Industria y Comercio
SUPERINTENDENCIA

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

No. 11-178794- -00005-0000

Fecha: 2012-07-31 16:52:50 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 379 FASENACIONALII
Act. 400 OPOSIOBSERVTER Folios: 35

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 RICAURTE

Título de la Nueva Creación: FORMULACION SUBCUTANEA DE ANTICUERPO ANTI-HER2

Gaceta: 643 de 30 de abril de 2012, No. de publicación 421

2. Datos del Opositor

[ ] Persona Natural [X] Persona Jurídica

LAFRANCOL S.A.

Nombre o denominación /Razón social completa

Documento de identificación: C.C. [ ] C.E. [ ] NIT [X] Otro [ ] Cuál

Número 890 301 463-8

Dirección Carrera 1 No. 46-84 Ciudad Cali

Teléfono: (092) 449.30.51 Fax: E-mail:

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

3. Datos del representante o apoderado:

REYES VILLAMIZAR JOSE LUIS	79`152.473	44655
Apellidos y Nombre	**Documento de identificación**	**T.P. No.**

Dirección del representante

Cra 17 No. 88-23 Of.205

Dirección electrónica	No. Fax	Número telefónico
info@reyes-abogados.com	621.25.42	621.26.31

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

6. Fundamentos de la oposición

Causal: Incumplimiento de requisitos de patentabilidad (Título II, Capítulo I, Decisión 486 de 2000)

Justificación: VER ANEXO

Prórroga: **SI** ☐ **NO** ☒

7. Anexos

- [x] Comprobante de pago de la tasa para la presentación de la solicitud No. 12007880 6 Fecha: JULIO 31 / 12
- [x] Poder, si fuera el caso con el que se acredita la representación.
- [x] Pruebas de los fundamentos de la oposición
- [x] Copias para traslado

8. Firma

JOSÉ LUIS REYES VILLAMIZAR

Nombre y apellidos

Firma

79.152.473 **C.C.**

44655 **T.P.**

REYES & REYES
A B O G A D O S

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

REF:	**Expediente 11-178-794**
TÍTULO SOLICITADO:	**Formulación subcutánea de anticuerpo ANTI-HER2**
PUBLICACIÓN:	**Gaceta 643 de 30/04/2012, Nº de publicación 421.**
PRIORIDAD:	**09167025.7 de 31/07/2009**
SOLICITANTE:	**F. HOFFMAN-LA ROCHE AG**
APODERADO:	**ALICIA LLOREDA RICAURTE**
TRÁMITE:	**PRESENTACION DE OPOSICION**

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

I. PETICIÓN

Negar el beneficio patentario a la solicitud contenida en el expediente **11-178-794,** por no cumplir con los requisitos de patentabilidad a que se refieren los artículos 14, 16 y 18 de la Decisión 486 de la Comunidad Andina de Naciones, con base en los fundamentos de hecho y de derecho que me permito exponer a continuación.

II. LEGÍTIMO INTERÉS

Asiste a **LAFRANCOL S.A.**, legítimo interés para remitir esta Información, pues la eventual concesión de la solicitud restringiría injustificadamente el empleo de

ciertas sustancias, derivados y procesos que a nuestro juicio carecen de nivel inventivo, en detrimento de sus legítimos intereses comerciales. De igual manera mis clientes están interesados, por su misma condición de industrias farmacéuticas, en evitar la concesión de privilegios de patente que incumplan los requisitos materiales de patentabilidad establecidos en las normas vigentes, y que supongan un riesgo para la libre competencia.

III. COMPLEMENTO DE INFORMACION

La solicitud colombiana en trámite **11-178-794** que se refiere a formulaciones farmacéuticas inyectables subcutáneas, altamente concentradas y estables conformadas por un anticuerpo antiHERr2 como Trastuzumab, Pertuzumab o T-DM1, o una mezcla de las mismas, no reúne los requisitos necesarios para otorgársele el privilegio de patente de invención, como se demuestra a través de los siguientes argumentos:

Con respecto a la **novedad** de la solicitud colombiana en trámite, podemos afirmar con total seguridad que se conocían con anterioridad los mismos tipos formulaciones farmacéuticas de acuerdo a la **reivindicación 15**, útiles en el tratamiento de los mismos trastornos tumorales relacionados en dicha solicitud y con el total de las características técnicas reivindicadas por lo tanto no es nueva.

Tampoco son nuevas, la vía de administración (**reivindicación 16**), la forma farmacéutica (**reivindicaciones 16 y 17**), el dispositivo de inyección (**reivindicación 19**), ni el equipo y/o el dispositivo de inyecciones para administración subcutánea (**reivindicaciones 20 y 21**). Cabe reiterar que la utilización de estos anticuerpos en el tratamiento de trastornos tumorales ya se conocía con anterioridad.

Veamos lo que reclama la reivindicación (15) de la solicitud colombiana en trámite:

> Una formulación farmacéutica de anticuerpo anti-HER2 altamente concentrada y estable de acuerdo con cualquiera de las reivindicaciones 1 a 13 que carecen de la enzima hialuronidasa.

Este tipo de formulaciones farmacéuticas que carecen de la enzima hialuronidasa para el tratamiento de trastornos tumorales, vía subcutánea, de alta concentración,

con buena estabilidad y los mismos componentes, ya se conocían previamente, tal y como lo expone la anterioridad **WO 2006/044908 de 27/04/2006** titulada **Antibody formulations**

Thus, in a first aspect, the invention concerns a stable pharmaceutical formulation comprising a monoclonal antibody in histidine-acetate buffer, pH 5.5 to 6.5. The monoclonal antibody preferably binds an antigen selected from the group consisting of HER2, CD20, DR5, BR3, IgE, and VEGF.

In addition, the invention concerns a method of treating a disease or disorder in a subject comprising administering the formulation to a subject in an amount effective to treat the disease or disorder.

In another aspect, the invention concerns a pharmaceutical formulation comprising: (a) a full length IgG1 antibody susceptible to deamidation or aggregation in an amount from about 10mg/mL to about 250mg/mL; (b) histidine-acetate buffer, pH 5.5 to 6.5; (c) saccharide selected from the group consisting of trehalose and sucrose, in an amount from about 60mM to about 250mM; and (d) polysorbate 20 in an amount from about 0.01% to about 0.1%.

The invention also provides a method for reducing deamidation or aggregation of a therapeutic monoclonal antibody, comprising formulating the antibody in a histidine-acetate buffer, pH 5.5 to 6.5.

In yet a further aspect, the invention concerns a pharmaceutical formulation comprising an antibody that binds to domain II of HER2 in a histidine buffer at a pH from about 5.5 to about 6.5, a saccharide and a surfactant.

The invention also relates to a pharmaceutical formulation comprising Pertuzumab in an amount from about 20mg/mL to about 40mg/mL, histidine-acetate buffer, sucrose, and polysorbate 20, wherein the pH of the formulation is from about 5.5 to about 6.5.

Comparando un poco más en detalle la formulación reivindicada en la reivindicación 15 contra dicha anterioridad, ya había sido enseñada previamente en el estado de la técnica y sus características se recalcan a lo largo de todo el documento como se comprueba a simple vista con la siguiente tabla:

CARACTERÍSTICAS REIVINDICADAS (Rv 15) CO 11-178-794	**WO 2006/044908**
Formulación estable	Pág 9 ln 1, 2; pág 55 ln 33, pág 58, ln 19, 20
Altamente concentrada	pág 34, ln 39, pág 57 ln 38, 39, Pág 58, ln 4-6;
Anticuerpo anti – HER2	Pág. 8 ln 27, pág 53, ln 17; pág 55 ln 34; Pág 56, ln 5
CARECE de Enzima hialuronidasa	**TAMBIEN CARECE DE ENZIMA HIALURONIDASA**

Adicionalmente podemos observar que:

(pág 58 ln 10– 13):

The formulation for administration is preferably an aqueous formulation (not lyophilized) and has not been subjected to prior lyophilization. While the formulation may be lyophilized, preferably it is not. However, freezing of the aqueous formulation, without simultaneous drying that occurs during freeze-drying, is specifically contemplated herein, facilitating longer term storage thereof, for instance in a stainless steel tank.

En el anterior párrafo se sugieren cuáles son las diferentes formas farmacéuticas en las que puede presentarse este tipo de formulaciones y además indica cuándo hacerlo para que las condiciones de estabilidad en almacenamiento sean las adecuadas, de tal forma que **lo reivindicado en los ítems 13, 17 y 18** de la solicitud ya era conocido en el estado de la técnica.

(pág 58, ln 4,5)

Where the antibody is for SQ or IM administration (*e.g.* for an anti-IgE antibody) higher concentrations of the antibody may be desired. Such substantially high antibody concentrations may be from about 50mg/mL to about 250mg/mL, or from about 80mg/mL to about 250mg/mL, or from about 100mg/mL to about 200mg/mL.

En éste aparte claramente se observan las vías de administración más adecuadas para una formulación con altas concentraciones de anticuerpo tal como se pretende proteger en la **reivindicación 16** de la solicitud, por lo tanto tampoco no es nueva.

(pág 62, ln 6, 16)

V. Articles of Manufacture

In another embodiment of the invention, an article of manufacture is provided which contains the pharmaceutical formulation of the present invention and provides instructions for its use. The article of manufacture comprises a container. Suitable containers include, for example, bottles, vials (*e.g.* dual chamber vials), syringes (such as dual chamber syringes) and test tubes. The container may be formed from a variety of materials such as glass or plastic. The container holds the formulation and the label on, or associated with, the container may indicate directions for use. The container holding the formulation may be a multi-use vial, which allows for repeat administrations (*e.g.* from 2-6 administrations) of the reconstituted formulation. The article of manufacture may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use as noted in the previous section.

De acuerdo a lo enseñado en el párrafo anterior, tampoco serían nuevas las **reivindicaciones 19, 20 y 21** ya que un dispositivo de inyección que comprende una formulación farmacéutica de anticuerpo, un equipo que comprende uno o más viales con sus respectivas instrucciones y un equipo que comprende un dispositivo de inyecciones para administración subcutánea tal como se reivindican, además de pertenecer a diferentes campos técnicos careciendo de unidad de invención, ya eran conocidos en la anterioridad de referencia **WO 2006/044908** y no tienen ninguna característica técnica diferencial, por lo tanto no son nuevos frente a lo ya conocido.

En conclusión, **la reivindicación 15** no se considera nueva ya que no hay características técnicas esenciales que la diferencien de lo enseñado por el estado de la técnica de acuerdo a lo divulgado exclusivamente en el documento US 2006/0088523, ni tampoco serían nuevas las **reivindicaciones 13, 16, 17, 18, 19, 20, 21** dado que no poseen ninguna característica adicional a lo previamente enseñado por el estado arte señalado.

Ahora refiriéndonos específicamente **al nivel inventivo** de la solicitud colombiana en trámite, la simple combinación de dos documentos que se expondrán a continuación, sugieren como llevar a cabo la formulación reivindicada según la reivindicación 1 y sus dependientes 2-12 y 14, ya que se enseñan la forma de resolver el problema técnico, en los mismos tipos de formulaciones para los mismos fines de la solicitud.

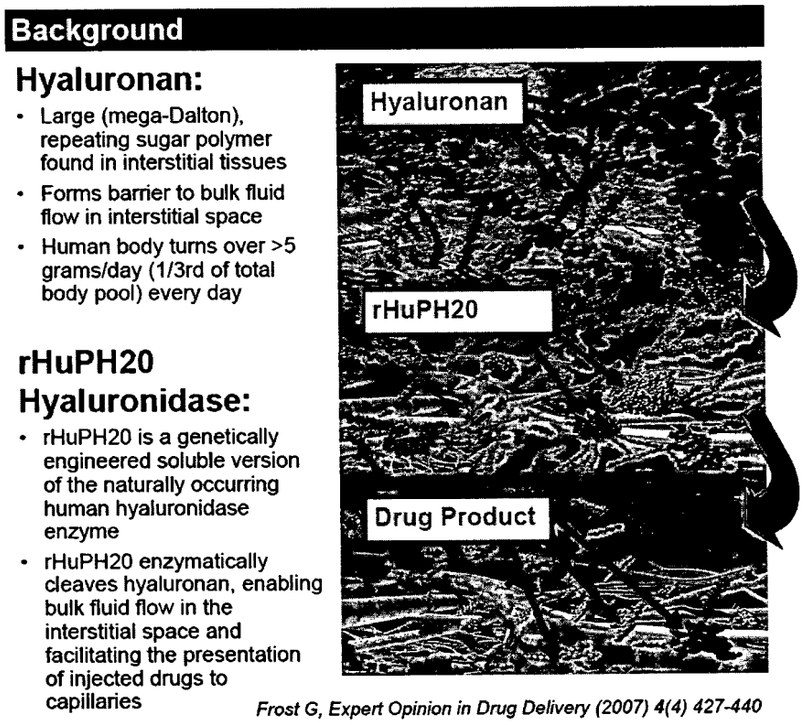
* La publicación (**D1**) **US WO 2006/044908 de 27/04/2006 "ANTIBODY FORMULATIONS"** Andya et al (***pág 8 summary of the invention***) expone lo siguiente:

> Thus, in a first aspect, the invention concerns a stable pharmaceutical formulation comprising a monoclonal antibody in histidine-acetate buffer, pH 5.5 to 6.5. The monoclonal antibody preferably binds an antigen selected from the group consisting of HER2, CD20, DR5, BR3, IgE, and VEGF.
>
> In addition, the invention concerns a method of treating a disease or disorder in a subject comprising administering the formulation to a subject in an amount effective to treat the disease or disorder.
>
> In another aspect, the invention concerns a pharmaceutical formulation comprising: (a) a full length IgG1 antibody susceptible to deamidation or aggregation in an amount from about 10mg/mL to about 250mg/mL; (b) histidine-acetate buffer, pH 5.5 to 6.5; (c) saccharide selected from the group consisting of trehalose and sucrose, in an amount from about 60mM to about 250mM; and (d) polysorbate 20 in an amount from about 0.01% to about 0.1%.
>
> The invention also provides a method for reducing deamidation or aggregation of a therapeutic monoclonal antibody, comprising formulating the antibody in a histidine-acetate buffer, pH 5.5 to 6.5.
>
> In yet a further aspect, the invention concerns a pharmaceutical formulation comprising an antibody that binds to domain II of HER2 in a histidine buffer at a pH from about 5.5 to about 6.5, a saccharide and a surfactant.
>
> The invention also relates to a pharmaceutical formulation comprising Pertuzumab in an amount from about 20mg/mL to about 40mg/mL, histidine-acetate buffer, sucrose, and polysorbate 20, wherein the pH of the formulation is from about 5.5 to about 6.5.

* Las publicaciones (**D2 y D3**) del Journal **EXPERT OPINION ON DRUG DELIVERY** Jul 2007, Vol. 4, No. 4: 427–440 *"Recombinant human hyaluronidase (rHuPH20): an enabling platform for subcutaneous drug and fluid administration"* **y** *"Addition of human hyaluronidase to Rapid Analog Insulin Reduces the Absolute Variability of Early Insulin Absorption across Infusion Set Life"* exponen los

conceptos de Gregory Frost publicados en Julio de 2007 tanto en el *abstract* como en el *background* respectivamente, acerca del incremento en la biodisponibilidad de gran cantidad de medicamentos al agregar un componente adicional a sus formulaciones: es decir, la presencia de la enzima hyaluronidasa (rHuPH20) y se se concluyó que incrementa el volumen inyectado y la disponibilidad del medicamento subcutáneamente, superando las limitaciones de dicha vía de administración, veamos:

ABSTRACT: *The extracellular matrix is a significant barrier to the effective* ***subcutaneous delivery of many drugs****, limiting both pharmacokinetic parameters and injection volumes. The space outside adipocytes in the hypodermis is not a fluid, but rather a solid extracellular matrix of collageneous fibrils embedded within a glycosaminoglycan-rich viscoelastic gel that buffers convective forces. The extracellular matrix limits the volume of drug that can be injected at a single site, as well as the rate and amount that reach the vascular compartment. A fully human recombinant DNA-derived* ***hyaluronidase enzyme (rHuPH20)*** *has been developed to leverage the historical efficacy of animal testes extract-derived spreading factors to reversibly modify the hypodermis, in light of discovery of the human hyaluronidase gene family.* ***The application of this technology to increase both injection volumes and bioavailability from subcutaneous injection*** *may overcome some key limitations of this route of administration in multiple settings of care.*



Frost G, Expert Opinion in Drug Delivery (2007) 4(4) 427-440

Teniendo en cuenta lo anterior, procedamos a revisar los que reclama la reivindicación (1):

Una formulación farmacéutica estable, altamente concentrada de un anticuerpo farmacéuticamente activo anti- HER2 para inyección subcutánea que comprende:

(a) Entre alrededor de 50 y 350 mg/ml de anticuerpo anti-HER2

(i) Entre alrededor de 1 y 100 mM de un agente tamponador que proporciona un pH de 5.5 ± 2.0

(b) Entre alrededor de 1 y 500 mM de un estabilizante o una mezcla de dos o más estabilizantes

(c) Entre alrededor de 0.01 y 0.08% de un tensioactivo no iónico y

(d) Más de 150 y hasta alrededor de 16.000 U/ml, alrededor de 2.000 U/ml, o alrededor de 12.000 U/ml, respectivamente de una enzima hialuronidasa.

REIVINDICACION 1 SOLICITUD COLOMBIANA 11-178-794	WO 2006/044908 (D1)	EXPERT OPINION ON DRUG DELIVERY
Formulación estable	**SI se encuentra** Pág 9 ln 1, 2; pág 55 ln 33, pág 58, ln 19, 20	-
Altamente concentrada	**SI se encuentra** pág 34, ln 39, pág 57 ln 38, 39, Pág 58, ln 4-6;	-
Para inyección subcutánea	**SI se encuentra** Pág 58 ln 4	**SI se encuentra** *Ver abstract resaltado en negrilla (D2) y Background (D3)*
Anticuerpo anti – HER2 entre 50 – 350 mg/ml	**SI se encuentra** Pág. 8 ln 27, pág 53, ln 17; pág 55 ln 34; Pág 56, ln 5	-
Agente tamponador entre 1 – 100 mM para un pH de 5.5 ± 2.0	**SI se encuentra** Pág. 8 ln 27, 30; pág 34, ln 34, 39, Pág 56, ln 6, 7	-
Estabilizante o una mezcla de dos o más estabilizantes entre 1 – 500 mM	**SI se encuentra** Pág. 8 ln 27, pág 34, ln 39; Pág 55, ln 39; Pág 56, ln 1; pág 58, ln 14 – 18	-
Tensioactivo no iónico entre 0.01 – 0.08%	**SI se encuentra** Pág. 8 ln 30, Pág 56, ln 1, 5, pág 58 ln 19-24	-
Enzima hialuronidasa más de 150 y hasta alrededor de 16000 U/ml alrededor de 2.000 U/ml o alrededor de 12.000 U/ml	**NO se encuentra**	**SI se encuentra** *Ver abstract resaltado en negrilla (D2) y Background (D3)*

Claramente se evidencia que una formulación con anticuerpos del mismo tipo, en las mismas condiciones, con los mismos componentes y rangos de concentración, tal y como lo reivindica la solicitud colombiana en cuestión, resultaría obvia para un experto medianamente versado en la materia, pues la única característica esencial que diferencia a la formulación de lo sugerido por Andya et al es una de las características técnicas, la cual precisamente había sido previamente sugerida en el abstract del journal expert opinión on drug delibera 2007 4(4) 427-440.

Así las cosas, la reivindicación 1 no tiene nivel inventivo ya que de acuerdo a lo divulgado en el documento **WO 2006/044908** y de acuerdo al componente que se sugería en el journal expert opinión, la simple combinación de dos (2) documentos

(D1 y D2) da como resultado la formulación que se pretende proteger (D3 se anexa background donde se comenta sobre el mismo hallazgo de Frost realizado en el 2007).

Más detalladamente, las características esenciales reivindicadas de 1 a 12 y 14 se observan reveladas por el estado de la técnica en la **WO 2006/044908** así:

(pág 55 ln 39):

In another embodiment, the invention concerns a pharmaceutical formulation comprising, or consisting essentially of, a full length IgG1 antibody susceptible to deamidation or aggregation in an amount from about 10mg/mL to about 250mg/mL; histidine-acetate buffer, pH 5.5 to 6.5; saccharide selected from the group

(pág 57, ln 38-39)

The antibody concentration in the formulation is preferably in the range from about 10mg/mL to about 250mg/mL. The antibody concentration may be determined based on the intended use and mode of administration of the formulation. For example, where the formulation is for IV administration (*e.g.* a HER2

La anterioridad repetidamente enseña una concentración del anticuerpo que abarca el rango de 10 mg/ml a 250 mg/ml, tal como aparece en la **reivindicación 3** de la solicitud colombiana en trámite en donde se pretenden proteger concentraciones del anticuerpo anti-HER2 de 100 a 150 mg/ml, 120 ± 18 mg/ml, alrededor de 110 mg/ml, alrededor de 100 mg/ml o alrededor de 130 mg/ml, por lo tanto las concentraciones de anticuerpo preferidas para la formulación de la solicitud en trámite ya pertenecían al estado de la técnica para formulaciones que contienen anticuerpos.

(pág 57 ln 36)

35 The concentration of the buffer is dictated, at least in part, by the desired pH. Exemplary concentrations for the buffer are in the range from about 1mM to about 200mM, preferably from about 10mM to about 40mM, most preferably about 20mM.

En éste párrafo ya se enseñaba con antelación a la presente solicitud de patente, que la concentración del buffer se define de acuerdo al pH que se desea, de tal manera que resulta obvio para el experto medianamente versado en la materia, seleccionar una concentración de buffer que no sobrepase los 200 mM, tal y como aparece en la **reivindicación 4,** además se indica claramente que la concentración preferida está en los 20 mM tal y como se pretende proteger en **la reivindicación 6.**

y 11 de la presente solicitud, por lo tanto, es una práctica reconocida y común en el estado de la técnica.

(pág 54, ln 36-40),

(xi) Antibody variant compositions

The present invention, in at least one aspect, concerns formulations comprising a composition which comprises a mixture of a main species antibody and one or more variants thereof. Where the main species antibody binds HER2, preferably the HER2 antibody (either or both of the main species HER2 antibody and antibody variant thereof) is one which binds to Domain II of HER2, inhibits HER dimerization more effectively

-54-

En este aparte se demuestra que utilizar formulaciones que contienen mezclas de las principales especies de anticuerpos y una o más de una de sus variantes, de preferencia anticuerpos HER2 en sus principales especies y variantes tal y como se revela en **reivindicación 12** de la presente solicitud, era una práctica reconocida y común en el estado de la técnica;

(pág 4, ln 4-6),

Clin. Oncol. 14:737-744 (1996)). Trastuzumab received marketing approval from the Food and Drug Administration September 25, 1998 for the treatment of patients with metastatic breast cancer whose tumors overexpress the HER2 protein.

A manera de ejemplo el anterior párrafo demuestra que variantes del anticuerpo anti-HER2 como el trastuzumab ya había sido reconocido desde 1998 por la FDA por su función farmacológica en el tratamiento del cáncer de seno metastásico.

(pág 56, ln 5),

and a surfactant. For example, the formulation may comprise Pertuzumab in an amount from about 20mg/mL to about 40mg/mL, histidine-acetate buffer, sucrose, and polysorbate 20, wherein the pH of the formulation is from about 5.5 to about 6.5

Adicionalmente anticuerpos como el Pertuzumab ya hacían parte de las formulaciones que se habían revelado en la anterioridad.

En conclusión la solicitud colombiana en trámite incumple todos los requisitos fundamentales para otorgársele exclusividad ya que carece de novedad frente a algunas de sus modalidades y el total de la solicitud carece de nivel inventivo pues un experto medianamente versado en la materia basándose en la publicación **WO 2006/044908** desarrollaría formulaciones como las que se pretenden proteger, ya que no solamente la publicación resuelve el mismo problema técnico objetivo al obtener el efecto terapéutico deseado en una formulación de alta concentración de

12

y 11 de la presente solicitud, por lo tanto, es una práctica reconocida y común en el estado de la técnica.

(pág 54, ln 36-40),

(xi) Antibody variant compositions

The present invention, in at least one aspect, concerns formulations comprising a composition which comprises a mixture of a main species antibody and one or more variants thereof. Where the main species antibody binds HER2, preferably the HER2 antibody (either or both of the main species HER2 antibody and antibody variant thereof) is one which binds to Domain II of HER2, inhibits HER dimerization more effectively

-54-

En este aparte se demuestra que utilizar formulaciones que contienen mezclas de las principales especies de anticuerpos y una o más de una de sus variantes, de preferencia anticuerpos HER2 en sus principales especies y variantes tal y como se revela en **reivindicación 12** de la presente solicitud, era una práctica reconocida y común en el estado de la técnica;

(pág 4, ln 4-6),

Clin. Oncol. 14:737-744 (1996)). Trastuzumab received marketing approval from the Food and Drug Administration September 25, 1998 for the treatment of patients with metastatic breast cancer whose tumors overexpress the HER2 protein.

A manera de ejemplo el anterior párrafo demuestra que variantes del anticuerpo anti-HER2 como el trastuzumab ya había sido reconocido desde 1998 por la FDA por su función farmacológica en el tratamiento del cáncer de seno metastásico.

(pág 56, ln 5),

and a surfactant. For example, the formulation may comprise Pertuzumab in an amount from about 20mg/mL to about 40mg/mL, histidine-acetate buffer, sucrose, and polysorbate 20, wherein the pH of the formulation is from about 5.5 to about 6.5

Adicionalmente anticuerpos como el Pertuzumab ya hacían parte de las formulaciones que se habían revelado en la anterioridad.

En conclusión la solicitud colombiana en trámite incumple todos los requisitos fundamentales para otorgársele exclusividad ya que carece de novedad frente a algunas de sus modalidades y el total de la solicitud carece de nivel inventivo pues un experto medianamente versado en la materia basándose en la publicación **WO 2006/044908** desarrollaría formulaciones como las que se pretenden proteger, ya que no solamente la publicación resuelve el mismo problema técnico objetivo al obtener el efecto terapéutico deseado en una formulación de alta concentración de

13

un anticuerpo anti-HER2 o una mezcla de los mismos sino que además combinado con lo revelado en el "journal expert opinion on drug delivery", consigue mejorar su administración vía intravenosa que limitaba la biodisponibilidad por características físico químicas como la solubilidad y la estabilidad en formulación liquida. Además a partir de esa única publicación, se logran inferir la mayoría de sus características técnicas esenciales en donde si bien no enseñan específicamente formulaciones que contengan hialuronidasa humana o estabilizantes adicionales como metionina (**reivindicación 9**), el experto medianamente versado en la materia se vería motivado a agregar tales componentes dado que en dicha publicación optan también por usar estabilizantes y además por medio de las enseñanzas dadas en el "journal expert opinion on drug delivery" se sabrá que al agregar rHuPH20 a su formulación se superarán con gran expectativa de éxito de forma obvia y evidente las barreras que supone la administración subcutánea en la entrega del medicamento.

Por consiguiente resultaría obvio el diseño de este tipo de formulaciones para un experto medianamente versado en la materia que conozca el estado de la técnica y que realice una combinación de éstos documentos llegando de tal manera a la propuesta de la presente solicitud.

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

IV. PRUEBAS

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

- Publicación internacional WO 2006/044908 de 27/04/2006.
- Apartes de dos artículos del Journal Expert Opinion on Drug Delivery de Julio de 2007:

1. Recombinant human hyaluronidase (rHuPH20): an enabling platform for subcutaneous drug and fluid administration.

14

2. Addition of human hyaluronidase to Rapid Analog Insulin Reduces the absolute variability of Early Insulin Absorption across infusion set life

V. NOTIFICACIONES

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

Señor Superintendente,

JOSÉ LUIS REYES VILLAMIZAR
C.C. 79'152.473 de Usaquén
T.P.A. 44.655 del C.S.J.

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 April 2006 (27.04.2006)

PCT

(10) International Publication Number
WO 2006/044908 A2

(51) International Patent Classification: Not classified

(21) International Application Number:
PCT/US2005/037471

(22) International Filing Date: 19 October 2005 (19.10.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/620,413 20 October 2004 (20.10.2004) US

(71) Applicant *(for all designated States except US)*: **GENENTECH, INC.** [US/US]; 1 DNA Way, South San Francisco, CA 94080-4990 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ANDYA, James, D.** [US/US]; 227 Richmond Drive, Apt. D, Millbrae, CA 94030 (US). **GWEE, Shiang, C.** [US/US]; 565 Crespi Drive, Pacifica, CA 94044 (US). **LIU, Jun** [US/US]; 1108 Glacier Avenue, Pacifica, CA 94044 (US). **SHEN, Ye** [US/US]; 386 Faxon Avenue, San Francisco, CA 94112 (US).

(74) Agents: **LEE, Wendy, M.** et al.; Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080-4990 (US).

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

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

Published:

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

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

(54) Title: ANTIBODY FORMULATIONS

(57) Abstract: The present application describes antibody formulations, including monoclonal antibodies formulated in histidine-acetate buffer, as well as a formulation comprising an antibody that binds to domain II of HER2 (for example, Pertuzumab), and a formulation comprising an antibody that binds to DR5 (for example, Apomab).

WO 2006/044908 A2

May 8, 2003. Certain anti-DR5 antibodies have likewise been described, see, *e.g.*, WO 98/51793 published November 8, 1998; Griffith *et al., J. Immunol.*, 162:2597-2605 (1999); Ichikawa *et al., Nature Med.*, 7:954-960 (2001); Hylander *et al.*, "An Antibody to DR5 (TRAIL-Receptor 2) Suppresses the Growth of Patient Derived Gastrointestinal Tumors Grown in SCID mice", Abstract, 2d International Congress on Monoclonal Antibodies in Cancers, Aug. 29-Sept. 1, 2002, Banff, Alberta, Canada; WO 03/038043 published May 8, 2003; WO 03/037913 published May 8, 2003. In addition, certain antibodies having cross-reactivity to both DR4 and DR5 receptors have been described (see, *e.g.*, US patent 6,252,050 issued June 26, 2001).

Summary of the Invention

The invention herein relates, at least in part, to the identification of histidine-acetate, pH 5.5 to 6.5, as a particularly useful buffer for formulating monoclonal antibodies, especially full length IgG1 antibodies which are susceptible to deamidation and/or aggregation. The formulation retards degradation of the antibody product therein.

Thus, in a first aspect, the invention concerns a stable pharmaceutical formulation comprising a monoclonal antibody in histidine-acetate buffer, pH 5.5 to 6.5. The monoclonal antibody preferably binds an antigen selected from the group consisting of HER2, CD20, DR5, BR3, IgE, and VEGF.

In addition, the invention concerns a method of treating a disease or disorder in a subject comprising administering the formulation to a subject in an amount effective to treat the disease or disorder.

In another aspect, the invention concerns a pharmaceutical formulation comprising: (a) a full length IgG1 antibody susceptible to deamidation or aggregation in an amount from about 10mg/mL to about 250mg/mL; (b) histidine-acetate buffer, pH 5.5 to 6.5; (c) saccharide selected from the group consisting of trehalose and sucrose, in an amount from about 60mM to about 250mM; and (d) polysorbate 20 in an amount from about 0.01% to about 0.1%.

The invention also provides a method for reducing deamidation or aggregation of a therapeutic monoclonal antibody, comprising formulating the antibody in a histidine-acetate buffer, pH 5.5 to 6.5.

In yet a further aspect, the invention concerns a pharmaceutical formulation comprising an antibody that binds to domain II of HER2 in a histidine buffer at a pH from about 5.5 to about 6.5, a saccharide and a surfactant.

The invention also relates to a pharmaceutical formulation comprising Pertuzumab in an amount from about 20mg/mL to about 40mg/mL, histidine-acetate buffer, sucrose, and polysorbate 20, wherein the pH of the formulation is from about 5.5 to about 6.5.

The invention also pertains to a pharmaceutical formulation comprising a DR5 antibody in a histidine buffer at a pH from about 5.5 to about 6.5, a saccharide, and a surfactant.

In another aspect, the invention concerns a pharmaceutical formulation comprising Apomab in an amount from about 10mg/mL to about 30mg/mL, histidine-acetate buffer, trehalose, and polysorbate 20, wherein the pH of the formulation is from about 5.5 to about 6.5.

In yet another aspect, the invention provides a method of treating cancer in a subject, comprising administering the pharmaceutical formulation to the subject in an amount effective to treat the cancer.

The invention also concerns a vial with a stopper pierceable by a syringe or a stainless steel tank comprising the formulation inside the vial or tank, optionally in frozen form.

Moreover, the invention provides a method of making a pharmaceutical formulation comprising: (a) preparing the monoclonal antibody formulation; and (b) evaluating physical stability, chemical stability, or biological activity of the monoclonal antibody in the formulation.

Brief Description of the Drawings

Figure 1 depicts Domains I-IV (SEQ ID Nos.19-22, respectively) of the extracellular domain of HER2.

Figures 2A and 2B depict alignments of the amino acid sequences of the variable light (V_L) (Fig. 2A) and variable heavy (V_H) (Fig. 2B) domains of murine monoclonal antibody 2C4 (SEQ ID Nos. 1 and 2, respectively); V_L and V_H domains of humanized 2C4 version 574 (SEQ ID Nos. 3 and 4, respectively), and human V_L and V_H consensus frameworks (hum κ1, light kappa subgroup I; humIII, heavy subgroup III) (SEQ ID Nos. 5 and 6, respectively). Asterisks identify differences between humanized 2C4 version 574 and murine monoclonal antibody 2C4 or between humanized 2C4 version 574 and the human framework. Complementarity Determining Regions (CDRs) are in brackets.

Figures 3A and 3B show the amino acid sequences of Pertuzumab light chain and heavy chain (SEQ ID Nos. 15 and 16, respectively). CDRs are shown in bold. Calculated molecular mass of the light chain and heavy chain are 23,526.22 Da and 49,216.56 Da (cysteines in reduced form). The carbohydrate moiety is attached to Asn 299 of the heavy chain.

Figures 4A and 4B show the amino acid sequences of Pertuzumab light and heavy chain, each including an intact amino terminal signal peptide sequence (SEQ ID Nos. 17 and 18, respectively).

Figure 5 depicts, schematically, binding of 2C4 at the heterodimeric binding site of HER2, thereby preventing heterodimerization with activated EGFR or HER3.

Figure 6 depicts coupling of HER2/HER3 to the MAPK and Akt pathways.

Figure 7 compares activities of Trastuzumab and Pertuzumab.

Figure 8 depicts stability of Pertuzumab formulation by ion exchange (IEX) analyses.

Figure 9 shows stability of Pertuzumab formulation by size exclusion chromatography (SEC) analysis.

Figure 10 reflects physical stability Pertuzumab in different formulations.

Figure 11 is from an agitation study of Pertuzumab liquid formulations.

Figure 12 is from another agitation study of Pertuzumab liquid formulations.

Figure 13 is from a freeze-thawing study of Pertuzumab formulation.

Figures 14A and 14B show the amino acid sequences of Trastuzumab light chain (SEQ ID No. 13) and heavy chain (SEQ ID No. 14).

Figures 15A and 15B depict a variant Pertuzumab light chain sequence (SEQ ID No. 23) and a variant Pertuzumab heavy chain sequence (SEQ ID No. 24).

Figure 16A and 16B shows oligosaccharide structures commonly observed in IgG antibodies.

Figures 17A and 17B show the sequences of the light and heavy chains (SEQ ID Nos. 37-44) of specific anti-IgE antibodies E25, E26, HAE1 and Hu-901. In Fig. 17A, the variable light domain ends with the residues VEIK, residue 111. In Fig. 17B, the variable heavy domain ends with the residues VTVSS, around residue 120.

Figure 18A is a sequence alignment comparing the amino acid sequences of the variable light domain (V_L) of each of murine 2H7 (SEQ ID No. 25), humanized 2H7v16 variant (SEQ ID No. 26), and the human

Activator and CAML-Interactor" or "TACI", BCMA protein, DR4, DR5 (alternatively referred to as Apo-2; TRAIL-R2, TR6, Tango-63, hAPO8, TRICK2 or KILLER), DR6, DcR1 (also referred to as TRID, LIT or TRAIL-R3), DcR2 (also called TRAIL-R4 or TRUNDD), OPG, DcR3 (also called TR6 or M68), CAR1, HVEM (also called ATAR or TR2), GITR, ZTNFR-5, NTR-1, TNFL1, CD30, Lymphotoxin beta receptor (LTBr), 4-1BB receptor and TR9 (EP988, 371A1).

The terms "Apo-2 ligand", "Apo-2L", "Apo2L", Apo-2 ligand/TRAIL" and "TRAIL" are used herein interchangeably to refer to a polypeptide sequence which includes amino acid residues 114-281, inclusive, 95-281, inclusive, residues 92-281, inclusive, residues 91-281, inclusive, residues 41-281, inclusive, residues 39-281, inclusive, residues 15-281, inclusive, or residues 1-281, inclusive, of the amino acid sequence shown in Fig. 24 (SEQ ID No. 46), as well as biologically active fragments, deletional, insertional, and/or substitutional variants of the above sequences. In one embodiment, the polypeptide sequence comprises residues 114-281 of Fig. 24 (SEQ ID No. 46). Optionally, the polypeptide sequence comprises residues 92-281 or residues 91-281 of Fig. 24 (SEQ ID No. 46). The Apo-2L polypeptides may be encoded by the native nucleotide sequence shown in Fig. 24 (SEQ ID No. 45). Optionally, the codon which encodes residue Pro119 (Fig. 24; SEQ ID No. 45) may be "CCT" or "CCG". Optionally, the fragments or variants are biologically active and have at least about 80% amino acid sequence identity, or at least about 90% sequence identity, or at least 95%, 96%, 97%, 98%, or 99% sequence identity with any one of the above sequences. The definition encompasses substitutional variants of Apo-2 ligand in which at least one of its native amino acids are substituted by another amino acid such as an alanine residue. The definition also encompasses a native sequence Apo-2 ligand isolated from an Apo-2 ligand source or prepared by recombinant and/or synthetic methods. The Apo-2 ligand of the invention includes the polypeptides referred to as Apo-2 ligand or TRAIL disclosed in WO97/01633 published January 16, 1997, WO97/25428 published July 17, 1997, WO99/36535 published July 22, 1999, WO 01/00832 published January 4, 2001, WO02/09755 published February 7, 2002, WO 00/75191 published December 14, 2000, and U.S. Patent No. 6,030,945 issued February 29, 2000. The terms are used to refer generally to forms of the Apo-2 ligand which include monomer, dimer, trimer, hexamer or hight oligomer forms of the polypeptide. All numbering of amino acid residues referred to in the Apo-2L sequence use the numbering according to Fig. 24 (SEQ ID No. 46), unless specifically stated otherwise.

"Apo-2 ligand receptor" includes the receptors referred to in the art as "DR4" and "DR5." *Pan et al.* have described the TNF receptor family member referred to as "DR4" (Pan *et al., Science*, 276:111-113 (1997); see also WO98/32856 published July 30, 1998; WO 99/37684 published July 29, 1999; WO 00/73349 published December 7, 2000; US 6,433,147 issued August 13, 2002; US 6,461,823 issued October 8, 2002, and US 6,342,383 issued January 29, 2002). Sheridan *et al., Science*, 277:818-821 (1997) and Pan *et al., Science*, 277:815-818 (1997) described another receptor for Apo2L/TRAIL (see also, WO98/51793 published November 19, 1998; WO98/41629 published September 24, 1998). This receptor is referred to as DR5 (the receptor has also been alternatively referred to as Apo-2; TRAIL-R, TR6, Tango-63, hAPO8, TRICK2 or KILLER; Screaton *et al., Curr. Biol.*, 7:693-696 (1997); Walczak *et al., EMBO J.*, 16:5386-5387 (1997); Wu *et al., Nature Genetics*, 17:141-143 (1997); WO98/35986 published August 20, 1998; EP870,827 published October 14, 1998; WO98/46643 published October 22, 1998; WO99/02653 published January 21, 1999; WO99/09165 published February 25, 1999; WO99/11791 published March 11, 1999; US 2002/0072091 published August 13, 2002; US 2002/0098550 published December 7, 2001; US 6,313,269 issued December 6, 2001; US 2001/0010924

Engineered antibodies with three or more (preferably four) functional antigen binding sites are also contemplated (US Appln No. US2002/0004587 A1, Miller *et al.*).

The HER2 antibodies disclosed herein may also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein *et al., Proc. Natl. Acad. Sci. USA*, 82:3688 (1985); Hwang *et al., Proc. Natl Acad. Sci. USA*, 77:4030 (1980); U.S. Pat. Nos. 4,485,045 and 4,544,545; and WO97/38731 published October 23, 1997. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes as described in Martin *et al. J. Biol. Chem.* 257: 286-288 (1982) via a disulfide interchange reaction. A chemotherapeutic agent is optionally contained within the liposome. See Gabizon *et al. J. National Cancer Inst.*81(19)1484 (1989).

(ix) Exemplary Antibodies

Exemplary antibodies which can be formulated according to the present invention include, but are not limited to the following:

anti-ErbB antibodies, including anti-HER2 antibodies, such as those described in more detail herein;

antibodies that bind to a B-cell surface marker, such as CD19, CD20 (for example Rituximab (RITUXAN®) and humanized 2H7), CD22, CD40 or BR3;

antibodies that bind to IgE, including Omalizumab (XOLAIR®) commercially available from Genentech, E26 (Figs. 17A-B herein), HAE1 (Figs. 17A-B herein), IgE antibody with an amino acid substitution at position 265 of an Fc region thereof (US 2004/0191244 A1), Hu-901 (Figs. 17A-B herein), an IgE antibody as in WO2004/070011, or an antibody (including antibody fragments and full length antibodies) comprising the variable domains of any of those IgE antibodies. See, also, Presta *et al., J. Immunol.* 151:2623-2632 (1993); International Publication No. WO 95/19181; US Patent No. 5,714,338, issued February 3, 1998; US Patent No. 5,091,313, issued February 25, 1992; WO 93/04173 published March 4, 1993; WO 99/01556 published January 14, 1999; and US Patent No. 5,714,338;

antibodies that bind to vascular endothelial growth factor (VEGF) or a receptor thereof, including Bevacizumab (AVASTIN™), commercially available from Genentech, and Ranibizumab (LUCENTIS™);

anti-IL-8 antibodies (St John *et al., Chest*, 103:932 (1993), and International Publication No. WO 95/23865);

anti-PSCA antibodies (WO01/40309);

anti-CD40 antibodies, including S2C6 and humanized variants thereof (WO00/75348);

anti-CD11a antibodies, including efalizumab (RAPTIVA®) (US Patent No. 5,622,700, WO 98/23761, Steppe *et al., Transplant Intl.* 4:3-7 (1991), and Hourmant *et al., Transplantation* 58:377-380 (1994)); anti-CD18 antibodies (US Patent No. 5,622,700, issued April 22, 1997, or as in WO 97/26912, published July 31, 1997);

anti-Apo-2 receptor antibody (WO 98/51793 published November 19, 1998);

anti-TNF-alpha antibodies including cA2 (REMICADE®), CDP571 and MAK-195 (See, US Patent No. 5,672,347 issued September 30, 1997, Lorenz *et al. J. Immunol.* 156(4):1646-1653 (1996), and Dhainaut *et al. Crit. Care Med.* 23(9):1461-1469 (1995));

anti-Tissue Factor (TF) (European Patent No. 0 420 937 B1 granted November 9, 1994);

anti-human $\alpha 4\beta_7$ integrin (WO 98/06248 published February 19, 1998);
anti-EGFR antibodies, including chimerized or humanized 225 antibody as in WO 96/40210 published December 19, 1996;
anti-CD3 antibodies, such as OKT3 (US Patent No. 4,515,893 issued May 7, 1985);
anti-CD25 or anti-tac antibodies such as CHI-621 (SIMULECT®) and (ZENAPAX®) (See US Patent No. 5,693,762 issued December 2, 1997);
anti-CD4 antibodies such as the cM-7412 antibody (Choy *et al. Arthritis Rheum* 39(1):52-56 (1996));
anti-CD52 antibodies such as CAMPATH-1H (Riechmann *et al. Nature* 332:323-337 (1988);
anti-Fc receptor antibodies such as the M22 antibody directed against FcγRI as in Graziano *et al. J. Immunol.* 155(10):4996-5002 (1995);
anti-carcinoembryonic antigen (CEA) antibodies such as hMN-14 (Sharkey *et al. Cancer Res.* 55(23Suppl): 5935s-5945s (1995);
antibodies directed against breast epithelial cells including huBrE-3, hu-Mc 3 and CHL6 (Ceriani *et al. Cancer Res.* 55(23): 5852s-5856s (1995); and Richman *et al. Cancer Res.* 55(23 Supp): 5916s-5920s (1995));
antibodies that bind to colon carcinoma cells such as C242 (Litton *et al. Eur J. Immunol.* 26(1):1-9 (1996));
anti-CD38 antibodies, *e.g.* AT 13/5 (Ellis *et al. J. Immunol.* 155(2):925-937 (1995));
anti-CD33 antibodies such as Hu M195 (Jurcic *et al. Cancer Res* 55(23 Suppl):5908s-5910s (1995) and CMA-676 or CDP771;
anti-CD22 antibodies such as LL2 or LymphoCide (Juweid *et al. Cancer Res* 55(23 Suppl):5899s-5907s (1995);
anti-EpCAM antibodies such as 17-1A (PANOREX®);
anti-GpIIb/IIIa antibodies such as abciximab or c7E3 Fab (REOPRO®);
anti-RSV antibodies such as MEDI-493 (SYNAGIS®);
anti-CMV antibodies such as PROTOVIR®;
anti-HIV antibodies such as PRO542;
anti-hepatitis antibodies such as the anti-Hep B antibody OSTAVIR®;
anti-CA 125 antibody OvaRex;
anti-idiotypic GD3 epitope antibody BEC2;
anti-$\alpha v\beta 3$ antibody VITAXIN®;
anti-human renal cell carcinoma antibody such as ch-G250; ING-1;
anti-human 17-1A antibody (3622W94);
anti-human colorectal tumor antibody (A33);
anti-human melanoma antibody R24 directed against GD3 ganglioside;
anti-human squamous-cell carcinoma (SF-25); and
anti-human leukocyte antigen (HLA) antibodies such as Smart ID10 and the anti-HLA DR antibody Oncolym (Lym-1).

(xi) Antibody variant compositions

The present invention, in at least one aspect, concerns formulations comprising a composition which comprises a mixture of a main species antibody and one or more variants thereof. Where the main species antibody binds HER2, preferably the HER2 antibody (either or both of the main species HER2 antibody and antibody variant thereof) is one which binds to Domain II of HER2, inhibits HER dimerization more effectively

than Trastuzumab, and/or binds to a heterodimeric binding site of HER2. The preferred embodiment herein of the main species antibody is one comprising the variable light and variable heavy amino acid sequences in SEQ ID Nos. 3 and 4, and most preferably comprising a light chain amino acid sequence selected from SEQ ID No. 15 and 23, and a heavy chain amino acid sequence selected from SEQ ID No. 16 and 24.

In one embodiment, the formulated HER2 antibody composition comprises a mixture of the main species HER2 antibody and an amino acid sequence variant thereof comprising an amino-terminal leader extension. Preferably, the amino-terminal leader extension is on a light chain of the antibody variant (*e.g.* on one or two light chains of the antibody variant). The main species HER2 antibody or the antibody variant may be an full length antibody or antibody fragment (*e.g.* Fab of F(ab')2 fragments), but preferably both are full length antibodies. The antibody variant herein may comprise an amino-terminal leader extension on any one or more of the heavy or light chains thereof. Preferably, the amino-terminal leader extension is on one or two light chains of the antibody. The amino-terminal leader extension preferably comprises or consists of VHS-. Presence of the amino-terminal leader extension in the composition can be detected by various analytical techniques including, but not limited to, N-terminal sequence analysis, assay for charge heterogeneity (for instance, cation exchange chromatography or capillary zone electrophoresis), mass spectrometry, etc. The amount of the antibody variant in the composition generally ranges from an amount that constitutes the detection limit of any assay (preferably N-terminal sequence analysis) used to detect the variant to an amount less than the amount of the main species antibody. Generally, about 20% or less (*e.g.* from about 1% to about 15%, for instance from 5% to about 15%) of the antibody molecules in the composition comprise an amino-terminal leader extension. Such percentage amounts are preferably determined using quantitative N-terminal sequence analysis or cation exchange analysis (preferably using a high-resolution, weak cation-exchange column, such as a PROPAC WCX-10™ cation exchange column). Aside from the amino-terminal leader extension variant, further amino acid sequence alterations of the main species antibody and/or variant are contemplated, including but not limited to an antibody comprising a C-terminal lysine residue on one or both heavy chains thereof, a deamidated antibody variant, etc.

Moreover, the main species antibody or variant may further comprise glycosylation variations, non-limiting examples of which include HER2 antibody comprising a G1 or G2 oligosaccharide structure attached to the Fc region thereof, HER2 antibody comprising a carbohydrate moiety attached to a light chain thereof (*e.g.* one or two carbohydrate moieties attached to one or two light chains of the antibody), HER2 antibody comprising a non-glycosylated heavy chain.

III. Preparation of the Formulation

The present invention provides, in a first aspect, a stable pharmaceutical formulation comprising a monoclonal antibody, preferably a full length human or humanized IgG1 antibody, in histidine-acetate buffer, pH 5.5 to 6.5, preferably pH 5.8 to 6.2. However, the antibody in the formulation may be an antibody fragment comprising an antigen-binding region, such as a Fab or F(ab')2 fragment.

In another embodiment, the invention concerns a pharmaceutical formulation comprising, or consisting essentially of, a full length IgG1 antibody susceptible to deamidation or aggregation in an amount from about 10mg/mL to about 250mg/mL; histidine-acetate buffer, pH 5.5 to 6.5; saccharide selected from the group

consisting of trehalose and sucrose, in an amount from about 60mM to about 250mM; and polysorbate 20 in an amount from about 0.01% to about 0.1%.

In yet a further embodiment, the invention provides a pharmaceutical formulation comprising an antibody that binds to domain II of HER2 in a histidine buffer at a pH from about 5.5 to about 6.5, a saccharide and a surfactant. For example, the formulation may comprise Pertuzumab in an amount from about 20mg/mL to about 40mg/mL, histidine-acetate buffer, sucrose, and polysorbate 20, wherein the pH of the formulation is from about 5.5 to about 6.5

In another aspect, the invention provides a pharmaceutical formulation comprising a DR5 antibody in a histidine buffer at a pH from about 5.5 to about 6.5, a saccharide, and a surfactant. Such a formulation may, for example, comprise, Apomab in an amount from about 10mg/mL to about 30mg/mL, histidine-acetate buffer, trehalose, and polysorbate 20, wherein the pH of the formulation is from about 5.5 to about 6.5.

The formulation is especially useful for antibodies that are susceptible to deamidation and/or aggregation and/or fragmentation, in that the buffer retards deamidation and/or aggregation and/or fragmentation of the antibody formulated therein. In addition, unlike other histidine buffers prepared using HCl, the histidine-acetate buffer lacks the chloride ion which was found to be beneficial herein in that this buffer when combined with saccharide had the same protective effect on antibody as polysorbate 20, and was stable and compatible with storage in stainless steel tanks. Thus, in addition to the formulation *per se* comprising the antibody susceptible to deamidation, aggregation and/or fragmentation, the invention provides a method for reducing deamidation, aggregation and/or fragmentation of a therapeutic monoclonal antibody (for example, relative to a composition at a different pH or in a different buffer), comprising formulating the antibody in a histidine-acetate buffer, pH 5.5 to 6.5. In this embodiment, one may determine or measure deamidation, aggregation and/or fragmentation before and after the antibody is formulated, with the formulated antibody demonstrating acceptable deamidation, aggregation and/or fragmentation in the formulation and upon storage thereof.

The antibody in the formulation may bind an antigen including but not limited to: HER2, CD20, IgE, DR5, BR3 and VEGF.

Where the formulated antibody binds HER2, it preferably is one which binds to Domain II of HER2, inhibits HER dimerization more effectively than Trastuzumab, and/or binds to a heterodimeric binding site of HER2. The preferred embodiment herein of a formulated HER2 antibody is one comprising the variable light and variable heavy amino acid sequences in SEQ ID Nos. 3 and 4, and most preferably comprising the light chain and heavy chain amino acid sequences in SEQ ID Nos. 15 and 16 (Pertuzumab).

Examples of CD20 antibodies which can be formulated herein include: "C2B8" which is now called "Rituximab" ("RITUXAN®") commercially available from Genentech (see also US Patent No. 5,736,137, expressly incorporated herein by reference); the yttrium-[90]-labeled 2B8 murine antibody designated "Y2B8" or "Ibritumomab Tiuxetan" ZEVALIN® commercially available from Biogen-Idec (see also US Patent No. 5,736,137, expressly incorporated herein by reference); murine IgG2a "B1," also called "Tositumomab," optionally labeled with ^{131}I to generate the "131I-B1" antibody (iodine I131 tositumomab, BEXXAR™) (US Patent No. 5,595,721, expressly incorporated herein by reference); murine monoclonal antibody "1F5" (Press *et al. Blood* 69(2):584-591 (1987) and variants thereof including "framework patched" or humanized 1F5 (WO03/002607, Leung, S.); ATCC deposit HB-96450); murine 2H7 and chimeric 2H7 antibody (Clark *et al. PNAS* 82: 1766-1770 (1985); US Patent No. 5,500,362, expressly incorporated herein by reference); humanized

2H7; huMax-CD20 (WO 04/035607, Genmab, Denmark); AME-133 (Applied Molecular Evolution); A20 antibody or variants thereof such as chimeric or humanized A20 antibody (cA20, hA20, respectively) (US 2003/0219433, Immunomedics); and monoclonal antibodies L27, G28-2, 93-1B3, B-C1 or NU-B2 available from the International Leukocyte Typing Workshop (Valentine *et al.*, In: Leukocyte Typing III (McMichael, Ed., p. 440, Oxford University Press (1987)).

In the preferred embodiment of a formulated CD20 antibody, the CD20 antibody is a humanized 2H7 antibody. Prefered humanized 2H7 antibodies herein are 2H7v16 and 2H7v511. The humanized 2H7v16 may be an intact antibody or antibody fragment comprising the variable light and variable heavy sequences in Figs. 18A-B (SEQ ID Nos. 26 and 29). Where the humanized 2H7v16 antibody is a full length antibody, preferably it comprises the light and heavy chain amino acid sequences with SEQ ID Nos. 63 and 65.

Where the antibody binds VEGF, it preferably comprises the variable domain sequences as depicted in Fig. 19. The most preferred anti-VEGF antibody is full length humanized IgG1antibody, Bevacizumab (AVASTIN™), commercially available from Genentech.

Where the formulated antibody binds IgE, it is preferably selected from the group consisting of: E25, Omalizumab (XOLAIR®) commercially available from Genentech (see also Figs. 17A-B), E26 (Figs. 17A-B herein), HAE1 (Figs. 17A-B herein), IgE antibody with an amino acid substitution at position 265 of an Fc region thereof (US 2004/0191244 A1), Hu-901 (Figs. 17A-B herein), an IgE antibody as in WO2004/070011, or an antibody (including antibody fragments and full length antibodies) comprising the variable domains of any of those IgE antibodies.

Where the antibody binds to a receptor in the tumor necrosis factor (TNF) superfamily or to a death receptor, it preferably binds to DR5, and preferably is an agonist antibody. Publications in this area include Sheridan *et al., Science*, 277:818-821 (1997), Pan *et al., Science*, 277:815-818 (1997), WO98/51793 published November 19, 1998; WO98/41629 published September 24, 1998; Screaton *et al., Curr. Biol.*, 7:693-696 (1997); Walczak *et al., EMBO J.*, 16:5386-5387 (1997); Wu *et al., Nature Genetics*, 17:141-143 (1997); WO98/35986 published August 20, 1998; EP870,827 published October 14, 1998; WO98/46643 published October 22, 1998; WO99/02653 published January 21, 1999; WO99/09165 published February 25, 1999; WO99/11791 published March 11, 1999; US 2002/0072091 published August 13, 2002; US 2002/0098550 published December 7, 2001; US 6,313,269 issued December 6, 2001; US 2001/0010924 published August 2, 2001; US 2003/01255540 published July 3, 2003; US 2002/0160446 published October 31, 2002, US 2002/0048785 published April 25, 2002; US 6,342,369 issued February, 2002; US 6,569,642 issued May 27, 2003, US 6,072,047 issued June 6, 2000, US 6,642,358 issued November 4, 2003; US 6,743,625 issued June 1, 2004. The most preferred DR5 antibody is Apomab.

Each of the formulations noted above comprises a buffer, preferably a histidine buffer, and most preferably a histidine-acetate buffer with a pH of 5.5 to 6.5, preferably 5.8 to 6.2, for example approximately 6.0. The concentration of the buffer is dictated, at least in part, by the desired pH. Exemplary concentrations for the buffer are in the range from about 1mM to about 200mM, preferably from about 10mM to about 40mM, most preferably about 20mM.

The antibody concentration in the formulation is preferably in the range from about 10mg/mL to about 250mg/mL. The antibody concentration may be determined based on the intended use and mode of administration of the formulation. For example, where the formulation is for IV administration (*e.g.* a HER2

antibody), the antibody concentration in the formulation is preferably from about 20mg/mL to about 40mg/mL. In the exemplified Pertuzumab formulation intended for intravenous (IV) administration, the antibody concentration was from about 20mg/mL to about 40mg/mL, most preferably about 30mg/mL.

Where the antibody is for SQ or IM administration (*e.g.* for an anti-IgE antibody) higher concentrations of the antibody may be desired. Such substantially high antibody concentrations may be from about 50mg/mL to about 250mg/mL, or from about 80mg/mL to about 250mg/mL, or from about 100mg/mL to about 200mg/mL.

Where the formulation comprises a DR5 antibody, such as Apomab, exemplary antibody concentrations are from about 10mg/mL to about 30mg/mL, for example about 20mg/mL DR5 antibody; such formulation being useful for intravenous administration.

The formulation for administration is preferably an aqueous formulation (not lyophilized) and has not been subjected to prior lyophilization. While the formulation may be lyophilized, preferably it is not. However, freezing of the aqueous formulation, without simultaneous drying that occurs during freeze-drying, is specifically contemplated herein, facilitating longer term storage thereof, for instance in a stainless steel tank.

The formulation preferably further comprises a saccharide, most preferably a disaccharide, such as trehalose or sucrose. The saccharide is generally included in an amount which reduces soluble aggregate formation, such as that which occurs upon freeze/thaw. Exemplary saccharide concentrations are in the range from about 10mM to about 1M, for example from about 60mM to about 250mM, and most preferably about 120mM for a HER2 antibody formulation, and about 240mM for a DR5 antibody formulation.

While it was found herein that a formulation comprising histidine-acetate buffer and saccharide was stable, the formulation optionally further comprises surfactant, such as polysorbate, most preferably polysorbate 20. The surfactant is generally included in an amount which reduces insoluble aggregate formation (such as that which occurs upon shaking or shipping). The surfactant concentration is preferably from about 0.0001% to about 1.0%, most preferably from about 0.01% to about 0.1%, for example about 0.02%.

Optionally, the formulation does not contain a tonicifying amount of a salt such as sodium chloride.

The formulation is generally sterile, and this can be achieved according to the procedures known to the skilled person for generating sterile pharmaceutical formulations suitable for administration to human subjects, including filtration through sterile filtration membranes, prior to, or following, preparation of the formulation.

Moreover, the formulation is desirably one which has been demonstrated to be stable upon storage. Various stability assays are available to the skilled practitioner for confirming the stability of the formulation. For example, the formulation may be one which is found to be stable upon storage: at about 40°C for at least 4 weeks; at about 5°C or about 15°C for at least 3 months or at least 1 year; and/or about -20°C for at least 3 months. Stability can be tested by evaluating physical stability, chemical stability, and/or biological activity of the antibody in the formulation around the time of formulation as well as following storage at the noted temperatures. Physical and/or stability can be evaluated qualitatively and/or quantitatively in a variety of different ways, including evaluation of aggregate formation (for example using size exclusion chromatography, by measuring turbidity, and/or by visual inspection); by assessing charge heterogeneity using cation exchange chromatography or capillary zone electrophoresis; amino-terminal or carboxy-terminal sequence analysis; mass spectrometric analysis; SDS-PAGE analysis to compare reduced and intact antibody; peptide map (for example tryptic or LYS-C) analysis; evaluting biological activity or antigen binding function of the antibody; etc. Instability may result in aggregation, deamidation (*e.g.* Asn deamidation), oxidation (*e.g.* Met oxidation),

monoclonal antibody;a growth inhibitory agent; a cytotoxic agent; a chemotherapeutic agent; EGFR-targeted drug; tyrosine kinase inhibitor; anti-angiogenic agent; and/or cytokine; etc.

In addition to the above therapeutic regimes, the patient may be subjected to surgical removal of cancer cells and/or radiation therapy.

V. Articles of Manufacture

In another embodiment of the invention, an article of manufacture is provided which contains the pharmaceutical formulation of the present invention and provides instructions for its use. The article of manufacture comprises a container. Suitable containers include, for example, bottles, vials (*e.g.* dual chamber vials), syringes (such as dual chamber syringes) and test tubes. The container may be formed from a variety of materials such as glass or plastic. The container holds the formulation and the label on, or associated with, the container may indicate directions for use. The container holding the formulation may be a multi-use vial, which allows for repeat administrations (*e.g.* from 2-6 administrations) of the reconstituted formulation. The article of manufacture may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use as noted in the previous section.

The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention. All literature and patent citations are incorporated herein by reference.

EXAMPLES

Stable Pertuzumab Liquid Formulations

These examples describe the development and stability testing of stable liquid formulations comprising Pertuzumab at protein concentrations in the range from about 10 mg/mL – 180 mg/mL. The selected formulations had low turbidity, and were physically and chemically stable. A chloride ion was removed from the formulation to reduce the risk of corrosion. The formulation was isotonic, and suitable for subcutaneous or intramuscular delivery. Insoluble aggregate formation upon agitation stress was prevented using histidine-acetate and sucrose formulation, without the need to include polysorbate 20.

Analytical Methods

Color, Appearance and Clarity (CAC)

The color, appearance, and clarity of the samples were determined by visual inspection of vials against a white and black background under white fluorescence light at room temperature.

UV Concentration Measurements

The liquid product aliquot was first diluted with formulation buffer so that the A_{max} near 278 nm is within 0.5-1.0 absorbance unit. The UV absorbance of the diluted samples was measured in a quartz cuvette with 1 cm path length on an HP 8453 spectrophotometer. Absorbance was measured at 278 nm and 320 nm. The absorbance from 320 nm is used to correct background light scattering due to larger aggregates, bubbles and

WHAT IS CLAIMED IS:

1. A stable pharmaceutical formulation comprising a monoclonal antibody in histidine-acetate buffer, pH 5.5 to 6.5.

2. The formulation of claim 1 wherein the pH is from 5.8 to 6.2.

3. The formulation of claim 1 wherein the histidine-acetate buffer concentration is from about 1mM to about 200mM.

4. The formulation of claim 3 wherein the histidine-acetate buffer concentration is from about 10mM to about 40mM.

5. The formulation of claim 1wherein the antibody concentration is from about 10mg/mL to about 250mg/mL.

6. The formulation of claim 5 wherein the monoclonal antibody concentration is from about 20mg/mL to about 40mg/mL.

7. The formulation of claim 5 wherein the monoclonal antibody concentration is from about 80mg/mL to about 250mg/mL.

8. The formulation of claim 1 further comprising saccharide.

9. The formulation of claim 8 wherein the saccharide is a disaccharide.

10. The formulation of claim 8 wherein the saccharide is trehalose.

11. The formulation of claim 8 wherein the saccharide is sucrose.

12. The formulation of claim 8 wherein the saccharide concentration is from about 10mM to about 1M.

13. The formulation of claim 12 wherein the saccharide concentration is from about 60mM to about 250mM.

14. The formulation of claim 1 further comprising surfactant.

15. The formulation of claim 14 wherein the surfactant is polysorbate.

16. The formulation of claim 15 wherein the surfactant is polysorbate 20.

17. The formulation of claim 14 wherein the surfactant concentration is from about 0.0001% to about 1.0%.

18. The formulation of claim 17 wherein the surfactant concentration is from about 0.01% to about 0.1%.

19. The formulation of claim 1 wherein the monoclonal antibody is a full length antibody.

20. The formulation of claim 19 wherein the monoclonal antibody is an IgG1 antibody.

21. The formulation of claim 1 wherein the monoclonal antibody is a humanized antibody.

22. The formulation of claim 1 wherein the monoclonal antibody is an antibody fragment comprising an antigen-binding region.

23. The formulation of claim 22 wherein the antibody fragment is a Fab or F(ab')2 fragment.

24. The formulation of claim 1 which is sterile.

25. The formulation of claim 1 wherein the monoclonal antibody binds an antigen selected from the group consisting of HER2, CD20, DR5, BR3, IgE, and VEGF.

26. The formulation of claim 25 wherein the antigen is CD20 and the monoclonal antibody is humanized 2H7.

27. The formulation of claim 25 wherein the antigen is VEGF and the monoclonal antibody is Bevacizumab.

28. The formulation of claim 1 wherein the monoclonal antibody is susceptible to deamidation or aggregation.

29. The formulation of claim 1 which is stable upon storage at about 40°C for at least 4 weeks

30. The formulation of claim 1 which is stable upon storage at about 5°C or about 15°C for at least 3 months.

31. The formulation of claim 1 which is stable upon storage at about -20°C for at least 3 months.

32. The formulation of claim 1 which is stable upon freezing and thawing.

33. The formulation of claim 1 which is aqueous.

34. The formulation of claim 1 which is frozen.

35. The formulation of claim 1 which is not lyophilized and has not been subjected to prior lyophilization.

36. The formulation of claim 35 which is aqueous and is administered to a subject.

37. The formulation of claim 36 wherein the formulation is for intravenous (IV), subcutaneous (SQ) or intramuscular (IM) administration.

38. The formulation of claim 37 which is for IV administration and the antibody concentration is from about 20mg/mL to about 40mg/mL.

39. The formulation of claim 37 which is for SQ administration and the antibody concentration is from about 80mg/mL to about 250mg/mL.

40. A vial with a stopper pierceable by a syringe comprising the formulation of claim 1 inside the vial.

41. The vial of claim 40 which is stored at about 2-8°C.

42. The vial of claim 40 which is a 20cc or 50cc vial.

43. A stainless steel tank comprising the formulation of claim 1 inside the tank.

44. The tank of claim 43 wherein the formulation therein is frozen.

45. A method of treating a disease or disorder in a subject comprising administering the formulation of claim 1 to a subject in an amount effective to treat the disease or disorder.

46. A pharmaceutical formulation comprising:

(a) a full length IgG1 antibody susceptible to deamidation or aggregation in an amount from about 10mg/mL to about 250mg/mL;

(b) histidine-acetate buffer, pH 5.5 to 6.5;

(c) saccharide selected from the group consisting of trehalose and sucrose, in an amount from about 60mM to about 250mM; and

(d) polysorbate 20 in an amount from about 0.01% to about 0.1%.

47. A method for reducing deamidation or aggregation of a therapeutic monoclonal antibody, comprising formulating the antibody in a histidine-acetate buffer, pH 5.5 to 6.5

48. The method of claim 47 comprising evaluating any antibody deamidation or aggregation before and after the antibody is formulated.

49. A pharmaceutical formulation comprising an antibody that binds to domain II of HER2 in a histidine buffer at a pH from about 5.5 to about 6.5, a saccharide, and a surfactant.

50. The formulation of claim 49 wherein the buffer is histidine-acetate.

51. The formulation of claim 49 wherein the HER2 antibody comprises the variable light and variable heavy amino acid sequences in SEQ ID Nos. 3 and 4, respectively.

52. The formulation of claim 51 wherein the HER2 antibody comprises a light chain amino acid sequence selected from SEQ ID No. 15 and 23, and a heavy chain amino acid sequence selected from SEQ ID No. 16 and 24.

53. The formulation of claim 49 wherein the pH of the formulation is from about 5.8 to about 6.2.

54. The formulation of claim 49 wherein the antibody binds to the junction between domains I, II and III of HER2.

55. The formulation of claim 49 wherein the antibody is a full length antibody.

56. The formulation of claim 49 wherein the antibody concentration is from about 20mg/mL to about 40mg/mL.

57. A pharmaceutical formulation comprising Pertuzumab in an amount from about 20mg/mL to about 40mg/mL, histidine-acetate buffer, sucrose, and polysorbate 20, wherein the pH of the formulation is from about 5.5 to about 6.5.

58. The formulation of claim 57 comprising about 30mg/mL Pertuzumab, about 20mM histidine-acetate, about 120mM sucrose, and about 0.02% polysorbate 20, wherein the pH of the formulation is about 6.0.

59. A vial with a stopper pierceable by a syringe comprising the formulation of claim 49.

60. A stainless steel tank comprising the formulation of claim 49 in the tank.

61. A method of treating HER2-expressing cancer in a subject, comprising administering the pharmaceutical formulation of claim 49 to the subject in an amount effective to treat the cancer.

62. The method of claim 61 wherein the formulation is administered to the subject intravenously, subcutaneously, or intramuscularly.

63. A method of making a pharmaceutical formulation comprising:

(a) preparing the formulation of claim 1; and

(b) evaluating physical stability, chemical stability, or biological activity of the monoclonal antibody in the formulation.

64. A pharmaceutical formulation comprising a DR5 antibody in a histidine buffer at a pH from about 5.5 to about 6.5, a saccharide, and a surfactant.

65. The formulation of claim 64 wherein the buffer is histidine-acetate.

66. The formulation of claim 64 wherein the DR5 antibody is an agonist antibody.

67. The formulation of claim 64 wherein the DR5 antibody is Apomab.

68. The formulation of claim 67 wherein the DR5 antibody comprises the heavy chain amino acid sequence of SEQ ID No. 51, and light chain amino acid sequence of SEQ ID No. 52.

69. The formulation of claim 64 wherein the pH of the formulation is from about 5.8 to about 6.2.

70. The formulation of claim 64 wherein the antibody is a full length antibody.

71. The formulation of claim 64 wherein the antibody concentration is from about 10mg/mL to about 30mg/mL.

72. A pharmaceutical formulation comprising Apomab in an amount from about 10mg/mL to about 30mg/mL, histidine-acetate buffer, trehalose, and polysorbate 20, wherein the pH of the formulation is from about 5.5 to about 6.5.

73. The formulation of claim 72 comprising about 20mg/mL Apomab, about 20mM histidine acetate, about 240mM trehalose, and about 0.02% polysorbate 20, wherein the pH of the formulation is about 6.0.

74. A vial with a stopper pierceable by a syringe comprising the formulation of claim 64.

75. A stainless steel tank comprising the formulation of claim 64 in the tank.

76. A method of treating cancer in a subject, comprising administering the pharmaceutical formulation of claim 64 to the subject in an amount effective to treat the cancer.

77. The method of claim 76 wherein the cancer is a solid tumor.

78. The method of claim 76 wherein the cancer is non-Hodgkin's lymphoma.

79. The method of claim 76 wherein the formulation is administered to the subject intravenously, subcutaneously, or intramuscularly.

Recombinant human hyaluronidase (rHuPH20): an enabling platform for subcutaneous drug and fluid administration

July 2007, Vol. 4, No. 4 , Pages 427-440 (doi:10.1517/17425247.4.4.427)
Gregory I Frost PhD,

Chief Scientific Officer, Halozyme Therapeutics, Inc., 11588 Sorrento Valley Road, CA, 92121, San Diego, +858 794 8889; +858 794 5730; gfrost@halozyme.com

The extracellular matrix is a significant barrier to the effective **subcutaneous delivery of many drugs**, limiting both pharmacokinetic parameters and injection volumes. The space outside adipocytes in the hypodermis is not a fluid, but rather a solid extracellular matrix of collageneous fibrils embedded within a glycosaminoglycan-rich viscoelastic gel that buffers convective forces. The extracellular matrix limits the volume of drug that can be injected at a single site, as well as the rate and amount that reach the vascular compartment. A fully human recombinant DNA-derived **hyaluronidase enzyme (rHuPH20)** has been developed to leverage the historical efficacy of animal testes extract-derived spreading factors to reversibly modify the hypodermis, in light of discovery of the human hyaluronidase gene family. **The application of this technology to increase both injection volumes and bioavailability from subcutaneous injection** may overcome some key limitations of this route of administration in multiple settings of care.

Recombinant human hyaluronidase (rHuPH20): an enabling platform for subcutaneous drug and fluid administration

July 2007, Vol. 4, No. 4 , Pages 427-440 (doi:10.1517/17425247.4.4.427)

Gregory I Frost PhD,
Chief Scientific Officer, Halozyme Therapeutics, Inc., 11588 Sorrento Valley Road, CA, 92121, San Diego, +858 794 8889; +858 794 5730; gfrost@halozyme.com

The extracellular matrix is a significant barrier to the effective subcutaneous delivery of many drugs, limiting both pharmacokinetic parameters and injection volumes. The space outside adipocytes in the hypodermis is not a fluid, but rather a solid extracellular matrix of collageneous fibrils embedded within a glycosaminoglycan-rich viscoelastic gel that buffers convective forces. The extracellular matrix limits the volume of drug that can be injected at a single site, as well as the rate and amount that reach the vascular compartment. A fully human recombinant DNA-derived hyaluronidase enzyme (rHuPH20) has been developed to leverage the historical efficacy of animal testes extract-derived spreading factors to reversibly modify the hypodermis, in light of discovery of the human hyaluronidase gene family. The application of this technology to increase both injection volumes and bioavailability from subcutaneous injection may overcome some key limitations of this route of administration in multiple settings of care.

Keywords

hyaluronan, hyaluronic acid, hyaluronidase, Hylenex, rHuPH20

27-LB

Addition of Human Hyaluronidase to Rapid Analog Insulin Reduces the Absolute Variability of Early Insulin Absorption across Infusion Set Life

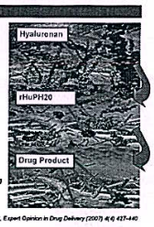
LINDA MORROW[1], DOUGLAS MUCHMORE[2], MARCUS HOMPESCH[1], PING JIANG[2], LAURENCE JADIN[2], AND DANIEL VAUGHN[2]

[1]Profil Institute for Clinical Research, Chula Vista, CA, [2]Halozyme Therapeutics, Inc., San Diego, CA

Background

Hyaluronan:

- Large (mega-Dalton), repeating sugar polymer found in interstitial tissues
- Forms barrier to bulk fluid flow in interstitial space
- Human body turns over >5 grams/day (10%of total body pool) every day



rHuPH20 Hyaluronidase:

- rHuPH20 is a genetically engineered soluble version of the naturally occurring human hyaluronidase enzyme
- rHuPH20 enzymatically cleaves hyaluronan, enabling bulk fluid flow in the interstitial space and facilitating the presentation of injected drugs to capillaries

Frost G, Expert Opinion in Drug Delivery (2007) 4(4) 427-440

Transient, locally acting permeation enhancer

- Injected dye dispersion is enhanced by rHuPH20 in a murine model
- rHuPH20 is rapidly catabolized at injection site ($t_{\frac{1}{2}}$ in skin ~30 min)
- rHuPH20 is immediately metabolized in blood ($t_{\frac{1}{2}}$ ~1-2 min)
- Permeation enhancement is transient; interstitial permeability is fully restored within 6-18 hours

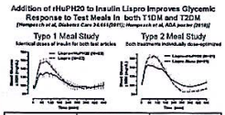
Bookbinder L et al, Journal of Controlled Release 114 (2006) 230-241

Addition of rHuPH20 to Insulin Lispro Improves Glycemic Response to Test Meals in both T1DM and T2DM

(Hompesch et al, Diabetes Care 34:915(2011); Hompesch et al, ADA poster (2010))

Type 1 Meal Study
Identical doses of insulin for both test articles

Type 2 Meal Study
Both treatments individually dose-optimized



	T1DM Meal	T2DM Meal
% in Glycemic Goal (ADA)	73% More (76% v. 43%)	48% More (71% v. 48%)
Hyperglycemic Excursions (>140 mg/dL)	79% Less	44% Less
Hypoglycemic Events	No Change	25 to 30% Fewer

Abstract

Pharmacokinetics(PK) and glucodynamics of an insulin aspart formulation (Aspart-PH20) with human hyaluronidase (rHuPH20) were compared to the commercial insulin aspart formulation (NovoLog®) approximately ½ and 2½ days following initiation of continuous subcutaneous infusion (CSII). Data are available for 13 (9 male, 4 female; mean age 39.7 (±9.7); mean BMI 26.2 (± 3.4)) of planned 18 T1DM patients who regularly use insulin pumps. Euglycemic clamp experiments followed a 0.15 U/kg bolus; usual basal rate was continued during clamps and PK results are thus baseline-subtracted.

For commercial aspart, insulin absorption was dramatically accelerated after 2½ days relative to ½ day CSII, with insulin exposure in the 1st h increasing 67% from 22±6 to 37±15% of total AUC (p=.009) and exposure beyond 2 h decreasing 38% from 47±10 to 29±13% (p=.0015). With rHuPH20, aspart exposure also increased after 2 ½ days CSII compared to ½ day (percentage increase in 1st hr % of total AUC of 40%, p=.02 with decrease beyond 2 hr of 33%, p=.05). The absolute shift in early exposure for aspart with rHuPH20 was reduced compared to aspart alone (AUC_{0-60} 22,400 v. 28,700 min*pmol/L, p<.02 for aspart-PH20; AUC_{0-60} 11,900 v. 22,300 min*pmol/L, p<.02 for aspart alone). With rHuPH20, aspart absorption is accelerated compared to aspart alone after both ½ day CSII (1st h exposure 36±9 v. 22±6 % of total AUC, p<.0001; exposure beyond 2 h 28±13 v. 47±10%, p=.0003) and 2½ days CSII (1st hr exposure 50±15 v. 37±15% of total AUC, p=.04; exposure beyond 2 h 18±11 v. 29±13%, p=.04). Insulin action profiles reflected the PK trends and both formulations were well tolerated. This study identifies temporal insulin absorption variability over infusion set life as a unique challenge to CSII therapy.

Study Design

Study Population: 20 generally healthy T1DM pumpers (16 completers, constituting efficacy evaluable population)

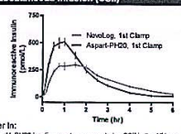
Study Drugs:

- NovoLog™
- Aspart-PH20 (Aspart 100 U/mL, rHuPH20 5 µg/mL)

Procedure:

- Admit to clinical research center 17:00 Hrs Day 1
  - New infusion set at admission
  - Treat with either NovoLog or Aspart-rHuPH20 (random sequence)
  - Adjust basal to target FBS = 110 mg/dL (adjust with IV insulin between 05:00 and 06:30 as needed)
- Perform euglycemic glucose clamp at 07:00 Hrs on Day 2 and again on Day 4
  - Administer bolus (0.15 U/kg) of either Aspart-rHuPH20 or NovoLog alone
- Assess Safety and Tolerability for 72 hours CSII
  - Local tolerability by Adverse Event reporting
  - Skin biopsy (6 mm full thickness punch biopsy) immediately after infusion (3 subjects), 18-24 hrs after infusion (3 subjects) or 36-48 hr after infusion (3 subjects). Biopsies stained for hyaluronan content and standard H&E
- Perform standardized test meal studies throughout clinic stay to assess glycemic response to mixed meals
- Readmit to CRC 5-14 days later for repeat procedures on the other study drug

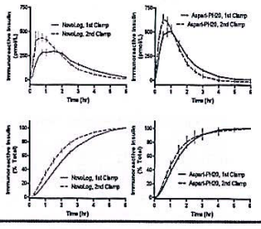
Pharmacokinetic Results: rHuPH20 accelerates exposure to rapid acting analog insulin bolus delivered by subcutaneous infusion (CSII)



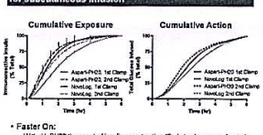
- Faster In:
  - –With rHuPH20 insulin aspart exposure during CSII in the 1st hour was 191% of control ($P < 0.0001$)
- Faster Out:
  - –With CSII, insulin aspart exposure beyond 2 hours decreased by 35% with rHuPH20 coinfusion ($P = 0.005$)

Effect of length of time infusion set is in use:

- Insulin absorption accelerates as infusion set ages
- rHuPH20 reduces this variability in insulin exposure



Pharmacodynamic Results: Acceleration of insulin action with rHuPH20 mirrors pharmacokinetic results for subcutaneous infusion



- Faster On:
  - –With rHuPH20 the onset of insulin aspart action (Early $I_{50\%}$) was accelerated from 47 to 35 minutes ($P = 0.05$)
- Faster Off:
  - –With rHuPH20 the mean duration of insulin aspart action was reduced from 164 to 147 minutes ($P = 0.003$)

Mixed Test Meal Results: Ultrafast profiles lead to improved early glycemic control

- Addition of rHuPH20 to Aspart reduces early post meal glycemic excursion
- As infusion set ages this difference between study drugs diminishes

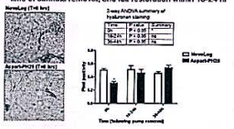
	NovoLog → Aspart-PH20			
Blood Glucose	**Day 1**	**Day 2**	**Day 3**	**All Days**
1 hr (mg/dL)	165-9128 (p=.0053)	136-9123 (p=.056)	135-9125 (p=.40)	145-9125 (p=.004)
90 min (mg/dL)	157-9112 (p=.0047)	140-9117 (p=.012)	137-9126 (p=.43)	140-9118 (p=.0062)
2 hr (mg/dL)	155-9108 (p=.2054)	153-9119 (p=.0115)	131-9137 (p=.66)	140-9121 (p=.017)

Safety Results: Adverse Events

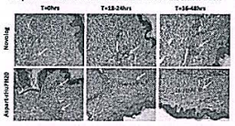
	Aspart-PH20 (N=18)	NovoLog alone (N=20)
Subjects w/ an Adverse Event	13 (72%)	16 (80%)
Adverse Events judged related by study drug	5 events in 3 subjects	7 events in 4 subjects
Procedure Related (IV or Joint Site Reactions, etc.)	14 events in 8 subjects	7 events in 4 subjects
Reported Uncommon AE's (Anemia, Nasal Pain, Scalp Hemorrhage, Cough, Nasal Congestion, Rhinitis, Nausea, Diarrhea, Abdominal Pain, Headache, Dizziness)	5 events in 5 subjects	20 events in 12 subjects
Skin biopsy site (Incision Site Hemorrhage, Erythema)	4 events in 4 subjects	0 events in 4 subjects

Safety: Skin biopsies

- Staining for hyaluronan demonstrated local depletion at time of cannula removal, and full restoration within 18-24 hr



- H&E staining revealed comparable local mild inflammatory response to both treatments at the cannula insertion track



Representative micrographs demonstrate histological changes at infusion sites. All biopsies had a superficial and deep inflammatory infiltrate (see arrows) of predominantly lymphocytes (non-specific chronic inflammation) with a smaller number of neutrophils. There are no significant differences between infusion of NovoLog alone and Aspart-PH20.

Summary and Conclusions

- rHuPH20 accelerates exposure to and action of rapid acting analog insulin both by pump and by SC injection
- Insulin absorption accelerates as infusion sets age; this variability is reduced when rHuPH20 is present
- Test meal data support previous work indicating reduction in glycemic excursion when rHuPH20 is used
- Skin biopsy data demonstrate the transient and local effects of rHuPH20, supporting previous safety findings

Contact Information: Douglas Muchmore, MD (858.704.8268) June 28, 2011

27

1658 B

AA 8567146

33

REPUBLICA DE COLOMBIA

NOTARIA 33 DE BOGOTA D.C.

ESCRITURA PUBLICA NUMERO: 1 6 5 8

- - MIL SEISCIENTOS CINCUENTA Y OCHO - -

FECHA DE OTORGAMIENTO: CUATRO (4) DE JULIO - - - - - - - -

DEL AÑO DOS MIL DOS (2002). ----------

ACTO: ----------

1.- PODER ESPECIAL.----------

OTORGADO POR: LAFRANCOL S.A., con Nit. No. - - -

890.301.463-8 - - - - - -

a favor de: JOSE LUIS REYES VILLAMIZAR, con C.C.No. 79.152.473 de Usaquén - Bogotá.---------

DIANA BEATRIZ LOPEZ

NOTARIA TREINTA Y TRES (33) DE BOGOTA D.C.

EN LA CIUDAD DE BOGOTA, DISTRITO CAPITAL, DEPARTAMENTO DE CUNDINAMARCA, REPUBLICA DE COLOMBIA, ANTE MI, DIANA BEATRIZ LOPEZ, - - - - - -

NOTARIA TREINTA Y TRES (33) - - - - -

DE ESTE CIRCULO SE OTORGA LA ESCRITURA PUBLICA QUE SE CONSIGNA EN LOS SIGUIENTES TERMINOS:----------

COMPARECIO: JUAN MARIA RENDON, mayor de edad, vecino de Bogotá, D.C, identificado con la cédula de ciudadanía número 17.125.100 de - - - - - -

Expedida en Bogotá - - - - - - - -

Y manifestó:----------

PRIMERO: Que obra en su carácter de Representante Legal de en nombre y representación de LAFRANCOL S.A, sociedad comercial organizada y existente conforme a las leyes colombianas, con domicilio y sede principal en Cali - Valle, constituida mediante escritura pública número

De la Notaría

De fecha

Reformada en distintas oportunidades, según consta en el certificado expedido por la Cámara de Comercio de Cali – Valle.----------

SEGUNDO: Que en tal carácter, por este público instrumento, concede PODER ESPECIAL, PERO AMPLIO Y SUFICIENTE a **JOSE LUIS REYES VILLAMIZAR**, mayor de edad, vecino de Bogotá, D.C, identificado con la cédula de ciudadanía número 79.152.473 de Usaquén, abogado en ejercicio, portador de la T.P.No. 446 55 del C.S. de la J., domiciliado en Bogotá, D.C, República de Colombia, con dirección en la carrera 17 No. 88-23, Oficina 205, de la misma ciudad, como su representante con poder especial, amplio y suficiente, para obtener la protección los derechos de propiedad industrial y de libre competencia de Lafrancol S.A., a cuyo efecto lo autorizo, par que nos represente ante cualesquiera entidad o persona, ejerza o no jurisdicción, especialmente ante las el orden administrativos, contencioso, administrativo y judicial, en todo lo concerniente a los siguientes asuntos:----

a.- Obtención, formulación de oberservaciones y oposición a patentes de invención, modelos y dibujos industriales y el registro de licencias de uso de esos derechos.----------

b.- Las acciones de oposición, cancelación, nulidad, medidas cautelares, concesión de licencias y en general los procesos civiles, penales, administrativos o contencioso- administrativos relacionados con estos derechosa u otros de diferente naturaleza, acciones y procesos que

promueva Lafrancol S.A., o se promuevan contra esta y.---------

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

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

HASTA AQUI LA MINUTA PRESENTADA

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

Se utilizaron las hojas de papel Notarial Números:

AA 8567146, 8567152.

COPIA NOTARIO NOVENO DE BOGOTÁ, D.C. después de la debida confrontación, auténtico esta copia por corresponder a la copia auténtica del documento, de la cual se ha tomado y que se ha tenido a la vista

Bogotá, D.C. 16 ABR. 2008

REPÚBLICA DE COLOMBIA NOTARIO NOVENO BOGOTÁ D.C.

ESTE PAPEL HACE PARTE DEL CUERPO DEL INSTRUMENTO

DERECHOS NOTARIALES: $ 49.600.oo

RESOLUCION No. 4188 del 28 de Diciembre del 2001

JUAN MARIA RENDON

C.C.No. 17.125.1oc de Bogotá

LAFRANCOL S.A.

.Nit. No. 890.301.463-8

TEL: 622-04-66.

ACEPTO :

JOSE LUIS REYES VILLAMIZAR

C.C. No. 79.152.473

TEL: 621-2631

Como NOTARIO NOVENO DE BOGOTA, D.C. después de ha debida confrontación auténtico esta copia con el documento de la cual se ha tomado y que se ha tenido a la vista.

Bogotá, D.C. 6 ABR. 2008

REPUBLICA DE COLOMBIA NOTARIA 9 CARLOS PEREZ TORRES BOGOTA D.C.

DIANA BEATRIZ LOPEZ

DIANA BEATRIZ LOPEZ

NOTARIA 33 DE BOGOTA D.C.

n.c.a.

Es Segunda COPIA (FOTOCOPIA) tomada de su original que se expide conforme al Artículo 79 del Decreto 960 de 1970 en 06 hojas útiles con destino a Jose Luis Reyes Villamizar.

NOS: Dcto. 1772/79

Santafé de ... D.C. 05 JUL. 2002

REPUBLICA DE COLOMBIA NOTARIA 33 Notaria Encargada BOGOTA, D.C.

35

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

Industria y Comercio
SUPERINTENDENCIA

RECIBO DE CAJA

No. 12 - 0078806

Bogotá D.C., Julio 31 de 2012 - 16:43:29

RECIBIDO DE : REYES Y REYES ABOGADOS NI 900.048.328

*** Soporte del Pago ***

TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	VR.PAGO
CONSIGNACION	BANCO DE BOGOTA	062754387	451803940	31/07/2012	315.000.00

*** 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
No. 11-178794- -00005-0000
Fecha: 2012-07-31 16:52:50 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 379 FASENACIONALII
Act. 400 OPOSIOBSERVTER Folios: 35