacatg gagactatac tgaatcctta cataatcaac	1500
gtcacactag cagccaaaat gtgtagccaa gtgctttgcc aggagcaagg agtgtgtata	1560
aggaaaaact ggaattcaag tgactatctt cacctcaacc cagataattt tgctattcaa	1620
cttgagaaag gtggaaagtt cacagtacgt ggaaaaccga cacttgaaga cctggagcaa	1680
ttttctgaaa aattttattg cagctgttat agcacccaga gttgtaagga gaaagctgat	1740
gtaaaagaca ctgatgctgt tgatgtgtgt attgctgatg gtgtctgtat agatgctttt	1800
ctaaaacctc ccatggagac agaagaacct caaattttct actatgcttc accctccaca	1860
ctatctgcca caatgttcat ttggaggctg gtagtctggg atcaaggtat tagcagaatt	1920
ggtttcttct gagagtcatc aggcaaaaat gtgtttcagg cctcttccct tggcttaccg	1980
gaactgacca accaatgact atcatcacca ccatcctagg gtcttacctg cagtcactct	2040
cctagatgct gtgggagacg ggcaagttac caagatagac cttccctcaa gataatcaga	2100
tatttcatgt attatcctta actttcttga catcatgggg gctttggcaa cctgatgaag	2160
cctatcaatt ttctttcaga agatatttat ataagattat aagaaaaatt aagtacacag	2220
ctattttctt tgcattggat caacatgcca tttatcaaga attatgcctt ttccatcact	2280
tttagcatgg aaaaataatt tcatgcaata tgcttaaaaa ttgggggaag acaaaagaaa	2340
tcctatatgt gtaaataaac ataaatttag gttttgctca aaaaaaaaaa aaaaa	2395

1. A substantially purified glycoprotein, comprising a neutral active soluble hyaluronidase polypeptide and at least one N-linked sugar moiety, wherein the N-linked sugar moiety is covalently attached to an asparagine residue of the polypeptide.

2. The polypeptide of claim 1, wherein the polypeptide is selected from of:

(a) a polypeptide that comprises a sequence of amino acids encoded by the sequence that includes at least about 74% amino acid sequence identity with the sequence of amino acids set forth in SEQ ID NO. 1;

(b) a polypeptide that comprises a sequence of amino acids encoded by the sequence of nucleotides set forth in SEQ ID NO. 6;

(c) a polypeptide that comprises a sequence of amino acids encoded by a sequence of nucleotides that hybridizes along at least 85% of its full-length under conditions of high stringency to the sequence of nucleotides set forth in SEQ ID NO. 6; or

(d) a polypeptide that is encoded by a sequence of nucleotides that is a splice variant of the sequence of nucleotides that comprises the sequence set forth in SEQ ID NO. 50.

3. The polypeptide of claim 1, wherein said sugar moiety is covalently attached to an asparagine residue selected from amino acids 82, 166, 235, 254, 368, 393 or 490 as set forth in SEQ ID NO. 1.

4. The polypeptide of claim 1, wherein said sugar moiety is covalently linked to said polypeptide through a PNGase sensitive bond.

5. The polypeptide of claim 1, wherein said sugar moiety is of the high mannose type.

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 terminal sialic acid.

9. The polypeptide of claim 1, wherein the polypeptide consists essentially of the hyaluronidase content 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 15, wherein the hyaluronidase domain comprises the sequence of amino acids set forth as amino acids 1-438 of SEQ ID NO. 3.

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. (canceled)

21. (canceled)

22. The polypeptide of claim 1, wherein the sHASEGP is a human polypeptide.

23. The polypeptide of claim 1, wherein the polypeptide is a soluble polypeptide as set forth in SEQ ID NO. 4.

24. (canceled)

25. (canceled)

26. (canceled)

27. (canceled)

28. (canceled)

29. (canceled)

30. The polypeptide of claim 1, wherein a signal sequence capable of directing the polypeptide outside the cell is operably attached to the sHASEGP polypeptide.

31. An isolated soluble human hyaluronidase glycoprotein (sHASEGP), wherein the potency of the sHASEGP is greater than 40,000 USP Units/mg protein.

32. The sHASEGP of claim 31, wherein the hyaluronidase is sialated.

33. (canceled)

34. (canceled)

35. The sHASEGP of claim 31, wherein the polypeptide is modified with a polymer.

36. The sHASEGP of claim 35, wherein the polymer is PEG or dextran.

37. (canceled)

38. A nucleic acid molecule, encoding the polypeptide of claim 1.

39. A nucleic acid molecule, comprising a sequence selected from:

(a) a sequence as set forth in SEQ ID NO.6;

(b) a sequence that hybridizes under high stringency to at least about 70% of the full-length to the sequence of nucleotides set forth in SEQ ID NO. 6;

(c) a sequence of nucleotides that encodes the polypeptide set forth in SEQ ID No. 1, 3, 4, 5 and 50;

(d) a sequence that is a splice variant of (a), (b) or (c);

(e) a sequence that encodes the hyaluronidase domain or a catalytically active portion thereof that includes a sequence of nucleotides having at least about 60% sequence identity with the sequence set forth in SEQ ID No. 6 or 50; and

(f) a sequence comprising degenerate codons of (a), (b), (c), (d) or (e).

40. A vector having a sequence as set forth in SEQ ID NO: 51.

41. A vector comprising the nucleic acid molecule of claim 38.

42. (canceled)

43. (canceled)

44. (canceled)

45. (canceled)

46. (canceled)

47. An isolated cell, comprising the vector of claim 41.

48. (canceled)

49. (canceled)

50. (canceled)

51. The cell of claim 47 that is a mammalian cell.

52. An antibody that binds to the sHASEGP of claim 1, wherein said antibody is free of reactivity to bovine, ovine or bacterial hyaluronidase.

53. A transgenic non-human animal, wherein an endogenous gene that encodes a polypeptide of claim 1 has been disrupted by homologous recombination or insertional mutagenesis of the animal or an ancestor thereof.

54. A method for producing a soluble polypeptide that contains sHASEGP, comprising:

introducing a nucleic acid as set forth in SEQ ID NO. 6 operably linked to a promoter into a cell that incorporates N-linked sugar moieties into sHASEGP;

culturing the cell under conditions whereby the encoded polypeptide is expressed by the cell; and

recovering the expressed polypeptide.

55. A method for producing a soluble polypeptide that contains sHASEGP, comprising:

introducing a nucleic acid that encodes the polypeptide of claim 1 operably linked to a suitable promoter into a cell capable of incorporating N-linked sugar moieties into sHASEGP; culturing the cell under conditions whereby the encoded polypeptide is expressed by the cell; and recovering the expressed polypeptide.

56. (canceled)

57. (canceled)

58. (canceled)

59. A method for producing a soluble polypeptide that contains sHASEGP, comprising:

PODER ESPECIAL

Yo, **ORLANDO MUÑOZ MARTINEZ,** mayor de edad y vecino de la ciudad de Bogotá (Colombia), identificado como aparece al pie de mi firma, quien en el presente documento actúa en calidad de Representante Legal de la sociedad **LABORATORIOS SYNTHESIS S.A.S.** sociedad legalmente constituida y domiciliada en la ciudad de Bogotá (Colombia), por el presente documento otorgo a la doctora **ELIANA ISAURA MORENO BOHÓRQUEZ**, abogada en ejercicio, identificada como aparece al pie de su firma, con tarjeta profesional No. 83823 **del C.S. de la J.**, poder especial, amplio y suficiente, para formular oposiciones u observaciones a la solicitud de patente No. **11 178.794,** titulada **"FORMULACIÓN SUBCUTÁNEA DE ANTICUERPO ANTI-HER2",** cuyo titular ***F. HOFFMANN-LAROCHE AG.*** publicada en la gaceta No. 643 del 30 de abril de 2012.

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

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

Dado y Firmado en Bogotá (Colombia) a los 06 días del mes de julio de 2012.

LABORATORIOS SYNTHESIS S.A.S.

C.C. No. 8.317.044 de Medellín
NIT: 860.000.760-1
Representante Legal

Acepto:

ELIANA ISAURA MORENO BOHÓRQUEZ
C.C. 51.975.664
T.P. 83823 del C.S.J

Munoz Orlando
Martinez
8317044
Medellin.

09 JUL 2012

ALBERTO VARGAS

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Cámara de Comercio de Bogotá

CAMARA DE COMERCIO DE BOGOTA

SEDE KENNEDY

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CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O INSCRIPCION DE DOCUMENTOS

LA CAMARA DE COMERCIO DE BOGOTA, CON FUNDAMENTO EN LAS MATRICULAS E INSCRIPCIONES DEL REGISTRO MERCANTIL

CERTIFICA:

NOMBRE : LABORATORIOS SYNTHESIS S A S
N.I.T. : 860000760-1
DOMICILIO : BOGOTA D.C.

CERTIFICA:

MATRICULA NO: 00008059 DEL 23 DE MARZO DE 1972

CERTIFICA:

RENOVACION DE LA MATRICULA :29 DE FEBRERO DE 2012
ULTIMO AÑO RENOVADO: 2012
ACTIVO TOTAL REPORTADO:$78,678,599,000

CERTIFICA:

DIRECCION DE NOTIFICACION JUDICIAL : CRA 44 NO 20C 73
MUNICIPIO : BOGOTA D.C.
EMAIL DE NOTIFICACION JUDICIAL : lmbautista@synthesis.com.co
DIRECCION COMERCIAL : CRA 44 NO 20C 73
MUNICIPIO : BOGOTA D.C.
EMAIL COMERCIAL : lmbautista@synthesis.com.co

CERTIFICA:

CONSTITUCION: ESCRITURA PUBLICA NO.2078, NOTARIA 8 BOGOTA, DEL 27 DE JULIO DE 1.950, INSCRITA EL 10 DE AGOSTO DE 1.950, BAJO EL NO. 21.766 DEL LIBRO RESPECTIVO ACLARADA POR ESCRITURA NO. 3608 DEL 8 DE OCTUBRE DE 1.956 NOTARIA 8 DE BOGOTA INSCRITA EN ESTA - CAMARA DE COMERCIO EL 19 DE OCTUBRE DE 1.956 BAJO EL NO. 35.547 DEL LIBRO RESPECTIVO, SE CONSTITUYO LA SOCIEDAD DENOMINADA: "LA-BORATORIOS BALBAG".

CERTIFICA:

QUE POR ESCRITURA PUBLICA NUMERO 3997 OTORGADA EN LA NOTARIA 8 DE BOGOTA EL 24 DE NOVIEMBRE DE 1.952, INSCRITA EN ESTA CAMARA DE CO MERCIO EL 1 DE DICIEMBRE DE 1.952, BAJO EL NUMERO 26.158 DEL LI-- BRO RESPECTIVO, LA SOCIEDAD CAMBIO SU NOMBRE DE:"LABORATORIOS --- BALBAG" POR EL DE "LABORATORIO SYNTHESIS" E INTRODUJO OTRAS RE-- FORMAS.

CERTIFICA :

QUE POR ESCRITURA PUBLICA NUMERO 6491 OTORGADA EN LA NOTARIA 1. - DE BOGOTA EL 24 DE DICIEMBRE DE 1.981, INSCRITA EN ESTA CAMARA DE COMERCIO EL 12 DE MARZO DE 1.982, BAJO EL NUMERO 113.297 DEL LI-BRO IX, LA SOCIEDAD CAMBIO SU NOMBRE DE "LABORATORIOS SYNTHESIS - BALAVOINE Y CIA. S.C.A" POR EL DE "LABORATORIOS SYNTHESIS LTDA. Y CIA. S.C.A" E INTRODUJO OTRAS REFORMAS.

CERTIFICA:

QUE POR ESCRITURA PUBLICA NUMERO 8580 OTORGADA EN LA NOTARIA 3 DE

BOGOTA EL 4 DE DICIEMBRE DE 1.973, INSCRITA EN ESTA CAMARA DE COMERCIO EL 7 DE ENERO DE 1.974, BAJO EL NUMERO 14.478 DEL LIBRO IX, LA SOCIEDAD SE TRASFORMO DE LIMITADA EN COMANDITA POR ACCIONES BAJO EL NOMBRE DE LABORATORIOS SYNTHESIS BALAVOINE Y CIA. S.C.A.

CERTIFICA:

QUE POR ACTA NO. 117 DE LA ASAMBLEA DE ACCIONISTAS, DEL 30 DE ABRIL DE 2010, INSCRITA EL 10 DE JUNIO DE 2010 BAJO EL NÚMERO 1390261 DEL LIBRO IX, LA SOCIEDAD DE LA REFERENCIA SE TRANSFORMO DE SOCIEDAD EN COMANDITA POR ACCIONES A SOCIEDAD POR ACCIONES SIMPLIFICADA BAJO EL NOMBRE DE: LABORATORIOS SYNTHESIS S.A.S.

CERTIFICA:

QUE POR E.P. NO.5.435 DEL 17 DE SEPTIEMBRE DE 1.996, NOTARIA PRIMERA DE SANTAFE DE BOGOTA, INSCRITA EL 30 DE SEPTIEMBRE DE 1.996, BAJO EL NO.556.909 DEL LIBRO IX, ADICIONADA POR LOS REGISTROS 557 146 DEL 1 DE OCTUBRE DE 1.996, Y 557.301 DEL 2 DE OCTUBRE DE 1996 DEL LIBRO IX, LA SOCIEDAD DE LA REFERENCIA COMUNICO QUE SE FUSIONO, ABSORBIENDO A LA SOCIEDAD "SYNTHESIS VETERINARIA LTDA Y CIA $ C A"

CERTIFICA:

QUE POR E.P. NO.1024 DEL 28 DE FEBRERO DE 1997, DE LA NOTARIA 1. DE SANTAFE DE BOGOTA, INSCRITA EL 31 DE MARZO DE 1997, BAJO EL NUMERO 579245 DEL LIBRO IX, CELEBRO ACUERDO DE FUSIÓN CON LA SOCIEDAD PRODUCTOS DIETÉTICAS DIETESYN S.A, DONDE LA SOCIEDAD DE LA REFERENCIA FUE LA ABSORBENTE.

CERTIFICA:

QUE POR ACTA NO. 120 DE LA ASAMBLEA DE ACCIONISTAS DEL 29 DE JULIO DE 2010, INSCRITA EL 19 DE NOVIEMBRE DE 2010 BAJO EL NUMERO 01429837 DEL LIBRO IX, LA SOCIEDAD DE LA REFERENCIA (ABSORBENTE) ABSORBE MEDIANTE FUSIÓN A LAS SOCIEDADES INMUNOSYN SAS Y GYNOPHARM SAS LAS CUALES SE DISUELVEN SIN LIQUIDARSE.

CERTIFICA:

REFORMAS:

ESCRITURAS NO.	FECHA	NOTARIA	INSCRIPCION
4471	18-XII-1.956	8 BTA.	22-XII-1.956 NO. 36.077
3322	31-XII-1.958	8 BTA.	12-I-1.959 NO. 42.034
2322	22-IX-1.961	8 BTA.	29-IX-1.961 NO. 51.161
290	2-II-1.967	6 BTA.	24-II-1.967 NO. 71.304
8545	16-XII-1.967	6 BTA.	26-I-1.968 NO. 75.041
4679	20-XII-1.967	8 BTA.	26-I-1.968 NO. 75.042
3010	14-V-1.968	6 BTA.	30-V-1.968 NO. 76.244
2931	4-V-1.970	6 BTA.	18-V-1.970 NO. 84.983
5656	13-VII-1.970	6 BTA.	13-VIII-1.970 NO. 86.126
3487	15-VII-1.971	3 BTA.	29-VII-1.971 NO. 90.980
4638	10-VII-1.984	11 BTA.	27-VIII-1.984 NO.157.001
1443	14-III-1.985	11 BTA.	26- III-1.985 NO.167.627
724	11- II-1.985	1 BTA.	21- II-1.985 NO.165.940
4836	11-VIII-1986	1 BTA.	23- IX -1.986 NO.197.784
5659	15- IX -1986	1 BTA.	23- IX -1.986 NO.197.784
6326	16- X -1987	1 BTA.	23- X -1.987 NO.221.604
5164	21-VIII-1990	1 BTA.	3- IX -1.990 NO.303.579
9244	23-XII- 1991	1 STAFE.BTA.	8-I -1.992 NO.351.255
8145	15- X - 1992	1 STAFE.BTA.	16- X-1.992 NO.382.488
1374	26- III-1993	1 STAFE.BTA.	19- III-1993 NO.399972
3120	01-VI-1.995	1A.STAFE BTA	23-VI- 1.995 NO.498.060
5435	17-IX-1.996	1A.STAFE BTA	30-IX- 1.996 NO.556.909
5435	17-IX-1.996	1A.STAFE BTA	01- X- 1.996 NO.557.146
5435	17-IX-1.996	1A.STAFE BTA	02- X- 1.996 NO.557.301

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Cámara de Comercio de Bogotá

CAMARA DE COMERCIO DE BOGOTA

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5942 7-X -1.996 1A.STAFE BTA 11- X- 1.996 NO.558.222
6305 24-X--1.996 1A.STAFE BTA 07-XI--1.996 NO.561.207
6929 25-XI-1.996 1A.STAFE BTA 12-XII-1.996 NO.565.961
787 19-II-1.997 1A.STAFE BTA 25-II -1.997 NO.575.141
1024 28-II-1.997 1A.STAFE BTA 31-III-1.997 NO.579.245

CERTIFICA:

REFORMAS:

E.P. NO.	FECHA	NOTARIA	CIUDAD	FECHA	NO.INSC.
0007110	1997/10/28	0001	BOGOTA D.C.	1997/11/06	00609204
0001896	1998/03/16	0001	BOGOTA D.C.	1998/04/01	00628368
0002924	1998/04/21	0001	BOGOTA D.C.	1998/05/18	00634309
0002377	2000/06/14	0001	BOGOTA D.C.	2000/06/20	00733626
0000SIN	2004/06/22	0000	BOGOTA D.C.	2004/06/28	00940785
117	2010/04/30	0000	BOGOTA D.C.	2010/06/10	01390261
120	2010/08/18	0000	BOGOTA D.C.	2010/08/26	01409241
120	2010/08/30	0000	BOGOTA D.C.	2010/09/13	01413659
120	2010/07/29	0000	BOGOTA D.C.	2010/11/19	01429837
139	2011/06/07	0000	BOGOTA D.C.	2011/06/20	01489422
146	2011/10/24	0000	BOGOTA D.C.	2011/11/30	01531828

CERTIFICA:

VIGENCIA: QUE LA SOCIEDAD NO SE HALLA DISUELTA. DURACION HASTA EL 27 DE JULIO DE 2040

CERTIFICA:

OBJETO SOCIAL: EL OBJETO DE LA SOCIEDAD ES EL EJERCICIO DE TODAS LAS ACTIVIDADES COMERCIALES PARA: 1) LA IMPORTACIÓN, EXPORTACIÓN, FABRICACIÓN, COMERCIALIZACIÓN, DISTRIBUCIÓN Y VENTA DE PRODUCTOS FARMACÉUTICOS, HIGIÉNICOS, VETERINARIOS E IMPLEMENTOS MÉDICOS. 2) CUALQUIER OTRA ACTIVIDAD LÍCITA DE COMERCIO RELACIONADA CON SU OBJETO SOCIAL PRINCIPAL Y TODO LO QUE SE RELACIONA CON LA IMPORTACIÓN DE LOS BIENES Y EQUIPOS NECESARIOS PARA EL DESARROLLO DE SU OBJETO SOCIAL. 3) TENER LA REPRESENTACIÓN EN COLOMBIA DE EMPRESAS O SOCIEDADES ESTABLECIDAS FUERA DEL TERRITORIO COLOMBIANO, CUYO OBJETO SEA SIMILAR O COMPLEMENTARIO. EN DESARROLLO DE SU OBJETO SOCIAL PRINCIPAL, LA SOCIEDAD PODRÁ ADELANTAR Y CELEBRAR LOS SIGUIENTES ACTOS Y CONTRATOS: A) EXPORTAR E IMPORTAR, AGENCIAR, DISTRIBUIR, COMERCIALIZAR, COMPRAR O VENDER A COMISIÓN O CONSIGNACIÓN O DE CUALQUIER OTRA FORMA O MODALIDAD, TODA CLASE DE EQUIPOS, APARATOS, BIENES AL POR MAYOR O AL DETAL, Y SERVICIOS RELACIONADOS CON SU ACTIVIDAD. B) GRAVAR EN CUALQUIER FORMA LOS BIENES DE SU PROPIEDAD MUEBLES O INMUEBLES, DAR EN PRENDA LOS PRIMEROS O HIPOTECAR LOS SEGUNDOS PARA GARANTIZAR OBLIGACIONES DE LA SOCIEDAD. C) CONSTITUIR APODERADOS QUE LE REPRESENTEN, ASOCIARSE CON OTRAS COMPAÑÍAS DE LA MISMA O PARECIDA NATURALEZA Y EN GENERAL REALIZAR TODA CLASE DE OPERACIONES COMERCIALES O CIVILES Y EJECUTAR O CELEBRAR TODA CLASE DE CONTRATOS O ACTOS DEL MISMO ORDEN, YA SEAN INDUSTRIALES, COMERCIALES O FINANCIEROS

ENCAMINADOS A ALCANZAR LOS FINES QUE SE PROPONEN. D) ADQUIRIR, ENAJENAR, ADMINISTRAR, RECIBIR O DAR EN ARRENDATARIO O A CUALQUIER TÍTULO, TODA CLASE DE BIENES MUEBLES E INMUEBLES. E) INTERVENIR ANTE TERCEROS O ANTE LOS ACCIONISTAS MISMOS COMO ACREEDORA O COMO DEUDORA EN TODA CLASE DE OPERACIONES DE CRÉDITO, DANDO O RECIBIENDO LAS GARANTÍAS DEL CASO, CUANDO HAYA LUGAR A ELLAS; F) EFECTUAR CUALQUIER CLASE DE OPERACIONES DE CRÉDITO ACTIVO O PASIVO, TALES COMO CONSTITUIR DEPÓSITOS, EFECTUAR PRESTAMOS. OTORGAR O RECIBIR DOCUMENTOS NEGOCIABLES, GIRAR, ACEPTAR, ENDOSAR, ASEGURAR Y NEGOCIAR EN GENERAL TÍTULOS VALORES RECIBIRLOS EN PAGO; G) CELEBRAR Y EJECUTAR EN GENERAL TODOS LOS ACTOS Y CONTRATOS PREPARATORIOS, COMPLEMENTARIOS O ACCESORIOS DE TODOS LOS ANTERIORES, LOS QUE SE RELACIONEN CON LA EXISTENCIA Y FUNCIONAMIENTO DE LA SOCIEDAD, Y LOS QUE SEAN CONDUCENTES AL BUEN LOGRO DE LOS FINES SOCIALES.

CERTIFICA:

CAPITAL:

** CAPITAL AUTORIZADO **

VALOR : $22,000,000,000.00
NO. DE ACCIONES : 22,000,000.00
VALOR NOMINAL : $1,000.00

** CAPITAL SUSCRITO **

VALOR : $20,877,288,000.00
NO. DE ACCIONES : 20,877,288.00
VALOR NOMINAL : $1,000.00

** CAPITAL PAGADO **

VALOR : $20,877,288,000.00
NO. DE ACCIONES : 20,877,288.00
VALOR NOMINAL : $1,000.00

CERTIFICA:

REPRESENTACION LEGAL: LA SOCIEDAD CONTARÁ CON UN GERENTE, QUIEN TENDRÁ DOS SUPLENTES DENOMINADOS SUBGERENTES, QUIENES LO REEMPLAZARÁN EN LAS FALTAS OCASIONALES, TEMPORALES O ABSOLUTAS DE AQUÉL.

CERTIFICA:

** NOMBRAMIENTOS **

QUE POR ACTA NO. 127 DE ASAMBLEA DE ACCIONISTAS DEL 12 DE NOVIEMBRE DE 2010, INSCRITA EL 23 DE NOVIEMBRE DE 2010 BAJO EL NUMERO 01430759 DEL LIBRO IX, FUE (RON) NOMBRADO (S):

NOMBRE	IDENTIFICACION
GERENTE	
PALACIOS LUIS ALBERTO	C.E. 000000000319788

QUE POR ACTA NO. 117 DE ASAMBLEA DE ACCIONISTAS DEL 30 DE ABRIL DE 2010, INSCRITA EL 10 DE JUNIO DE 2010 BAJO EL NUMERO 01390261 DEL LIBRO IX, FUE (RON) NOMBRADO (S):

NOMBRE	IDENTIFICACION
SUBGERENTE	
MUÑOZ MARTINEZ ORLANDO	C.C. 000000008317044

QUE POR ACTA NO. 139 DE ASAMBLEA DE ACCIONISTAS DEL 7 DE JUNIO DE 2011, INSCRITA EL 20 DE JUNIO DE 2011 BAJO EL NUMERO 01489425 DEL LIBRO IX, FUE (RON) NOMBRADO (S):

NOMBRE	IDENTIFICACION
SUBGERENTE	
PICART LAURENZ FRANCISCO JAVIER	C.E. 00000000E399661

CERTIFICA:

FACULTADES DEL REPRESENTANTE LEGAL: EL GERENTE DE LA SOCIEDAD TIENE A

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Cámara de Comercio de Bogotá

CAMARA DE COMERCIO DE BOGOTA

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SU CARGO LA ADMINISTRACIÓN INMEDIATA DE LA SOCIEDAD, ASÍ COMO LA REPRESENTACIÓN LEGAL DE LA MISMA Y EN TAL VIRTUD LE ESTÁN ASIGNADAS LAS SIGUIENTES FUNCIONES Y ATRIBUCIONES: A) LLEVAR LA REPRESENTACIÓN DE LA ENTIDAD, TANTO JUDICIAL COMO EXTRAJUDICIALMENTE. B) EJECUTAR LOS ACUERDOS Y DECISIONES DE LA ASAMBLEA GENERAL DE ACCIONISTAS. C) CONSTITUIR, PARA CASOS ESPECIALES, APODERADOS JUDICIALES O EXTRAJUDICIALES. D) CELEBRAR LOS ACTOS, OPERACIONES Y CONTRATOS CONDUCENTES AL BUEN LOGRO DEL OBJETO DE LA SOCIEDAD. E) CUIDAR DE LA RECAUDACIÓN E INVERSIÓN DE LOS FONDOS DE LA SOCIEDAD. F) PRESENTAR A LA REUNIÓN ORDINARIA ANUAL DE LA ASAMBLEA GENERAL DE ACCIONISTAS, EL INVENTARIO Y LOS ESTADOS FINANCIEROS DE PROPÓSITO GENERAL DE FIN DE EJERCICIO, JUNTO CON UN INFORME ESCRITO RELACIONADO CON LA SITUACIÓN Y LA MARCHA DE LA ENTIDAD. G) CREAR LOS EMPLEADOS NECESARIOS PARA LA DEBIDA MARCHA DE LA SOCIEDAD, SEÑALAR SUS FUNCIONES Y ASIGNACIONES Y HACER LOS NOMBRAMIENTOS CORRESPONDIENTES. H) CONVOCAR A LA ASAMBLEA GENERAL CUANDO PROCEDA HACERLO CONFORME A LA LEY O A ESTOS ESTATUTOS. I) PRESENTAR A LA ASAMBLEA GENERAL DE ACCIONISTAS, CUANDO ASÍ LO SOLICITE, ESTADOS FINANCIEROS MENSUALES DE PRUEBA E INFORMES. J) EJERCER LAS FUNCIONES QUE LE DELEGUE LA ASAMBLEA GENERAL DE ACCIONISTAS. K) CUMPLIR Y HACER QUE SE CUMPLAN EN OPORTUNIDAD Y DEBIDAMENTE TODAS LAS EXIGENCIAS DE LAS LEYES EN RELACIÓN CON EL FUNCIONAMIENTO Y LAS ACTIVIDADES DE LA INSTITUCIÓN. L) LAS DEMÁS QUE LE CORRESPONDAN CONFORME A LA LEY Y A ESTOS ESTATUTOS. EL GERENTE DE LA SOCIEDAD DEBERÁ CONTAR CON AUTORIZACIÓN PREVIA DE LA ASAMBLEA GENERAL DE ACCIONISTAS, CUANDO PRETENDA SUSCRIBIR EN NOMBRE DE LA SOCIEDAD (I) ACTOS O CONTRATOS PARA ADQUIRIR, ENAJENAR, RECIBIR O DAR EN ARRENDAMIENTO O A CUALQUIER OTRO TÍTULO, TODA CLASE DE BIENES INMUEBLES DE LA SOCIEDAD O BIENES MUEBLES DIFERENTES A LOS PRODUCTOS COMERCIALIZADOS ORDINARIAMENTE POR LA SOCIEDAD, ASÍ COMO (II) ACTOS O CONTRATOS, INDEPENDIENTE DE SU OBJETO, QUE SUPERE EN CUANTÍA EL EQUIVALENTE A 300 SMLMV (TRESCIENTOS SALARIOS MÍNIMOS MENSUALES LEGALES VIGENTES).

CERTIFICA:

QUE POR ESCRITURA PUBLICA NO. 3511 DE LA NOTARIA 18 DE BOGOTA D.C., DEL 8 DE SEPTIEMBRE DE 2010, INSCRITA EL 1 DE OCTUBRE DE 2010 BAJO EL NO. 00018604 DEL LIBRO V, COMPARECIO JOSE ANTONIO JAIME ESCOBAR IDENTIFICADO CON CEDULA DE CIUDADANÍA NO. 19.262.407 DE BOGOTA, EN SU CALIDAD DE GERENTE DE LABORATORIOS SYNTHESIS LTDA., POR MEDIO DE LA PRESENTE ESCRITURA PUBLICA, CONFIERE PODER GENERAL AL A OLGA VICTORIA PRIETO ZAMORA, IDENTIFICADA CON CEDULA DE CIUDADANÍA NO. 52.780.303 DE BOGOTA PARA QUE EN NOMBRE DE LA SOCIEDAD LABORATORIOS SYNTHESIS S.A.S., PROMUEVA Y DEFIENDA LOS INTERESES DE LA SOCIEDAD JUDICIAL Y EXTRAJUDICIALMENTE, EN TODA ESPECIE DE GESTIONES Y ACTUACIONES ANTE LAS AUTORIDADES ADMINISTRATIVAS O JURISDICCIONALES Y EN ESPECIAL PARA: A. DILIGENCIAR Y SUSCRIBIR LOS DOCUMENTOS NECESARIOS

PARA EL REGISTRO DE INVERSIONES EXTRANJERAS ANTE LAS AUTORIDADES COLOMBIANAS. B. TRANSIGIR, DESISTIR Y APELAR A DECISIONES DE ÁRBITROS O DE AMIGABLES COMPONEDORES EN LAS CUESTIONES EN QUE TENGA INTERÉS FRENTE A TERCEROS, A LOS ASOCIADOS MISMOS O A SUS ADMINISTRADORES. C. ADELANTAR CUALQUIER TIPO DE TRÁMITE ANTE EL INSTITUTO DE VIGILANCIA DE MEDICAMENTOS Y ALIMENTOS INVIMA O A LA ENTIDAD QUE HAGA SUS VECES. D. RECIBIR NOTIFICACIÓN DE TODAS LAS PROVIDENCIAS QUE SE EXPIDAN DENTRO DE LOS DIFERENTES TRÁMITES E INTERPONER LOS RECURSOS PROPIOS DE LA VÍA GUBERNATIVA. E. REPRESENTAR Y VIGILAR ANTE LOS FUNCIONARIOS, OFICINAS Y DEPENDENCIAS DEL INSTITUTO DE VIGILANCIA DE MEDICAMENTOS Y ALIMENTOS INVIMA O A LA ENTIDAD QUE HAGA SUS VECES, TODAS LAS SOLICITUDES QUE CONTENGAN TRÁMITES A NOMBRE DE LABORATORIOS SYNTHESIS S.A.S. F. SOLICITAR, OBTENER, REVISAR Y MODIFICAR REGISTROS SANITARIOS. G. OBTENER ANTE LAS OFICINAS Y AUTORIDADES NACIONALES QUE CORRESPONDAN Y EN ESPECIAL DE LA DIVISIÓN DE PROPIEDAD INDUSTRIAL DE LA SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO, REGISTROS DE MARCAS, RENOVACIÓN DE REGISTROS MARCARIOS, ANOTACIÓN DE TRASPASOS Y CAMBIO DE NOMBRE, PRESENTACIÓN Y TRÁMITE DE OPOSICIONES, PRESENTACIÓN Y TRÁMITE DE RECURSOS POR LA VÍA GUBERNATIVA Y DEMÁS DILIGENCIAS RELACIONADAS, A CUYO EFECTO LA FACULTA PARA DAR ANTE DICHAS AUTORIDADES, PARA ELEVAR SOLICITUDES, FORMULAR DESCRIPCIONES, APELACIONES Y RECLAMOS, SOLICITAR TESTIMONIO, RECIBIR, DESISTIR, SUSTITUIR, CANCELAR, TRANSIGIR, RENUNCIAR, ACEPTAR NOTIFICACIONES Y NOMBRAR APODERADOS JUDICIALES Y EXTRAJUDICIALES. H. ADQUIRIR MARCAS, PATENTES, PROCEDIMIENTOS DE FABRICACIÓN, EXPLOTADAS EN CUALQUIER FORMA, YA SEA UTILIZÁNDOLOS DIRECTAMENTE O PERMITIENDO SU EXPLOTACIÓN POR OTRAS PERSONAS NATURALES O JURÍDICAS CONTRA EL PAGO DE REGALÍAS O PARTICIPACIONES. I. CELEBRAR Y EJECUTAR EN GENERAL TODOS LOS ACTOS Y CONTRATOS PREPARATORIOS, COMPLEMENTARIOS O ACCESORIOS DE TODOS LOS ANTERIORES, LOS QUE SE RELACIONEN CON LA EXISTENCIA Y FUNCIONAMIENTO DE LA SOCIEDAD, Y LOS QUE SEAN CONDUCENTES AL BUEN LOGRO DE LOS FINES SOCIALES. J. RECIBIR NOTIFICACIONES DE CUALESQUIERA TIPO DE ACTOS EMANADOS DE LAS AUTORIDADES ADMINISTRATIVAS O JUDICIALES. K. PRESENTAR RECURSOS POR LA VÍA ADMINISTRATIVA O JUDICIAL, RENUNCIAR A TÉRMINOS, DESISTIR, CONCILIAR, TRANSIGIR Y MODIFICAR. I. ELEVAR PETICIONES Y SOLICITAR INFORMACIÓN A LAS AUTORIDADES ADMINISTRATIVAS Y JURISDICCIONALES. M. PRESENTAR DEMANDAS ANTE LAS AUTORIDADES ADMINISTRATIVAS Y JUDICIALES. N. SUSTITUIR EL PRESENTE PODER Y REVOCAR TAL SUSTITUCIÓN DENTRO DE LA REPÚBLICA DE COLOMBIA.

CERTIFICA:

** REVISOR FISCAL **

QUE POR CERTIFICACION DE REVISOR FISCAL DEL 4 DE OCTUBRE DE 2011, INSCRITA EL 10 DE OCTUBRE DE 2011 BAJO EL NUMERO 01519171 DEL LIBRO IX, FUE (RON) NOMBRADO (S):

NOMBRE	IDENTIFICACION
REVISOR FISCAL PRINCIPAL	
ARCILA SABOGAL DIANA YAMILE	C.C. 000000052828052

QUE POR DOCUMENTO PRIVADO DE REVISOR FISCAL DEL 6 DE FEBRERO DE 2012, INSCRITA EL 8 DE FEBRERO DE 2012 BAJO EL NUMERO 01605220 DEL LIBRO IX, FUE (RON) NOMBRADO (S):

NOMBRE	IDENTIFICACION
REVISOR FISCAL SUPLENTE	
VILLATE AVENDAÑO MARIA PILAR	C.C. 000001053605913

QUE POR ACTA NO. 119 DE ASAMBLEA DE ACCIONISTAS DEL 22 DE JUNIO DE 2010, INSCRITA EL 8 DE JULIO DE 2010 BAJO EL NUMERO 01397026 DEL LIBRO IX, FUE (RON) NOMBRADO (S):

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Cámara de Comercio de Bogotá

CAMARA DE COMERCIO DE BOGOTA

SEDE KENNEDY

14 DE JUNIO DE 2012 HORA 08:52:40

R035032303 PAGINA: 4 de 5

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NOMBRE IDENTIFICACION

REVISOR FISCAL PERSONA JURIDICA

DELOITTE & TOUCHE LTDA N.I.T. 000008600058134

CERTIFICA:

PERMISO DE FUNCIONAMIENTO:QUE POR RESOLUCION NO.05952 DEL 11 DE - JUNIO DE 1.985, INSCRITA EN ESTA CAMARA DE COMERCIO EL 8 DE JULIO DE 1.985 BAJO EL NO. 172.879 DEL LIBRO IX, LA SUPERINTENDENCIA - DE SOCIEDADES CONCEDIO PERMISO DEFINITIVO DE FUNCIONAMIENTO A LA SOCIEDAD.

CERTIFICA:

QUE POR RESOLUCION NO.09006 DEL 19 DE DICIEMBRE DE 1.986 SUPERIN-TENDENCIA DE SOCIEDADES INSCRITA EL 16 DE ENERO DE 1.987 BAJO EL NO.204.133 DEL LIBRO IX, SE AUTORIZO A LA SOCIEDAD PARA COLOCAR SETENTA Y CUATRO MIL ($74.000) ACCIONES DE LAS QUE TIENE EN RE-SERVA, A SU VALOR NOMINAL DE CIEN PESOS ($100.00) M/CTE., CADA UNA.

CERTIFICA:

QUE POR DOCUMENTO PRIVADO DE REPRESENTANTE LEGAL DEL 28 DE JUNIO DE 2011, INSCRITO EL 1 DE JULIO DE 2011 BAJO EL NUMERO 01492789 DEL LIBRO IX, SE COMUNICO QUE SE HA CONFIGURADO UNA SITUACION DE CONTROL POR PARTE DE LA SOCIEDAD MATRIZ: LABORATORIOS SYNTHESIS S A S, RESPECTO DE LAS SIGUIENTES SOCIEDADES SUBORDINADAS:

- SOCIEDAD PARA LA ENSEÑANZA DE LA HOMEOPATIA CLINICA S A S QUIEN SE IDENTIFICARA CON LA SIGLA S E C H

DOMICILIO: BOGOTA D.C.

FECHA DE CONFIGURACION DE LA SITUACION DE CONTROL : 2011-05-31

CERTIFICA:

QUE POR DOCUMENTO PRIVADO DE REPRESENTANTE LEGAL DEL 9 DE MARZO DE 2011, INSCRITO EL 18 DE MARZO DE 2011 BAJO EL NUMERO 01462396 DEL LIBRO IX, COMUNICO LA SOCIEDAD MATRIZ:

- EUROPEAN CHEMICALS & CO

DOMICILIO: (FUERA DEL PAIS)

QUE SE HA CONFIGURADO UNA SITUACION DE CONTROL CON LA SOCIEDAD DE LA REFERENCIA.

CERTIFICA:

*** ACLARACIÓN SITUACIÓN DE CONTROL Y DE GRUPO EMPRESARIAL ***

SE ACLARA LA SITUACIÓN DE CONTROL Y GRUPO EMPRESARIAL INSCRITA CON EL NO. 01462396 DEL 9 DE MARZO DE 2011 DEL LIBRO IX EN EL SENTIDO DE INDICAR QUE SE CONFIGURÓ DESDE EL 19 DE NOVIEMBRE DE 2010.

CERTIFICA:

QUE LA SOCIEDAD TIENE MATRICULADOS LOS SIGUIENTES ESTABLECIMIENTOS:

NOMBRE : CARDIOPHARM

MATRICULA NO : 00537358 DE 3 DE MARZO DE 1993

RENOVACION DE LA MATRICULA : EL 29 DE FEBRERO DE 2012

ULTIMO AÑO RENOVADO : 2012

****
NOMBRE : GENERICS
MATRICULA NO : 00664623 DE 18 DE SEPTIEMBRE DE 1995
RENOVACION DE LA MATRICULA : EL 29 DE FEBRERO DE 2012
ULTIMO AÑO RENOVADO : 2012
****
NOMBRE : MASCOTT
MATRICULA NO : 00664622 DE 18 DE SEPTIEMBRE DE 1995
RENOVACION DE LA MATRICULA : EL 29 DE FEBRERO DE 2012
ULTIMO AÑO RENOVADO : 2012
****
NOMBRE : ONCOPHARM
MATRICULA NO : 00625764 DE 13 DE DICIEMBRE DE 1994
RENOVACION DE LA MATRICULA : EL 29 DE FEBRERO DE 2012
ULTIMO AÑO RENOVADO : 2012
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NOMBRE : GASTROPHARM
MATRICULA NO : 00621442 DE 2 DE NOVIEMBRE DE 1994
RENOVACION DE LA MATRICULA : EL 29 DE FEBRERO DE 2012
ULTIMO AÑO RENOVADO : 2012
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NOMBRE : GYNECOPHARM
MATRICULA NO : 00621245 DE 1 DE NOVIEMBRE DE 1994
RENOVACION DE LA MATRICULA : EL 29 DE FEBRERO DE 2012
ULTIMO AÑO RENOVADO : 2012
****
NOMBRE : ENTEROPHARM
MATRICULA NO : 00621244 DE 1 DE NOVIEMBRE DE 1994
RENOVACION DE LA MATRICULA : EL 29 DE FEBRERO DE 2012
ULTIMO AÑO RENOVADO : 2012
****
NOMBRE : EQUIPHARMA
MATRICULA NO : 00556630 DE 21 DE JULIO DE 1993
RENOVACION DE LA MATRICULA : EL 29 DE FEBRERO DE 2012
ULTIMO AÑO RENOVADO : 2012
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CERTIFICA:

DE CONFORMIDAD CON LO ESTABLECIDO POR LA LEY 962 DE 2005, LOS ACTOS DE REGISTRO AQUI CERTIFICADOS QUEDAN EN FIRME CINCO (5) DIAS HABILES DESPUES DE LA FECHA DE INSCRIPCION, SIEMPRE QUE NO SEAN OBJETO DE RECURSOS EN LA VIA GUBERNATIVA.

* * * EL PRESENTE CERTIFICADO NO CONSTITUYE PERMISO DE * * *
* * * FUNCIONAMIENTO EN NINGUN CASO * * *

SEÑOR EMPRESARIO, SI SU EMPRESA TIENE ACTIVOS INFERIORES A 30.000 SMLMV Y UNA PLANTA DE PERSONAL DE MENOS DE 200 TRABAJADORES, USTED TIENE DERECHO A RECIBIR UN DESCUENTO EN EL PAGO DE LOS PARAFISCALES DE 75% EN EL PRIMER AÑO DE CONSTITUCION DE SU EMPRESA, DE 50% EN EL SEGUNDO AÑO Y DE 25% EN EL TERCER AÑO. LEY 590 DE 2000 Y DECRETO 525 DE 2009.

RECUERDE INGRESAR A www.supersociedades.gov.co PARA VERIFICAR SI SU EMPRESA ESTA OBLIGADA A REMITIR ESTADOS FINANCIEROS. EVITE SANCIONES.

EL SECRETARIO DE LA CAMARA DE COMERCIO,

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11757419 0

Cámara de Comercio de Bogotá

CAMARA DE COMERCIO DE BOGOTA

SEDE KENNEDY

14 DE JUNIO DE 2012 HORA 08:52:40

R035032303 PAGINA: 5 de 5

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VALOR : $ 4,000

DE CONFORMIDAD CON EL DECRETO 2150 DE 1995 Y LA AUTORIZACION IMPARTIDA POR LA SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO, MEDIANTE EL OFICIO DEL 18 DE NOVIEMBRE DE 1996, LA FIRMA MECANICA QUE APARECE A CONTINUACION TIENE PLENA VALIDEZ PARA TODOS LOS EFECTOS LEGALES