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SEÑOR

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

E. S. D.

JO

REF: **EXPEDIENTE 13-283797**

TÍTULO SOLICITADO: **"FORMULACIÓN SUBCUTÁNEA QUE COMPRENDE: ANTICUERPO ANTI-HER2"**

PUBLICACIÓN: **Gaceta 643 de 30 de abril de 2012**

PRIORIDAD: **EPO 09167025.7 DE 31/07/2009**

SOLICITANTE: **F. HOFFMANN-LA ROCHE AG.**

APODERADO: **ALICIA LLOREDA RICAURTE**

TRÁMITE: **REMISION DE INFORMACION**

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 **LABORATORIOS LEGRAND S.A.**, en el trámite de la referencia y con el propósito de coadyuvar en la decisión sobre la solicitud colombiana 13-283-797, referida a formulaciones farmacéuticas de anticuerpos anti-HER2 altamente concentradas, líquidas y estables que son adecuadas para inyección subcutánea y son estables durante el almacenamiento a largo plazo, de manera respetuosa me permito presentar argumentos para demostrar que la solicitud en cuestión y en especial el nuevo capítulo reivindicatorio propuesto por el solicitante en noviembre de 2017, incumple los requisitos de patentabilidad aplicables y no merece, por ello, recibir el beneficio patentario

I. COMPLEMENTO DE INFORMACIÓN.

Con el propósito de reiterar que la solicitud colombiana en trámite carece de los requisitos de patentabilidad establecidos por los artículos 14, 16 y 18 de la Decisión 486, me permito plantear los siguientes argumentos:

En primer término, vale la pena señalar que tanto la modificación realizada en el capítulo reivindicatorio (10 reivindicaciones) y que fue presentada en respuesta al Oficio 8288, como la modificación ahora objeto de análisis de Noviembre de 2017 y que fue presentada en respuesta al Oficio 8830 (8 reivindicaciones), corresponden a cambios que no deberían aceptarse puesto que reivindican materia que había sido estudiada en la solicitud parental 11-178-794 y por tal motivo –de ser aceptado dicho capítulo–, estaría retrocediéndose en el trámite surtido en la Resolución 90501 del 23 de noviembre de 2015, en donde se procedió a aceptar la solicitud divisional del expediente 13-283-797 basándose ese Despacho en que:

> «la materia definida en el capítulo reivindicatorio de la solicitud divisional no es idéntica a la estudiada en la solicitud parental, toda vez que el capítulo reivindicatorio de la solicitud parental reclamaba una composición farmacéutica de trastuzumab en combinación con rHuPH20 o un dispositivo para la administración de dicha composición, mientras que en la solicitud divisional se reivindica una formulación farmacéutica de trastuzumab que no contiene la enzima hialuronidasa». *(Subrayado fuera del texto)*

No obstante, dicho capítulo reivindicatorio modificado (10 reivindicaciones) presentado en esa oportunidad en respuesta al Oficio 8288 y el actual, conformado por 8 reivindicaciones, se observa que la formulación propuesta contiene trastuzumab, trehalosa y la enzima hialuronidasa- pero ahora como parte de un kit- (ver las respectiva reivindicación # 7 en cada caso).

1.1. La solicitud parental 11-178-794 referida a una composición que contiene i) trastuzumab, ii) un tampón de histidina, iii) trehalosa, iv) metionina, v) tensoactivo no iónico y vi) enzima hialuronidasa, fue denegada mediante la Resolución 49854 de 22/08/2014, decisión que fue confirmada mediante la Resolución 82283 de 20/10/2015.

El 26 de diciembre de 2011 se presentó ante ese Despacho la solicitud titulada «Formulación subcutánea de anticuerpo Anti-HER2», reivindicando prioridad EP 09167025.7 del 31 de agosto de 2009, cuya solicitud internacional es PCT/EP2010/060930 y su publicación internacional WO2011/012637, referida a:

> «La presente invención está relacionada con una **formulación farmacéutica** altamente concentrada y estable de un anticuerpo anti-HER2 farmacéuticamente activo, como por ejemplo **el Trastuzumab** (HERCEPIN ™), Pertuzumab o T-DM1, o una mezcla de tales moléculas de anticuerpo para la inyección subcutánea. En particular, la presente invención está relacionada con formulaciones que comprenden, además de una cantidad adecuada del anticuerpo anti-HER2, **una cantidad efectiva de al menos una enzima hialuronisada** como una formulación combinada o para su utilización en forma de una co-formulación. Dichas formulaciones comprenden adicionalmente al menos un agente tamponante, como por ejemplo un **tampón de histidina**, un estabilizante o una mezcla de dos o más estabilizantes (por ejemplo, un sacárido, como por ejemplo la a, a-**trehalosa dihidratada o la sacarosa**, y opcionalmente metionina como segundo estabilizante), **un tensoactivo no iónico** y una cantidad efectiva de al menos una enzima hialuronisada. También se proporcionan los métodos para preparar tales formulaciones y la utilización de las mismas» (Resumen, negrita fuera del texto)

A continuación presentamos el capítulo reivindicatorio inicial, en donde se observa que la formulación denegada contenía la enzima hialuronidasa:

> «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;
> b. entre alrededor de 1 y 100 Mm de un agente tamponador que proporciona un pH de 5,5 ± 2,0;
> c. entre alrededor de 1 y 500 Mm de un estabilizante o una mezcla de dos o mas estabilizantes;
> d. entre alrededor de 0,01 y 0,08% de un tensoactivo no ionico, y
> e. más de 150 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»
> (p. 103, capítulo reivindicatorio inicial, Expediente 11-178794)

Posteriormente, ese Despacho mediante la Resolución 49854 deniega dicha solicitud de patente debido a su carencia de nivel inventivo a la luz de los documentos D1 -US6267958- «*Protein formulation*» publicado el 31/Julio/2001 y D2 -US2007/071675- «*Dual variable domain immunoglobulin and uses thereof*» publicado el 29/Marzo/2007, argumentando:

> «...el documento D1 es considerado el estado de la técnica cercano a la invención definida en la reivindicación 1 porque divulga una formulación liofilizada estable, para administración subcutánea que comprende un anticuerpo anti HER2 (erlizumab) en una cantidad de entre 5 – 40mg, un buffer de histidina con un pH de 6.0, sacarosa entre 10 – 100mM y un surfactante no iónico como polisorbato (Col. 3, ln 39 – 42).
>
> ...
>
> el documento D2 divulga una composición farmacéutica liofilizada que comprende anticuerpo trastuzumab o pertuzumab en concentración de 0.1 – 250mg/ml, un buffer de histidina 1 – 50mM, sacarosa o trehalosa, metionina 5 – 10mM, polisorbato 80 entre 0 – 0.05% e hialuronidasa (Pág. 18, párr. [0112]; pág. 31 y 32, párr. [0184])
>
> Por lo tanto, una persona normalmente versada en la materia y conocedora de los conceptos de los documentos D1 y D2, sin ningún esfuerzo modificaría la composición descrita en el documento D1, incrementando la concentración del anticuerpo anti HER2 (trastuzumab, pertuzumab) e incluyendo la enzima hialurodinasa recombinante humana Hylenex® para mejorar la biodisponibilidad según lo sugiere el documento D2, para obtener la composición propuesta en la solicitud de manera obvia o evidente.
>
> En consecuencia, la reivindicación 1, de acuerdo a lo divulgado en los documentos D1 y D2, carece de Nivel Inventivo.»

Frente a dicha decisión, la solicitante presenta un recurso de reposición el 3 de octubre de 2014 en el que modifica la materia reclamada, así:

> «1. Una formulación farmacéutica liquida, estable, altamente concentrada de un anticuerpo farmacéuticamente activo anti-HER2 para administración subcutánea, que comprende:
>
> a. desde 120 ± 18 mg/ml de Trastuzumab;
>
> b. entre 1 a 50 mM de un agente tamponador de histidina que proporciona un pH de 5,5 ± 0,6;

c. entre 15 a 250 mM de α,α-trehalosa dihidratada o sacarosa y opcionalmente entre 5 a 25 mM de metionina;
d. entre 0,01 a 0,08% de un tensioactivo no iónico; y
e. de más de 150 y hasta 16.000 U/ml de una enzima hialuronidasa» (p. 12, recurso de reposición.

Vale la pena resaltar que los elementos que caracterizan al producto de esta reivindicación son:

i) trastuzumab, en una cantidad de 120 ± 18 mg/ml
ii) tampón de histidina pH de 5,5 ± 0,6, entre 1 a 50 mM
iii) α,α-trehalosa dihidratada o sacarosa, entre 15 a 250 mM
iv) metionina, entre 5 a 25 mM
v) un tensioactivo no iónico, entre 0,01 a 0,08%
vi) enzima hialuronidasa, entre 160 a 16.000 U/ml

1.2. La solicitud divisional 13-283-797 se refiere a los mismos elementos de la solicitud parental 11-178-794 los cuales son: i) trastuzumab, ii) un tampón de histidina, iii) trehalosa, iv) metionina, v) tensoactivo no iónico y vi) enzima hialuronidasa.

El de 03 de diciembre de 2013 la solicitante presenta una solicitud divisional con número de expediente 13-283-797, la cual es rechazada por ese Despacho mediante Resolución 56499 del 23 de septiembre de 2014, debido a que la materia reclamada fue la misma ya analizada para el Exp. 11-178-794, así:

«CUARTO: Que en este caso la división de la solicitud propuesta 13-283797-00000-0000 no procede comoquiera que dicha solicitud fraccionaria contiene la misma materia que el capítulo reivindicatorio presentado parta la solicitud original radicado bajo el N° 11-178794-00009-0000, en consecuencia carece de objeto para que proceda a un nuevo estudio de patentabilidad, dado que replica la materia contenida en el pliego reivindicatorio inicialmente aportado para estudio, mediante escrito radicado bajo el N° 11-178794-00007-0000 del 19 de noviembre de 2012, frente al cual este Despacho realizó el examen de patentabilidad mediante oficio 15882 del 27 de agosto de 2013, por lo que no es procedente realizar un segundo estudio.»

En respuesta a este acto administrativo, la solicitante modifica el capítulo reivindicatorio **excluyendo la enzima hialuronidasa** y por ende ese Despacho mediante la Resolución 90501 de 23/11/2015 revoca la Negación y ordena continuar con el trámite de dicha solicitud divisional:

> «SEGUNDO: Que mediante escrito radicado en esta Entidad el 06 de Noviembre de 2014, con el No. 13-283797-00006-0000, encontrándose dentro del término establecido para el efecto, la sociedad F. HOFFMANN-LA ROCHE AG., interpuso recurso de reposición contra la citada Resolución, con fundamento en los argumentos que a continuación se sintetizan:
> En primer lugar se trae a colación que el señor solicitante allega junto al memorial del recurso un capitulo reivindicatorio constituido por 14 cláusulas, que se refieren específicamente a una formulación liquida para inyección subcutánea de trastuzumab, la cual no contiene la enzima hialuronidasa, por lo que no existe solapamiento de materia con la solicitud original y no amplia el objeto de la invención divulgada inicialmente.
> La recurrente manifiesta que: "(...) la materia reclamada en el capítulo reivindicatorio de la presente solicitud divisional no contiene la misma materia del capítulo reivindicatorio originalmente presentado para la solicitud No. 11-178794 y más aun teniendo en cuenta las modificaciones presentadas a la divisional en el punto 1 del presente escrito" y continúa señalando que: "no se entienden las razones por las cuales la solicitud divisional de la referencia, ha sido rechazada y no sido objeto de un estudio de fondo como lo debe tener cualquier solicitud divisional (...)".
> [...]
> Por lo tanto, este Despacho encuentra que, tal como lo argumenta la recurrente, la materia reivindicada en la solicitud divisional radicada bajo el No. 13-283797-00000- 0000 de 06 de Noviembre de 2014, no corresponde a la materia de la solicitud parental radicada bajo el número 11-178794-00007-0000 del 19 de Noviembre de 2012, estudiada mediante Oficio 15882 y notificado el 27 de Agosto de 2013, toda vez que el capítulo reivindicatorio analizado en dicha solicitud reclamaba una composición farmacéutica de trastuzumab en combinación con una enzima hialuronidasa o un dispositivo para la administración de dicha composición, mientras que en la solicitud divisional se reivindica una formulación farmacéutica de trastuzumab que comprende: un tampón de histidina pH de 5,5 ± 0,6, trehalosa dihidratada o sacarosa, un tensioactivo no iónico y opcionalmente metionina, un dispositivo para la administración de dicha composición y un kit que contiene la formulación de trastuzumab más uno o más viales de rHuPH20.

[...]

Con relación al argumento de la opositora donde señala que las reivindicaciones modificadas sin enzima hialuronidasa no pueden ser aceptadas porque comprende una amplitud mayor en la materia a protegerse, este Despacho reitera a la opositora que dentro de las modalidades de la invención se incorpora una formulación farmacéutica de un anticuerpo anti-HER2, que carecen de la enzima hialuronidasa (Pág. 65, Líneas 11 a 22), la cual encuentra soporte en la formulación G del ejemplo 1 (Pág. 76 y 77). Por lo tanto, este Despacho encuentra que la materia definida en el capítulo reivindicatorio de la solicitud divisional cumple con el requisito definido en el artículo 36 de la Decisión 486, ya que no implica una ampliación de la materia inicialmente divulgada.»

En resumen, la solicitante allegó un nuevo capítulo reivindicatorio en el recurso interpuesto contra la Resolución 56499 – el cual se refiere a una composición que no contiene hialuronidasa –aceptándose dicha divisional por la SIC, aclarando a la opositora que dentro de las modalidades de la invención se incorpora una formulación de un anticuerpo anti-HER2 que no contienen la enzima hialuronidasa.

Posteriormente, en el Oficio 8288 de 2016 la SIC citó el documento D1, US6267958, «*Protein formulation*», publicado el 31/Julio/2001 –también citado para la solicitud parental– y el documento D2, Lam X., «*Antioxidants for Prevention of Methionine Oxidation in Recombinant Monoclonal Antibody HER2*» - 11/Noviembre/1997, como anterioridades que anticipaban la materia reclamada – el 6 de noviembre de 2014–concluyendo la ausencia de novedad y nivel inventivo.

En respuesta al Oficio 8288, la solicitante presentó un nuevo capitulo reivindicatorio (10 reivindicaciones), que reincide en el hecho de reclamar los elementos característicos de la solicitud parental, así:

«1. Una formulación farmacéutica liquida, estable, altamente concentrada de Trastuzumab para inyección subcutánea que consiste esencialmente de:
a. entre 120 ± 18 mg/ml de Trastuzumab;
b. entre 1 a 50 mM de un tampón de histidina que proporciona un pH de 5,5 ± 0,6;
c. entre 15 a 250 mM de α,α-trehalosa dihidratada o sacarosa y entre 5 a 25 mM de metionina; y
d. entre 0,01 a 0,08% de un tensioactivo no iónico;»

«9. Un kit de acuerdo con la reivindicación 8 que comprende adicionalmente uno o más viales que contienen una enzima hialuronidasa. »

«10. El kit de acuerdo con la reivindicación 9 que comprende adicionalmente uno o más viales que contienen una enzima hialuronidasa»

Como se puede observar, la solicitante reclama la composición libre de hialuronidasa y en las reivindicaciones 9 y 10 se describe que el kit contiene enzima hialuronidasa, por tanto, si se agrupan los elementos de la formulación de la solicitud parental, se tendría:

i) trastuzumab, en una cantidad de 120 ± 18 mg/ml
ii) tampón de histidina pH de 5,5 ± 0,6, entre 1 a 50 mM
iii) α,α-trehalosa dihidratada o sacarosa, entre 15 a 250 mM
iv) metionina, entre 5 a 25 mM
v) un tensioactivo no iónico, entre 0,01 a 0,08%
vi) enzima hialuronidasa, entre 160 a 16.000 U/ml

Luego, en el Oficio 8830 la SIC citó el documento D1, US6267958, «*Protein formulation*», publicado el 31/Julio/2001 –también citado para la solicitud parental–, el documento D3, WO2006/044908, «*Antibody formulation in histidine-acetate buffer*» - 27/Abril/2006, y D4, US20060104968, «*Soluble glycosamino glycanases and methods of preparing and using soluble glycosamiloglycanases*» - 18/Mayo/2006, como anterioridades que anticipaban la materia reclamada, concluyendo la ausencia de nivel inventivo.

En respuesta al Oficio 8830, la solicitante presentó un nuevo capitulo reivindicatorio (8 reivindicaciones) en Noviembre 2017, que reincide en el hecho de reclamar los elementos característicos de la solicitud parental, así:

«7. Un kit para inyección subcutánea de una formulación de anticuerpo anti-HER2 estable altamente concentrada que comprende:
A) Un vial que contiene:
a. 120 +/- 18 mg/ml Trastuzumab
b. De 1 a 50 mM de un tampón de histidina que proporciona un pH de 5.5 ± 0.6;

c. De 15 a 250 mM de α, α-trehalosa dihidratada o sacarosa y de 5 a 25 mM de metionina; y

d. 0.01 a 0.08% de un surfactante no iónico.

B) Un vial que contiene 150 a 16'000 U / ml, 2000 U / ml o 12'000 U / ml de una enzima hialuronidasa.»

«8. El kit según la reivindicación 7, en el que la enzima hialuronidasa es rHuPH20.»

Por su parte, la reivindicación 8, la enzima hialuronidasa es rHuPH20, es decir, hialuronidasa humana recombinante PH20, la cual —vale destacar— ya era conocida en el estado de la técnica:

«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 GI., 2007.

Aunque la solicitante enfoca la reivindicación 7 a un kit que contiene en un vial A que comprende la composición de trastuzumab libre de hialuronidasa, menciona que el kit tiene un vial B que puede contener hialuronidasa entre 150 a 16.000 U/ml.

Como es evidente, tal reivindicación 7 estaría agrupando nuevamente los elementos que ya habían sido evaluados en la solicitud parental. Veamos:

Elementos	Solicitud Parental, última modificación–denegada	Solicitud Divisional, penúltima modificación
Formulación	1. Una formulación farmacéutica liquida, estable, altamente concentrada de un anticuerpo farmacéuticamente activo anti-HER2 para administración subcutánea, que comprende:	7. Un kit para inyección subcutánea de una formulación de anticuerpo anti-HER2 estable altamente concentrada que comprende: A) Un vial que contiene:
trastuzumab, en una cantidad de 120 ± 18 mg/ml	a. desde 120 ± 18 mg/ml de Trastuzumab;	a. 120 +/- 18 mg/ml Trastuzumab
tampón de histidina pH de 5,5 ± 0,6, entre 1 a 50 mM	b. entre 1 a 50 mM de un agente tamponador de histidina que proporciona un pH de 5,5 ± 0,6;	b. De 1 a 50 mM de un tampón de histidina que proporciona un pH de 5.5 ± 0.6;
α,α-trehalosa dihidratada o sacarosa, entre 15 a 250 mM	c. entre 15 a 250 mM de α,α-trehalosa dihidratada o sacarosa	c. De 15 a 250 mM de α, α-trehalosa dihidratada o sacarosa
metionina, entre 5 a 25 mM	y opcionalmente entre 5 a 25 mM de metionina;	d. De 5 a 25 mM de metionina; y
un tensioactivo no iónico, entre 0,01 a 0,08%	d. entre 0,01 a 0,08% de un tensioactivo no iónico;	e. 0.01 a 0.08% de un surfactante no iónico.
enzima hialuronidasa, entre 160 a 16.000 U/ml	e. de más de 150 y hasta 16.000 U/ml de una enzima hialuronidasa	B) Un vial que contiene 150 a 16'000 U/ml, 2000 U/ml o 12'000 U/ml de una enzima hialuronidasa.»

Teniendo en cuenta el cuadro anterior, todos los elementos reclamados en la solicitud parental son tomados nuevamente en la presente solicitud divisional, en las reivindicaciones 7 y 8, por lo que no deberían ser aceptadas, pues es evidente que carece de nivel inventivo.

1.3. Las formulaciones de Trastuzumab habían sido anticipadas por documentos del estado de la técnica, en donde mencionan composiciones líquidas o liofilizadas que contienen trastuzumab, buffer de histidina, trehalosa, o sacarosa y un surfactante, así como la enzima hialuronidasa:

El documento US6267958 (reconocido como anterioridad por este Despacho), titulado «***Protein formulation***», se refiere a formulaciones que contienen anticuerpo anti-HER2, buffer de histidina, trehalosa o sacarosa, un surfactante. Veamos:

«A stable lyophilized protein formulation is described which can be reconstituted with a suitable diluent to generate a high protein concentration reconstituted formulation which is suitable for subcutaneous administration. For example, anti-IgE and anti-HER2 antibody formulations have been prepared by lyophilizing these antibodies in the presence of a lyoprotectant. The lyophilized mixture thus formed is reconstituted to a high protein concentration without apparent loss of stability of the protein

(...)

1. *A stable isotonic reconstituted formulation comprising an antibody in an amount of about 50 mg/mL to about 400 mg/mL and a diluent, which reconstituted formulation has been prepared from a lyophilized mixture of the antibody and a lyoprotectant which prevents or reduces chemical or physical instability of the antibody upon lyophilization and subsequent storage, wherein the molar ratio of lyoprotectant:antibody is about 100-510 mole lyoprotectant:1 mole of antibody, and wherein the antibody concentration in the reconstituted formulation is about 2-40 times greater than the antibody concentration in the mixture before lyophilization.*
2. *The formulation of claim 1 wherein the lyoprotectant is sucrose.*
3. *The formulation of claim 1 wherein the lyoprotectant is trehalose.*
4. *The formulation of claim 1 which further comprises a buffer.*
5. *The formulation of claim 4 wherein the buffer is histidine or succinate.*
6. *The formulation of claim 1 which farther comprises a surfactant.*
7. *The formulation of claim 1 which is sterile.»*

Por su parte, el documento US20070071675, titulado **«*Dual variable domain immunoglobulin and uses thereof*»**, revela que las composiciones de anticuerpos pueden contener hialuronidasa, como Hylenex®, una hialuronidasa recombinante humana[1]:

«[0184] (...) *Stabilizers can be used in both liquid and lyophilized dosage forms, principally 1-50 mM L-Methionine (optimally 5-10 mM). Other suitable bulking agents include glycine, arginine, can be included as 0-0.05% polysorbate-80 (optimally 0.005-0.01%). The pharmaceutical composition comprising the binding proteins of the invention prepared as an injectable solution for parenteral administration, can further comprise an agent useful as an adjuvant, such as those*

[1] http://hylenex.com/

used to increase the absorption, or dispersion of a therapeutic protein (e.g., antibody). A particularly useful adjuvant is hyaluronidase, such as Hylenex® (recombinant human hyaluronidase). Addition of hyaluronidase in the injectable solution improves human bioavailability following parenteral administration, particularly subcutaneous administration. It also allows for greater injection site volumes (i.e. greater than 1 ml) with less pain and discomfort, and minimum incidence of injection site reactions. (see WO2004078140, and US2006104968 incorporated herein by reference).»

Por su parte, Xanthe M. Lam et al., en ***"Antioxidants for Prevention of Methionine Oxidation in Recombinant Monoclonal Antibody HER2"*** revela a manera de ejemplos formulaciones líquidas de trastuzumab que contienen polisorbato, metionina y manitol:

Table 1—List of rhuMAb HER2 Liquid Formulations

Formulation	Usage	Protein Concentration, mg/mL	Buffer Excipients
A	Single dose	5	5 mM sodium acetate, pH 5.0 147 mM NaCl 0.01% polysorbate 20
B	Multiple dose	21	5 mM sodium acetate, pH 5.0 4% mannitol 0.01% polysorbate 20 1% benzyl alcohol
C	Multiple dose	21	5 mM sodium acetate, pH 5.0 4% mannitol 0.01% polysorbate 20 1% benzyl alcohol 14.5 mM methionine
D	Multiple dose	21	5 mM sodium acetate, pH 5.0 4% mannitol 0.01% polysorbate 20 0.01% benzethonium chloride 14.5 mM methionine

Así las cosas, se observa que los elementos reclamados por la solicitud divisional, ya habían sido anticipados por otros autores.

1.4. Según el estado de la técnica, la enzima hialuronidasa es empleada como un adyuvante para la administración de fármacos inyectados y así mismo brinda propiedades al medicamento que disminuyen la molestia en el sitio de aplicación en el paciente. Según la FDA y Hylenex sus indicaciones y usos son aplicables a múltiples fármacos; por lo tanto, su adición a una formulación de trastuzumab era obvia.

Como lo menciona el documento US20070071675, la enzima hialuronidasa es empleada como un adyuvante que mejora la biodisponibilidad de la administración de la formulación que contiene trastuzumab y facilita el caso de la administración subcutánea, así mismo ayuda a disminuir el dolor de la administración del mismo.

Dicho efecto también es soportado por los hallazgos de Frost GI., (2007), quien menciona que rHuPH20 incrementa el volumen de inyección y la biodisponibilidad de administraciones subcutáneas y ayuda a superar múltiples limitaciones de dicha vía de administración.

Según la FDA[2] (2005), la hialuronidasa recombinante humana es un modificador de la permeabilidad de tejidos, indicado como adyuvante en los casos de:

- Administración de fluidos subcutáneos para hidratar el producto
- Incrementar la dispersión y absorción de otros medicamentos inyectados
- En la mejora de la resorción de radiofármacos

De acuerdo con lo anterior, era obvio para una persona versada en la materia adicionar una enzima hialuronidasa a una composición —por ejemplo de trastuzumab– para facilitar su administración, en especial cuando dicha composición es altamente concentrada.

1.5. En resumen, el nuevo capítulo reivindicatorio de Noviembre de 2017 (8 reivindicaciones), carece de novedad y nivel inventivo a la luz de las enseñanzas de los documentos del estado de la técnica mencionados. La formulación de la nueva reivindicación principal ya era conocida y, los elementos del kit ya habían sido enseñados, en especial la indicación y formas de uso de la enzima hialuronidasa rHuPH20.

Como se observa en el siguiente cuadro, todos los elementos del nuevo capítulo reivindicatorio habían sido anticipados por el estado de la técnica:

[2] https://www.accessdata.fda.gov/drugsatfda_docs/label/2012/021859s009lbl.pdf

Solicitud Divisional 13 283797	solicitud parental 11-178797	US6267958	US2007/071675	Xanthe Lam X.	Frost GI
«1. Una formulación farmacéutica liquida, estable, altamente concentrada de Trastuzumab para inyección subcutánea que consiste esencialmente de:	SI	SI (Reiv.1)	SI [0040], [0167], R. 25	SI	
a. entre 120 ± 18 mg/ml de Trastuzumab;	SI	SI (Reiv.1)	SI	trastuzumab	
b. entre 1 a 50 mM de un tampón de histidina que proporciona un pH de 5,5 ± 0,6;	SI	SI (Reiv.5)	SI, [0181], [0184]	pH 5,0	
c. entre 15 a 250 mM de α,α-trehalosa dihidratada o sacarosa y entre 5 a 25 mM de metionina; y	SI	SI (Reiv.1)	SI, [0184]	Manitol	
d. entre 0,01 a 0,08% de un tensioactivo no iónico;»	SI	SI (Reiv.6)	SI, [0184]	SI	
«7. Un kit para inyección subcutánea de una formulación de anticuerpo anti-HER2 estable altamente concentrada que comprende:	SI	SI (Reiv.17)*	SI [0040], [0167], R. 25		
A) Un vial que contiene:	SI				
a. 120 +/- 18 mg/ml Trastuzumab	SI	SI	SI	trastuzumab	
b. De 1 a 50 mM de un tampón de histidina que proporciona un pH de 5.5 ± 0.6;	SI	SI	SI, [0181], [0184]	pH 5,0	
c. De 15 a 250 mM de α, α-trehalosa dihidratada o sacarosa y de 5 a 25 mM de metionina; y	SI	SI	SI, [0184]		
d. 0.01 a 0.08% de un surfactante no iónico.	SI	SI	SI, [0184]	SI	
B) Un vial que contiene 150 a 16'000 U / ml, 2000 U / ml o 12'000 U / ml de una enzima hialuronidasa.»	SI		SI, [0184]	SI	SI
«8. El kit según la reivindicación 7, en el que la enzima hialuronidasa es rHuPH20.»	SI			SI	SI
* Se refiere a que la formulación puede ser reconstituida en mas de un paso. Lo cual puede ser incluido en un kit.					

En primer lugar, se observa que la solicitud divisional 13-283-797 está reivindicando los mismos elementos de la solicitud parental, la cual fue denegada en su momento, por su falta de novedad y nivel inventivo.

En segundo lugar, los elementos incluidos en el capítulo reivindicatorio modificado habían sido anticipados previamente por US6267958, US2007/071675, Lam et.al., ya que enseñan formulaciones de trastuzumab que contienen los elementos reclamados y que claramente pueden sugerir a una persona versada en la materia a obtener formulaciones de trastuzumab a las que se les puede adicionar hialuronidasa con el fin de facilitar la administración del medicamento al paciente.

Finalmente, se desea destacar que no hay elementos dentro de la memoria descriptiva que evidencien la existencia de un efecto sorprendente o inesperado, por el contrario, de acuerdo a la información enseñada por la FDA sobre Hylenex (2005) y Frost GI. (2007), era evidente para una persona versada en la materia que las características inherentes a la hialuronidasa eran deseables para facilitar la administración de una formulación altamente concentrada de trastuzumab.

En consecuencia, y debido a que las características técnicas de la invención han sido anticipadas por los documentos mencionados, los anticuerpos reclamados carecen de **Novedad y Nivel Inventivo**.

Por todo lo anterior, ruego se analicen los argumentos expuestos con el rigor y detenimiento habituales y que en consecuencia se decrete, además de la negación de la solicitud en comento, el archivo del expediente que la contiene.

II. PRUEBAS.

Ruego que se tengan como tales, sin perjuicio de que el Despacho considere oficiosamente, las siguientes:

- Xanthe M. LAM, Janet Y. Yang, and Jeffrey L. Cleland. Journal of Pharmaceutical Sciences, Vol. 86, No. 11, Noviembre 1997. ***Antioxidants for Prevention of Methionine Oxidation in Recombinant Monoclonal Antibody HER2,*** p.1250-1255. Consulta: Todo el documento.

- Frost GI., ***"Recombinant human hyaluronidase (rHuPH20): an enabling platform for subcutaneous drug and fluid administration***" Expert Opin Drug Deliv. 2007 Jul;4(4):427-40. DOI: 10.1517/17425247.4.4.427. Consulta: Portada

- US2007/0071675A1, Chengbin Wu, Tariq Ghayur, Richard W. Dixon, Jochen G.Salfeld. ***Dual Variable Domain Immunoglobulin and Uses Thereof***. Consultado: Portada, Párr. [0025], [0040], [0167] y [0184]

- US6,267,958 B1, James Andya, Jeffrey L. Cleland, Chung C. Hsu, Xanthe

M. Lam, David E. Overcashier, Steven J. Shire, Janet Yu-Feng Yang, Sylvia Sau-Yan Wu. ***Protein formulation***. Consultado: Portada, col. 31 a 34.

- Highlights of Prescribing Information. ***HYLENEX recombinant (hyaluronidase human injection)*** Initial U.S. Approval: 2005. p. 3-5

Señor Superintendente,

JOSE LUIS REYES VILLAMIZAR
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Antioxidants for Prevention of Methionine Oxidation in Recombinant Monoclonal Antibody HER2

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Received April 10, 1997, from the *Department of Pharmaceutical Research and Development, Genentech, Inc., 460 Pt. San Bruno Blvd., MS 10, South San Francisco, CA 94080.* Accepted for publication August 3, 1997[⊗].

Abstract □ Recombinant humanized monoclonal antibody HER2, rhuMAb HER2, in liquid formulations undergoes oxidation when exposed to intense light and elevated temperatures (30 & 40 °C). Met-255 in the heavy chain of the Fc region of the antibody is the primary site of oxidation. Met-431 of the Fc fragment can also be oxidized under extreme conditions. The amount of oxidation was determined by cleaving the Fab and Fc fragments by papain digestion, and the oxidized Fc fragment was detected by hydrophobic interaction chromatography. Oxidation of rhuMAb HER2 was also formulation dependent. The presence of NaCl in the rhuMAb HER2 formulation caused an increase in oxidation at higher temperatures after contact with stainless steel containers or stainless steel components in the filling process. The corrosion of stainless steel by chloride ions at the low pH of the formulation buffer generated iron ions that catalyzed methionine oxidation in rhuMAb HER2. Temperature-induced oxidation of rhuMAb HER2 occurred by the formation of free radicals, and light-induced oxidation of rhuMAb HER2 occurred via singlet oxygen pathway. Antioxidants, such as methionine, sodium thiosulfate, catalase, or platinum, prevented Met oxidation in rhuMAb HER2, presumably as free radicals or oxygen scavengers. The minimum effective levels (molar ratios of protein to antioxidant) required to inhibit temperature-induced oxidation were 1:5 and 1:25 for methionine and thiosulfate, respectively. A thiosulfate adduct of rhuMAb HER2 was observed by cation-exchange chromatography. These studies demonstrate that stoichiometric amounts of methionine and thiosulfate are sufficient to eliminate temperature-induced oxidation of rhuMAb HER2 caused by free radicals that were generated by the presence of metal ions and peroxide impurities in the formulation.

Introduction

Oxidation of methionine is one of the major degradation pathways in many protein pharmaceuticals, including interleukin-2, relaxin, parathyroid hormone, and human growth hormone.[1–7] Methionine residues in proteins are susceptible to oxidation, resulting in the formation of methionine sulfoxide and, under extreme conditions, sulfones. Methionine sulfoxide formation can occur during synthesis, purification, formulation, manufacturing, and storage of protein pharmaceuticals. During formulation and storage, oxidation of methionine-containing proteins can be caused by the presence of certain formulation excipients, including polyethylene glycols and nonionic polyether surfactants; these compounds can undergo autooxidation to form peroxides.[8] In fact, peroxides such as hydrogen peroxide have been widely used for studying the kinetics and mechanisms of methionine oxidation in proteins.[9–11] Peroxides react with metal ions to form free radicals that can initiate oxidation of proteins.[12,13] Methionine can also be photooxidized by a free radical pathway,[14] or via singlet oxygen intermediate formation.[15] For instance, photosensitizers can absorb radiation energy to form excited species that initiate the formation of free radicals for methionine oxidation. The excited species can also react with oxygen to form singlet oxygen, which in turn oxidizes methionine to yield methionine sulfoxide. Photolytic degradation of protein drug products can be influenced by many factors, including the buffer and its concentration, excipients, and formulation pH. Storage conditions, such as radiation intensity and duration and temperature, can also affect the rate of photolytic degradation.

The use of antioxidants in pharmaceutical products is one of the common ways to protect the drugs from oxidative degradation.[16,17] The unknown toxicity of many antioxidants and their incompatibility with proteins and/or excipients in parenteral formulations has hampered their use in protein pharmaceuticals. Nevertheless, several antioxidants have been identified for the prevention of methionine oxidation in recombinant human proteins,[18] including chelating agents, reducing agents, oxygen scavengers, and chain terminators.[19] Chelating agents bind to metal ions that catalyze oxidative reactions. Metal ions can react with peroxide impurities or the protein itself in the formulation to form free radicals that initiate oxidative degradation. Reducing agents reduce an oxidized drug product. For instance, the oxidized form of methionine, methionine sulfoxide, can be reduced to methionine by a reducing agent. Oxygen scavengers are substances that are more susceptible to oxidation than the drugs they are protecting. These substances are reducing agents that can react with oxygen by preferential oxidation, and thus remove the source of oxidation. Chain terminators are substances that can react with free radicals to produce intermediates that terminate the oxidation reactions via free radical pathways.

The overexpression of a 185 kDa glycoprotein ($p185^{HER2}$) has been found in tumor cells of breast and ovarian cancer, and rhuMAb HER2 binds to the extracellular domain of the glycoprotein to inhibit the growth of human breast carcinoma cells.[20,21] In the initial development of a liquid formulation for rhuMAb HER2 used in the treatment of breast cancer patients, methionine sulfoxide formation was detected using *tert*-butylhydroperoxide oxidant.[22] The goals of this study were to investigate the effect of temperature and light on oxidation of rhuMAb HER2 in liquid formulations, to determine the mechanisms of methionine oxidation, and to identify antioxidants for their prevention.

Experimental Section

Materials— rhuMAb HER2, expressed in Chinese hamster ovary cells, was purified by Process Recovery Operations at Genentech, Inc. All chemicals and reagents used were reagent grade. Solvents used for hydrophobic interaction and cation-exchange chromatography (HPLC) assays were filtered before use. *L*-Methionine, sodium thiosulfate, and catalase were purchased from Sigma Chemical Company (St. Louis, MO). Platinum was purchased from Aldrich Chemical Company (Milwaukee, WI). Carboxypetidase B and papain (from Carcia papaya) were obtained from Boehringer Mannheim Biochemicals (Indianapolis, IN).

rhuMAb HER2 Formulations—The thermal stability and photostability studies of rhuMAb HER2 were assessed for the single and multiple dose formulations listed in Table 1. Formulation A was designed for use in single dosing, and Formulations B–D were

[⊗] Abstract published in *Advance ACS Abstracts,* October 1, 1997.

S0022-3549(97)00143-3 CCC: $14.00

Table 1—List of rhuMAb HER2 Liquid Formulations

Formulation	Usage	Protein Concentration, mg/mL	Buffer Excipients
A	Single dose	5	5 mM sodium acetate, pH 5.0 147 mM NaCl 0.01% polysorbate 20
B	Multiple dose	21	5 mM sodium acetate, pH 5.0 4% mannitol 0.01% polysorbate 20 1% benzyl alcohol
C	Multiple dose	21	5 mM sodium acetate, pH 5.0 4% mannitol 0.01% polysorbate 20 1% benzyl alcohol 14.5 mM methionine
D	Multiple dose	21	5 mM sodium acetate, pH 5.0 4% mannitol 0.01% polysorbate 20 0.01% benzethonium chloride 14.5 mM methionine

designed for multiple does and therefore contain preservatives. All formulations were filled under aseptic conditions into 10-mL glass vials with a fill volume of 10 mL.

HPLC Assays—A Hewelett Packard HP-1090L HPLC instrument equipped with a diode array detector and solvent delivery system was used to assess the stability of rhuMAb HER2 by hydrophobic interaction, size exclusion, and cation-exchange chromatography methods.

Hydrophobic interaction HPLC (HIC) was employed to quantify the amount of oxidized Fc in rhuMAb HER2. For separating the Fc domain from the Fab fragment of the monoclonal antibody, rhuMAb HER2 samples were first digested with carboxypeptidase B at a concentration of 1:100 (w/w; carboxypeptidase B: rhuMAb HER2) at 37 °C for 20 min, followed by papain digestion at a concentration of 1:200 (w/w; papain: rhuMAb HER2) at 37 °C for 2 h. Digested samples were injected directly onto a TSK Butyl-NPR column (4.6 × 35 mm, Tosohaas) and eluted at 0.5 mL/min with a mobile phase consisting of buffer A (20 mM Tris at pH 7.0) and buffer B (2 M ammonium sulfate in buffer A). A linear gradient from 10 to 100% buffer A was performed over 37 min at ambient temperature. Peak detection was performed at 214 nm.

Native size exclusion chromatography (SEC) was used to determine the amount of soluble aggregates and monomer present in the rhuMAb HER2 formulations. Samples were eluted isocratically at ambient temperature using a TSK G3000SWXL column (7.8 × 300 mm, Tosohaas) at 1 mL/min with phosphate buffered saline (PBS) at pH 7.2 as mobile phase. The runtime was 20 min, and peaks were detected at 280 nm.

Ion exchange HPLC (IEC) was employed with a Bakerbond WP Carboxy Sulfon column (4.6 × 250 mm, J. T. Baker) to characterize deamidation of rhuMAb HER2 in the formulations. Samples were eluted at 1 mL/min with mobile phase consisting of buffer A (20 mM sodium phosphate at pH 6.9) and buffer B (0.2 M sodium chloride in buffer A). A linear gradient from 10 to 45% buffer B was performed over 55 min. The column was maintained at 40 °C, and peaks were detected at 214 nm.

Extracellular Domain (ECD) Plate Binding Assay—This assay assessed the ability of rhuMAb HER2 to bind to the ECD of the $p185^{HER2}$ glycoprotein. rhuMAb HER2 samples were diluted into assay buffer to concentrations within the linear range (10–70 ng/mL) of the standard curve for the assay. Diluted samples were incubated with recombinant $p185^{HER2}$ ECD protein in 96 wells of a microtiter plate for 1 h at ambient temperature. After incubation, the wells were washed with assay buffer, and a HRP-conjugated anti-human Fc goat antibody was added. After additional washing, a substrate of *o*-phenylenediamine was added for a 10 min incubation at ambient temperature in the dark. Absorbances at 490–492 nm were read, and sample concentrations were determined from a standard curve using a four-parameter logistic curve fitting program.

Bioassay—This assay quantitated the bioactivity of rhuMAb HER2 by measuring the antiproliferative effects of the antibody on BT-474 cells derived from human breast ductal carcinoma. rhuMAb HER2 samples were diluted into assay buffer to a concentration of 0.25 μg/mL. Diluted samples (100 μL) were incubated with BT-474 cells in a 96-well tissue culture plate at 37 °C for 96 h. After incubation, medium was removed and the plate was stained with Crystal Violet stain. Bioactivity of rhuMAb HER2 was quantitated by measuring the absorbance at 540 nm. Sample concentrations were determined from a four-parameter fit equation generated from the standard curve data. The range for sample quantitation was 20% from the low and high asymptote of the curve generated.

Thermal Stability Studies—The effect of temperature on oxidation of rhuMAb HER2 was studied by incubating samples of Formulation A (single dose) and Formulation B (multiple dose) at 5, 30, and 40 °C for 2 weeks. At each timepoint, samples were analyzed for oxidation by HIC, aggregation by SEC, deamidation by IEC, and activity by ECD plate binding assay and bioassay.

Photostability Studies—Two vials of each liquid formulation (A–D) were stored in a light box (Forma Scientific, model 3890) under high intensity fluorescent light maintained at 20 000 lux, which is ~15–20 times that of indoor fluorescent light. Another two vials wrapped with aluminum foil were also stored in the light box as controls for the same period of time. The temperature of the light box was at 27 °C. After 2 weeks, samples were assayed by the same analytical methods as those used for the thermal stability studies.

Antioxidant Studies—To study the inhibitory effect of antioxidants on light-induced oxidation of rhuMAb HER2, methionine and sodium thiosulfate were added to formulation A at final concentrations of 3.5 mM (0.05% w/v) and 6.3 mM (0.1% w/v), respectively. The antioxidant-containing formulations were filtered into 10-mL glass vials with a fill volume of 10 mL, and two vials from each formulation were kept in the light box. As controls, two other vials were shielded from light by wrapping with aluminum foil and stored in the same light box. At 2 weeks, samples were analyzed for oxidation by the HIC assay.

The inhibitory effect of antioxidants on temperature-induced oxidation of rhuMAb HER2 was evaluated by adding 3.5 mM methionine (0.05%), 6.3 mM sodium thiosulfate (0.1%), catalase (0.002%), and platinum (0.005%) to Formulation A before filling into 10-mL glass vials. Samples were stored at 40 °C for 2 weeks and then assayed by the HIC method. The effect of molecular oxygen on oxidation of rhuMAb HER2 was also examined by replacing headspace oxygen from two 10-mL filled vials of Formulation A with nitrogen before they were stored at 40 °C for 2 weeks. Samples were analyzed by the HIC assay.

To determine the minimum effective levels required to inhibit rhuMAb HER2 oxidation, methionine and sodium thiosulfate at molar ratios between 1:1 and 1:180 (protein: antioxidant) were added to Formulation A. Antioxidant-containing rhuMAb HER2 material was then filled into 10-mL glass vials. Samples were stored at 40 °C for 2 weeks, and then assayed for oxidation by the HIC method.

Results and Discussion

Thermal Stability of rhuMAb HER2—The effect of temperature on oxidation of rhuMAb HER2 was studied in Formulation A (single dose) and Formulation B (multiple dose) at 5, 30, and 40 °C. As shown in Figure 1, the increase in oxidation of rhuMAb HER2 was temperature and formulation dependent. After incubation for 2 weeks, the rhuMAb HER2 in Formulation A (5 mg/mL protein in 5 mM sodium acetate, 147 mM NaCl, 0.01% polysorbate 20, pH 5.0) had 10, 17, and 52% oxidized Fc at 5, 30, and 40 °C, respectively. The percentage of oxidized Fc analyzed by HIC at each timepoint was defined as the sum of the peak areas of the two oxidized peaks (1 & 2) divided by the total peak areas of the Fc peaks (Figure 2). Previous studies demonstrated that peak 1 contained oxidized Met-255 and peak 2 consisted of rhuMAb HER2 oxidized at both Met-255 and Met-431 in the Fc.[22] Although these two methionine residues located on the surface

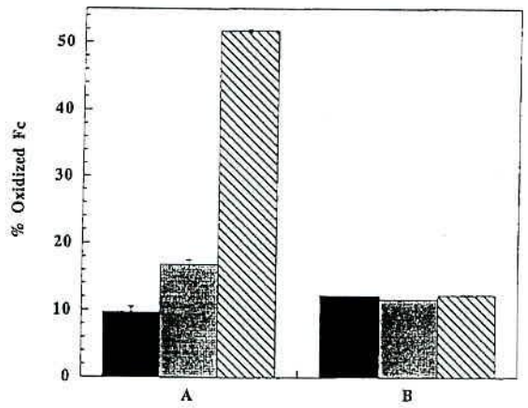


Figure 1—Effect of temperature on oxidation of rhuMAb HER2 in Formulations A and B. Samples were analyzed for methionine oxidation by HIC after 2 weeks of incubation at 5 °C (black), 30 °C (gray), and 40 °C (striped).

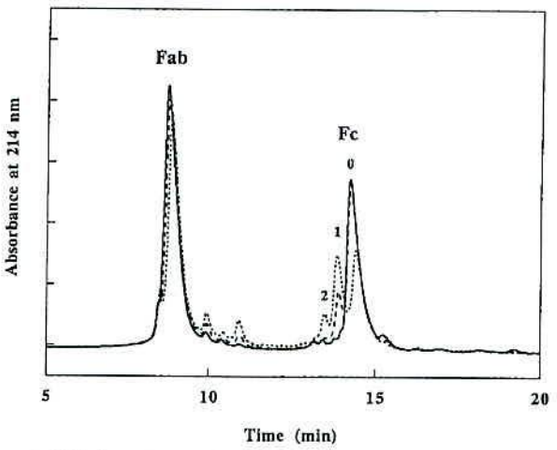


Figure 2—HIC chromatograms showing the effect of temperature on oxidation of the two methionine residues on the Fc domain of rhuMAb HER2 (Formulation A). Samples were incubated at 5 °C (solid line), 30 °C (dashed line), and 40 °C (dotted line) for 2 weeks. The three main peaks of the rhuMAb HER2 Fc domain are non-oxidized Fc (peak 0), Fc fragment with oxidized Met-255 (peak 1), and Fc fragment with oxidized Met-255 and Met-431 (peak 2). After incubation, the 5, 30, and 40 °C samples contained 10, 17, and 52% oxidized Fc (peaks 1 & 2), respectively.

of the antibody are more susceptible to oxidation than other methionine sites that are buried in the variable domains, they are neither within the Fcγ receptor region nor the complement C1q epitopes in the Fc of the heavy chain. In Formulation A, oxidation of these two methionine residues increased with temperature. In contrast, Formulation B (multiple dose), which consisted of different excipients, did not exhibit an increase in methionine oxidation at high temperatures (30 & 40 °C) after 2 weeks of incubation. In addition, when the surfactant (polysorbate 20) was not added in Formulaiton A, <10% oxidized Fc was detected in the samples that were strored at 30–40 °C. These results indicate that oxidation of rhuMAb HER2 was also formulation dependent.

The difference in the degree of oxidation of rhuMAb HER2 in Formulations A and B at high temperatures was also process related. The NaCl-containing rhuMAb HER2 Formulation A had 52% oxidized Fc at 40 °C for 2 weeks when it was filled into 10-mL glass vials by a stainless steel filler, as compared with 18% oxidized Fc when filled by a nonstainless steel filler. The replacement of NaCl by mannitol as a tonicifier yielded Formulation B that did not induce rhuMAb HER2 oxidation at high temperatures, even though it was filled by the same stainless steel filler. These results reveal that the excipient, NaCl, in Formulation A played an important role in rhuMAb HER2 oxidation. One hypothesis for the role of salt in the oxidation of rhuMAb HER2 was the corrosion of the stainless steel components of the filler by chloride ions at low pH, generating metal ions such as iron that catalyzed oxidation. When rhuMAb HER2 in Formulation A was tested for metal ions by induced coupled plasma (ICP) spectrophotometry, the amount of iron increased from <0.1 ppm in the control sample (glass vial) to 3.1 ppm in a sample stored at 5 °C for 3 months in a 30-mL stainless steel container (Type 316L, Fluid Line Technology). Furthermore, when the NaCl-containing rhuMAb HER2 Formulation A was prepared by diluting its concentrated bulk with a formulation buffer that had been made in a stainless steel tank, the protein had a 26% increase in oxidation after 2 weeks at 40 °C. No increase in oxidation was observed for the formulation buffer prepared in glass container. These results further reveal that the presence of NaCl in a formulation and contact with stainless steel both contributed to the increase in rhuMAb HER2 oxidation, probably through the generation of metal ions.

To further confirm that the formation of iron ions as a result of corrosion of stainless steel by the salt was the major cause for the oxidation of rhuMAb HER2 at high temperatures, a metal chelating agent (EDTA) was added to the sample vials filled by stainless steel filler prior to incubation. After incubation at 40 °C for 2 weeks, samples containing either 0.02 or 0.05 mM EDTA did not show an increase in methionine oxidation, as compared with a 42% increase in the control sample without the chelating agent. This result suggests that the formation of iron ions in the rhuMAb HER2 Formulation A due to the contact with stainless steel was responsible for the oxidation of the protein at high temperatures.

The oxidation of methionine residues in peptides and proteins is often initiated through the generation of reactive oxygen species,[23] and the rate and amount of methionine sulfoxide formation are affected by exogenous factors such as metal ions.[24] Although many oxidative pathways are possible for methionine, the mechanism for the temperature-induced oxidation of methionine residues of rhuMAb HER2 in the NaCl-containing Formulation A may involve metal-ion-catalyzed free radical formation. The formulation contained polysorbate 20, a nonionic polyether surfactant that can undergo autooxidation upon storage to form alkyl hydroperoxides. These peroxide impurities can be further decomposed by the presence of heavy metal ions, such as iron formed by the contact between stainless steel, and chloride ion at low pH to initiate alkoxyl free radical (RO•). This alkoxyl free radical can then react with methionine residues of rhuMAb HER2 to form a positively charged methionine free radical that can further react with the hydroxide ion to generate hydroxyl methionine free radical in the propagation phase. The alkoxyl free radical can also react with molecular oxygen to generate hydroxyl radical ($HO_2^{\bullet}$), which can be terminated by reacting with the hydroxyl methionine free radical to form methionine sulfoxide, as shown in the following proposed mechanism:

Initiation
$$ROOH + Fe^{2+} \rightarrow RO^{\bullet} + Fe^{3+} + OH^{-} \quad (1)$$

Propagation
$$RO^{\bullet} + R'\text{-}S\text{-}CH_3 \rightarrow R'\text{-}S^{\bullet}_{+}\text{-}CH_3 + RO^{-}$$
$$R'\text{-}S^{\bullet}_{+}\text{-}CH_3 + OH^{-} \rightarrow R\text{-}S^{\bullet}(OH)\text{-}CH_3 \quad (2)$$
$$RO^{\bullet} + O_2 + H_2O \rightarrow HO_2^{\bullet} + ROOH$$

Termination
$$R\text{-}S^{\bullet}(OH)\text{-}CH_3 + HO_2^{\bullet} \rightarrow R\text{-}S(O)\text{-}CH_3 + H_2O_2 \quad (3)$$

Table 2—Effect of Temperature on Stability of rhuMAb HER2 Liquid Formulations

Temp (°C)	Days	Formulation	% Monomer (SEC)	% Deamidation (IEC)	% Specific Activity (ECD Assay)	% Specific Activity (Bioasay)
5	0	A	100	9.4	104.6 ± 2.4	94.9 ± 10.6
		B	100	12.0	94.8 ± 2.1	113.0 ± 18.1
30	14	A	99.4	8.6	98.0 ± 3.1	93.5 ± 6.1
		B	99.4	8.3	107.7 ± 3.2	104.0 ± 4.5
40	14	A	99.0	8.4	91.9 ± 3.9	96.8 ± 3.0
		B	99.6	6.7	98.5 ± 0.1	97.5 ± 8.3

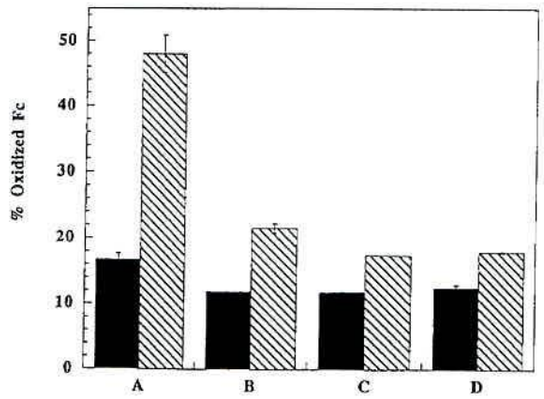


Figure 3—Effect of light on oxidation of rhuMAb HER2 in various formulations. Wrapped (black) and unwrapped (striped) samples were stored in a ligth box with light intensity of 20 000 lux for 2 weeks. The light box temperature was 27 °C. After light exposure, samples were analyzed for methionine oxidation by HIC.

The polysorbate 20 was necessary to prevent agitation-induced aggregation of the protein, so it could not be excluded so as to eliminate oxidation by the pathways described in eqs 1–3. Although temperature and excipient in the formulation could cause methionine oxidation of rhuMAb HER2, they had no significant effect on aggregation, deamidation, and biological activity of the protein (Table 2). In fact, rhuMAb HER2 in Formulation A, which contained 52% oxidized Fc, still retained >90% of its binding activity and bioactivity, as determined by the plate binding assay and bioassay, respectively. This result is probably due to the fact that the potential oxidation sites are neither within the complementarity-determining regions (CDRs) nor the Fc receptor binding sites of the antibody. The major concern about methionine oxidation in rhuMAb HER2 is related to regulatory issue. To fulfill the FDA requirement for a stable formulation, one must demonstrate that the pharmaceutical product is not >10% degraded and that the degradation products do not have any adverse effects on the safety and efficacy of the drug.

Photostability Studies—To study an alternative oxidation pathway for rhuMAb HER2, the effect of light on methionine oxidation in rhuMAb HER2 was assessed in various formulations. The results are shown in Figure 3. After 2 weeks of storage in the light box, the unwrapped vials of rhuMAb HER2 in formulations B, C, and D all had a slight increase in oxidized Fc (17–22%) as compared with ~12% in their wrapped control vials. However, the amount of oxidation induced by light in these formulations was still much less than that observed in Formulation A (48% oxidized Fc in the unwrapped vials and 16.5% in the wrapped control vials). These results suggest that rhuMAb HER2 was susceptible to photooxidation in all formulations tested, and that the greater extent of methionine oxidation in Formulation A was probably due to the combined effects of light and the increase in temperature of the light box (27 °C).

Photolytic oxidation of rhuMAb HER2 can occur when the protein itself absorbs energy from the radiation source (fluorescence light in the light box). This absorbed energy can be dissipated in the form of thermal energy, which produces an increase in temperature for inducing methionine oxidation of rhuMAb HER2. This sequence may also be an explanation for the greater increase in methionine oxidation in Formulation A than in Formulations B, C, and D after light exposure, because Formulation A was shown to be temperature sensitive (Figure 1). Another mechanism for photolytic degradation of rhuMAb HER2 in these formulations may involve the formation of singlet oxygen, which has shown to be one of the photooxidation pathways for methionine-containing compounds.[15] The formation of singlet oxygen was probably due to the presence of polysorbate 20, a photosensitive agent, in the rhuMAb HER2 formulations. After the absorption of radiation energy from the light box, polysorbate may dissipate its energy by reacting with molecular oxygen to generate singlet oxygen. This singlet oxygen can react with methionine to form an intermediate that oxidizes a second methionine molecule to form sulfoxide, as shown in the following equations:

$$\text{Polysorbate} \xrightarrow{h\nu} \text{Polysorbate}^* \quad (4)$$

$$\text{Polysorbate}^* + O_2 \rightarrow \text{Polysorbate} + {}^1O_2 \quad (5)$$

$$\text{R-S-CH}_3 + {}^1O_2 \rightarrow \text{R-S(OO)-CH}_3 \quad (6)$$

$$\text{R-S(OO)-CH}_3 + \text{R-S-CH}_3 \rightarrow \text{R-S(O)-CH}_3 + \text{R-S(O)-CH}_3 \quad (7)$$

The photo-induced oxidation of rhuMAb HER2 was prevented by the presence of methionine in the formulation. As shown in Figure 3, Formulation B (21 mg/mL rhuMAb HER2 in 5 mM sodium acetate, pH 5.0, 4% mannitol, 0.01% polysorbate 20, and 1% benzyl alcohol) contained 22% oxidized Fc after 2 weeks of light exposure as compared with 17% in Formulation C, which consisted of the excipients used in Formulation B plus 14.5 mM methionine. Formulation D, which contained 0.01% benzethonium chloride as preservative, photo-oxidized at a similar rate as Formulation C, which contained 1% benzyl alcohol. Thus, the preservative did not affect the rate or extent of oxidation. These results suggest that the presence of a reducing agent such as methionine can act as an antioxidant to reduce photooxidation of methionine in the protein. In fact, methionine has been shown to be effective in reducing methionine sulfoxide formation in peroxide-mediated oxidation of recombinant human ciliary neurotrophic factor and nerve growth factor.[18]

In summary, methionine oxidation was the major degradation pathway of rhuMAb HER2 caused by light. The oxidized rhuMAb HER2 (light-induced) appeared to retain its full binding activity and bioactivity in the plate binding assay and bioassay, respectively. Light had no significant effect on

Table 3—Effect of Light on Stability of rhuMAb HER2 Liquid Formulations

Condition	Days	Formulation	% Monomer (SEC)	% Deamidation (IEC)	% Specific Activity (ECD Assay)	% Specific Activity (Bioasay)
Dark	14	A	99.4	8.3	118.0 ± 6.8	NT[a]
		B	100	9.4	93.1 ± 9.5	104.7 ± 4.5
		C	100	9.8	88.7 ± 5.4	106.4 ± 4.7
		D	100	9.6	90.1 ± 0.9	111.7 ± 5.8
Light	14	A	98.6	8.0	113.7 ± 5.2	NT
		B	100	8.2	95.0 ± 7.6	93.2± 6.3
		C	100	8.2	100.6 ± 1.4	98.8 ± 4.6
		D	100	8.2	98.6 ± 6.9	95.0 ± 0.7

[a] NT = not tested.

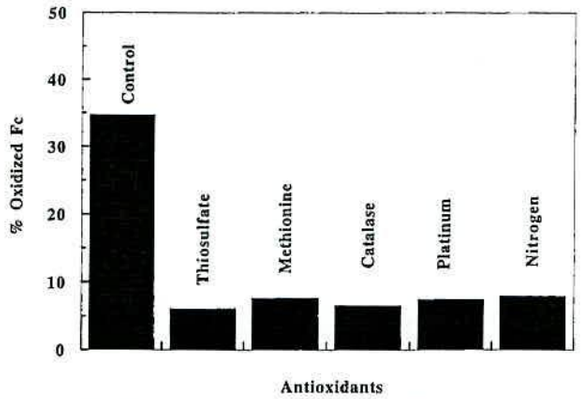


Figure 4—Methods to prevent temperature-induced oxidation of rhuMAb HER2 in Formulation A. Antioxidants were added to the formulation before filling into sample vials for incubation at 40 °C for 2 weeks. After incubation, samples were assayed by HIC to assess methionine oxidation. The amount of oxidation was compared with a control sample containing no antioxidant stored under the same conditions.

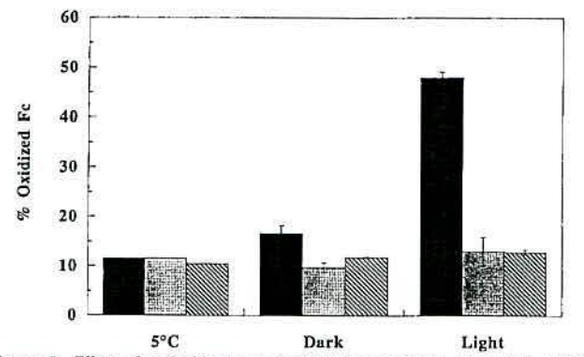
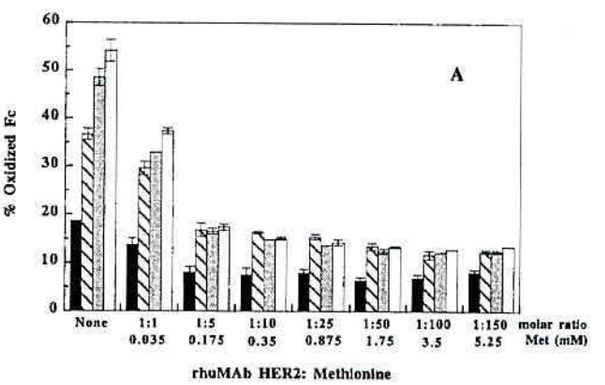


Figure 5—Effect of antioxidants on light-induced oxidation of rhuMAb HER2 Formulation A. Antioxidants were added to the formulation before filling into sample vials. Sample containing no antioxidant (black), 6.3 mM sodium thiosulfate (gray), and 3.5 mM methionine (striped) were stored wrapped (Dark) and unwrapped (Light) in a light box with light intensity of 20 000 lux for 2 weeks. The light box temperature was 27 °C. After light exposure, samples were assessed for methionine oxidation of rhuMAb HER2 by HIC. Results were also compared with the control samples stored in the dark at 5 °C for 2 week.

aggregate formation and deamidation of the protein as determined by native SEC and IEC (Table 3).

Mechanisms for Prevention of Methionine Oxidation by Antioxidants—Although methionine oxidation did not affect the activity of rhuMAb HER2, it may impact its immunogenicity or shelf-life. Potential methods to reduce temperature-induced methionine oxidation of rhuMAb HER2 were investigated to select inhibitors of oxidation and understand the mechanism of oxidation. As shown in Figure 4, rhuMAb HER2 in Formulation A containing added antioxidants, such as methionine, sodium thiosulfate, catalase, or platinum, did not oxidize after 2 weeks at 40 °C. In contrast, the control sample without antioxidant consisted of 52% oxidized Fc under the same storage conditions. Interestingly, removal of oxygen in the sample vials by repeated pulling vacuum and replacing with nitrogen was also effective in reducing rhuMAb HER2 oxidation. These results support the proposed mechanism that temperature-induced methionine oxidation of rhuMAb HER2 in the NaCl-containing Formulation A occurred by free radical formation in the presence of molecular oxygen. Methionine and thiosulfate can either inhibit free radical-induced oxidation by terminating the chain reaction or simply by competing with the methionine residues in rhuMAb HER2 for reaction with the free hydroxyl radicals. The free radical scavengers, catalase and platinum, also prevented methionine oxidation in rhuMAb HER2.



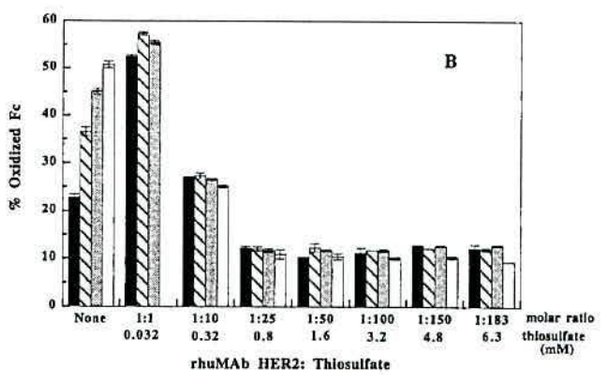


Figure 6—(A) Determination of the minimum effective level of methionine required to prevent oxidation of rhuMAb HER2. (B) Determination of the minimum effective level of sodium thiosulfate required to prevent oxidation of rhuMAb HER2. Methionine or thiosulfate was added to the rhuMAb HER2 Formulation A at different protein-to-antioxidant molar ratios before filling. Samples were stored at 40 °C for 1 week (black), 2 weeks (striped), 3 weeks (gray), and 4 weeks (white) and analyzed for oxidation of rhuMAb HER2 by HIC at the end of each storage period.

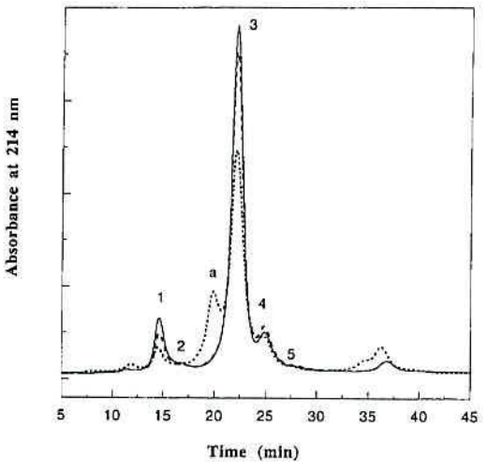


Figure 7—Interaction of sodium thiosulfate with rhuMAb HER2 detected by cation-exchange chromatography (IEC). After storage in a light box for 2 weeks, a degradation species (peak a) was observed in samples containing 6.3 mM thiosulfate (dotted line) as compared with the samples containing 3.5 mM methionine (dashed line) or no antioxidant (solid line). Peaks 1–5 are the five major isoforms of rhuMAb HER2 analyzed by IEC.

The effect of antioxidants on light-induced methionine oxidation in rhuMAb HER2 Formulation A was also studied (Figure 5). Unwrapped sample vials, containing either 6.3 mM sodium thiosulfate or 3.5 mM methionine (concentrations commonly used in parenteral pharmaceuticals), did not show an increase in oxidation when stored in a light box for 2 weeks. In contrast, oxidized Fc was increased to 48% for the unwrapped sample without antioxidants. These results suggest that methionine and sodium thiosulfate can be used for inhibiting photolytic oxidation in addition to temperature-induced oxidation of rhuMAb HER2. These antioxidants may prevent photolytic oxidation of rhuMAb HER2 by competing with methionine residues on the protein to react with molecular oxygen or singlet oxygen.

Different concentrations of methionine and thiosulfate were added to the rhuMAb HER2 Formulation A to determine the minimum effective level required to inhibit protein oxidation. Methionine at a final concentration of 0.175 mM, equivalent to a protein-to-methionine molar ratio of 1:5, was the minimum effective level for reduction of rhuMAb HER2 oxidation caused by temperature (Figure 6a). Thiosulfate at a minimum concentration of 0.8 mM, protein-to-thiosulfate molar ratio of 1:25, was required to inhibit rhuMAb HER2 oxidation in Formulation A (Figure 6b). Unlike methionine, thiosulfate reacted with rhuMAb HER2 as detected by cation-exchange chromatography (Figure 7). At a 1:100 molar ratio of protein-to-thiosulfate, an unknown degradation species (peak a) was observed in the sample. This peak may be an adduct between rhuMAb HER2 and thiosulfate yielding a new charged species. This species was more acidic than the native rhuMAb HER2 and eluted before the main peak of the native protein (peak 3). These data demonstrate that methionine is the preferred antioxidant for preventing rhuMAb HER2 oxidation.

Conclusions—Based on these studies, we conclude that temperature and light induce methionine oxidation of rhuMAb HER2. The rate of oxidation increases with temperature and is formulation dependent. The corrosion of stainless steel by sodium chloride present in rhuMAb HER2 liquid formulation to generate iron ions was the major catalyst for the protein oxidation at high temperatures. Temperature-induced oxidation of rhuMAb HER2 may occur by the formation of free radicals, and photo-induced oxidation may occur via singlet oxygen pathway. Methionine oxidation of rhuMAb HER2 either caused by temperature or light can be reduced by adding a stoichiometric amount of methionine or thiosulfate (antioxidants) to the formulation, but thiosulfate reacted with the protein. A protein-to-methionine molar ratio of 1:5 can inhibit rhuMAb HER2 oxidation.

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

Frost GI[1].

Author information

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.

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(54) **DUAL VARIABLE DOMAIN IMMUNOGLOBULIN AND USES THEREOF**

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(57) **ABSTRACT**

The present invention relates to engineered multivalent and multispecific binding proteins, methods of making, and specifically to their uses in the prevention and/or treatment of acute and chronic inflammatory and other diseases.

DUAL VARIABLE DOMAIN IMMUNOGLOBULIN AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of priority to U.S. provisional application No. 60/709,911 filed Aug. 19, 2005, and to U.S. provisional application No. 60/732,892 filed Nov. 2, 2005.

FIELD OF THE INVENTION

[0002] The present invention relates to multivalent and multispecific binding proteins, methods of making, and specifically to their uses in the prevention and/or treatment of acute and chronic inflammatory, cancer, and other diseases.

BACKGROUND OF THE INVENTION

[0003] Engineered proteins, such as multispecific antibodies capable of binding two or more antigens are known in the art. Such multispecific binding proteins can be generated using cell fusion, chemical conjugation, or recombinant DNA techniques.

[0004] Bispecific antibodies have been produced using the quadroma technology (see Milstein, C. and A. C. Cuello, Nature, 1983. 305(5934): p. 537-40) based on the somatic fusion of two different hybridoma cell lines expressing murine monoclonal antibodies with the desired specificities of the bispecific antibody. Because of the random pairing of two different Ig heavy and light chains within the resulting hybrid-hybridoma (or quadroma) cell line, up to ten different immunogloblin species are generated of which only one is the functional bispecific antibody. The presence of mispaired by-products, and significantly reduced production yields, means sophisticated purification procedures are required.

[0005] Bispecific antibodies can be produced by chemical conjugation of two different mAbs (see Staerz, U. D., et al., Nature, 1985. 314(6012): p. 628-31). This approach does not yield homogeneous preparation. Other approaches have used chemical conjugation of two different monoclonal antibodies or smaller antibody fragments (see Brennan, M., et al., Science, 1985. 229(4708): p. 81-3). Another method is the coupling of two parental antibodies with a heterobifunctional crosslinker, but the resulting preparations of bispecific antibodies suffer from significant molecular heterogeneity because reaction of the crosslinker with the parental antibodies is not site-directed. To obtain more homogeneous preparations of bispecific antibodies two different Fab fragments have been chemically crosslinked at their hinge cysteine residues in a site-directed manner (see Glennie, M. J., et al., J Immunol, 1987. 139(7): p. 2367-75). But this method results in Fab'2 fragments, not full IgG molecule.

[0006] A wide variety of other recombinant bispecific antibody formats have been developed in the recent past (see Kriangkum, J., et al., Biomol Eng, 2001. 18(2): p. 3140). Amongst them tandem single-chain Fv molecules and diabodies, and various derivatives there of, are the most widely used formats for the construction of recombinant bispecific antibodies. Routinely, construction of these molecules starts from two single-chain Fv (scFv) fragments that recognize different antigens (see Economides, A. N., et al., Nat Med, 2003. 9(1): p. 47-52). Tandem scFv molecules (taFv) represent a straightforward format simply connecting the two scFv molecules with an additional peptide linker. The two scFv fragments present in these tandem scFv molecules form separate folding entities. Various linkers can be used to connect the two scFv fragments and linkers with a length of up to 63 residues (see Nakanishi, K., et al., Annu Rev Immunol, 2001. 19: p. 423-74). Although the parental scFv fragments can normally be expressed in soluble form in bacteria, it is, however, often observed that tandem scFv molecules form insoluble aggregates in bacteria. Hence, refolding protocols or the use of mammalian expression systems are routinely applied to produce soluble tandem scFv molecules. In a recent study, in vivo expression by transgenic rabbits and cattle of a tandem scFv directed against CD28 and a melanoma-associated proteoglycan was reported (see Gracie, J. A., et al., J Clin Invest, 1999. 104(10): p. 1393-401). In this construct, the two scFv molecules were connected by a CH1 linker and serum concentrations of up to 100 mg/L of the bispecific antibody were found. Various strategies including variations of the domain order or using middle linkers with varying length or flexibility were employed to allow soluble expression in bacteria. A few studies have now reported expression of soluble tandem scFv molecules in bacteria (see Leung, B. P., et al., J Immunol, 2000. 164(12): p. 6495-502; Ito, A., et al., J Immunol, 2003. 170(9): p. 4802-9; Karni, A., et al., J Neuroimmunol, 2002. 125(1-2): p. 134-40) using either a very short Ala3 linker or long glycine/serine-rich linkers. In a recent study, phage display of a tandem scFv repertoire containing randomized middle linkers with a length of 3 or 6 residues was employed to enrich for those molecules that are produced in soluble and active form in bacteria. This approach resulted in the isolation of a preferred tandem scFv molecule with a 6 amino acid residue linker (see Arndt, M. and J. Krauss, Methods Mol Biol, 2003. 207: p. 305-21). It is unclear whether this linker sequence represents a general solution to the soluble expression of tandem scFv molecules. Nevertheless, this study demonstrated that phage display of tandem scFv molecules in combination with directed mutagenesis is a powerful tool to enrich for these molecules, which can be expressed in bacteria in an active form.

[0007] Bispecific diabodies (Db) utilize the diabody format for expression. Diabodies are produced from scFv fragments by reducing the length of the linker connecting the VH and VL domain to approximately 5 residues (see Peipp, M. and T. Valerius, Biochem Soc Trans, 2002. 30(4): p. 507-11). This reduction of linker size facilitates dimerization of two polypeptide chains by crossover pairing of the VH and VL domains. Bispecific diabodies are produced by expressing, two polypeptide chains with, either the structure VHA-VLB and VHB-VLA (VH-VL configuration), or VLA-VHB and VLB-VHA (VL-VH configuration) within the same cell. A large variety of different bispecific diabodies have been produced in the past and most of them cab be expressed in soluble form in bacteria. However, a recent comparative study demonstrates that the orientation of the variable domains can influence expression and formation of active binding sites (see Mack, M., G. Riethmuller, and P. Kufer, Proc Natl Acad Sci USA, 1995. 92(15): p. 7021-5). Nevertheless, soluble expression in bacteria represents an important advantage over tandem scFv molecules. However, since two different polypeptide chains are expressed within

1a), CCL4 (MIP-1b), CCL5 (RANTES), CCL7 (MCP-3), CCL8 (mcp-2), CCL11 (eotaxin), CCL13 (MCP-4), CCL15 (MIP-1d), CCL16 (HCC-4), CCL17 (TARC), CCL18 (PARC), CCL19 (MIP-3b), CCL20 (MIP-3a), CCL21 (SLC/exodus-2), CCL22 (MDC/STC-1), CCL23 (MPIF-1), CCL24 (MPIF-2/eotaxin-2), CCL25 (TECK), CCL26 (eotaxin-3), CCL27 (CTACK/ILC), CCL28, CXCL1(GRO1), CXCL2 (GRO2), CXCL3 (GRO3), CXCL5 (ENA-78), CXCL6 (GCP-2), CXCL9 (MIG), CXCL10 (IP 10), CXCL11 (I-TAC), CXCL12 (SDF1), CXCL13, CXCL14, CXCL16, PF4 (CXCL4), PPBP (CXCL7), CX3CL1 (SCYD1), SCYE1, XCL1 (lymphotactin), XCL2 (SCM-1b), BLR1 (MDR15), CCBP2 (D6/JAB61), CCR1 (CKR1/HM145), CCR2 (mcp-1RB/RA), CCR3 (CKR3/CMKBR3), CCR4, CCR5 (CMKBR5/ChemR13), CCR6 (CMKBR6/CKR-L3/STRL22/DRY6), CCR7 (CKR7/EBI1), CCR8 (CMKBR8/TER1/CKR-L1), CCR9 (GPR-9-6), CCRL1 (VSHK1), CCRL2 (L-CCR), XCR1 (GPR5/CCXCR1), CMKLR1, CMKOR1 (RDC1), CX3CR1 (V28), CXCR4, GPR2 (CCR10), GPR31, GPR81 (FKSG80), CXCR3 (GPR9/CKR-L2), CXCR6 (TYMSTR/STRL33/Bonzo), HM74, IL8RA (IL8Ra), IL8RB (IL8Rb), LTB4R (GPR16), TCP10, CKLFSF2, CKLFSF3, CKLFSF4, CKLFSF5, CKLFSF6, CKLFSF7, CKLFSF8, BDNF, C5R1, CSF3, GRCC10 (C10), EPO, FY (DARC), GDF5, HIF1A, IL8, PRL, RGS3, RGS13, SDF2, SLIT2, TLR2, TLR4, TREM1, TREM2, and VHL. The binding protein of the invention is capable of binding cell surface protein selected from the group consisting of integrins. The binding protein of the invention is capable of binding enzyme selected from the group consisting of kinases and proteases. The binding protein of the invention is capable of binding receptor selected from the group consisting of lymphokine receptor, monokine receptor, and polypeptide hormone receptor.

[0023] In a preferred embodiment the binding protein is multivalent. More preferably the binding protein is multispecific. The multivalent and or multispecific binding proteins described above have desirable properties particularly from a therapeutic standpoint. For instance, the multivalent and or multispecific binding protein may (1) be internalized (and/or catabolized) faster than a bivalent antibody by a cell expressing an antigen to which the antibodies bind; (2) be an agonist antibody; and/or (3) induce cell death and/or apoptosis of a cell expressing an antigen which the multivalent antibody is capable of binding to. The "parent antibody" which provides at least one antigen binding specificity of the multivalent and or multispecific binding proteins may be one which is internalized (and/or catabolized) by a cell expressing an antigen to which the antibody binds; and/or may be an agonist, cell death-inducing, and/or apoptosis-inducing antibody, and the multivalent and or multispecific binding protein as described herein may display improvement(s) in one or more of these properties. Moreover, the parent antibody may lack any one or more of these properties, but may be endowed with them when constructed as a multivalent binding protein as hereindescribed.

[0024] In another embodiment the binding protein of the invention has an on rate constant (Kon) to one or more targets selected from the group consisting of: at least about $10^2M^{-1}s^{-1}$; at least about $10^3M^{-1}s^{-1}$; at least about $10^4M^{-1}s^{-1}$; at least about $10^5M^{-1}s^{-1}$; and at least about $10^6M^{-1}s^{-1}$, as measured by surface plasmon resonance. Preferably, the binding protein of the invention has an on rate constant (Kon) to one or more targets between $10^2M^{-1}s^{-1}$ to $10^3M^{-1}s^{-1}$; between $10^3M^{-1}s^{-1}$ to $10^4M^{-1}s^{-1}$; between $10^4M^{-1}s^{-1}$ to $10^5M^{-1}s^{-1}$; or between $10^5M^{-1}s^{-1}$ to $10^6M^{-1}s^{-1}$, as measured by surface plasmon resonance.

[0025] In another embodiment the binding protein has an off rate constant (Koff) for one or more targets selected from the group consisting of: at most about $10^{-3}s^{-1}$; at most about $10^{-4}s^{-1}$; at most about $10^{-5}s^{-1}$; and at most about $10^{-6}s^{-1}$, as measured by surface plasmon resonance. Preferably, the binding protein of the invention has an off rate constant (Koff) to one or more targets of $10^{-3}s^{-1}$ to $10^{-4}s^{-1}$; of $10^{-4}s^{-1}$ to $10^{-5}s^{-1}$; or of $10^{-5}s^{-1}$ to $10^{-6}s^{-1}$, as measured by surface plasmon resonance.

[0026] In another embodiment the binding protein has a dissociation constant (K_D) to one or more targets selected from the group consisting of: at most about 10^{-7} M; at most about 10^{-8} M; at most about 10^{-9} M; at most about 10^{-10} M; at most about 10^{-11} M; at most about 10^{-12} M; and at most 10^{-13} M. Preferably, the binding protein of the invention has a dissociation constant (K_D) to IL-12 or IL-23 of 10^{-7} M to 10^{-8} M; of 10^{-8} M to 10^{-9} M; of 10^{-9} M to 10^{-10} M; of 10^{-10} to 10^{-11} M; of 11 M to 10^{-12} M; or of 10^{-12} to M 10^{-13} M.

[0027] In another embodiment the binding protein described above is a conjugate further comprising an agent selected from the group consisting of; an immunoadhension molecule, an imaging agent, a therapeutic agent, and a cytotoxic agent. Preferably the imaging agent is selected from the group consisting of a radiolabel, an enzyme, a fluorescent label, a luminescent label, a bioluminescent label, a magnetic label, and biotin. More preferably the imaging agent is a radiolabel selected from the group consisting of: ^{3}II, ^{14}C, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I, ^{177}Lu, ^{166}Ho, and ^{153}Sm. Preferably the therapeutic or cytotoxic agent is selected from the group consisting of; an antimetabolite, an alkylating agent, an antibiotic, a growth factor, a cytokine, an anti-angiogenic agent, an anti-mitotic agent, an anthracycline, toxin, and an apoptotic agent.

[0028] In another embodiment the binding protein described above is a crystallized binding protein and exists as a crystal. Preferably the crystal is a carrier-free pharmaceutical controlled release crystal. More preferably the crystallized binding protein has a greater half life in vivo than the soluble counterpart of said binding protein. Most preferably the crystallized binding protein retains biological activity.

[0029] In another embodiment the binding protein described above is glycosylated. Preferably the glycosylation is a human glycosylation pattern.

[0030] One aspect of the invention pertains to an isolated nucleic acid encoding any one of the binding protein disclosed above. A further embodiment provides a vector comprising the isolated nucleic acid disclosed above wherein said vector is selected from the group consisting of pcDNA; pTT (Durocher et al., *Nucleic Acids Research* 2002, Vol 30, No. 2); pTT3 (pTT with additional multiple cloning site; pEFBOS (Mizushima, S. and Nagata, S., (1990) *Nucleic acids Research* Vol 18, No. 17); pBV; pJV; pcDNA3.1 TOPO, pEF6 TOPO and pBJ.

[0031] In another aspect a host cell is transformed with the vector disclosed above. Preferably the host cell is a prokaryotic cell. More preferably the host cell is *E. Coli*. In a related embodiment the host cell is an eukaryotic cell. Preferably

ovascular hypertension, reperfusion injury, restrictive cardiomyopathy, sarcomas, scleroderma, senile chorea, Senile Dementia of Lewy body type, seronegative arthropathies, shock, sickle cell anemia, skin allograft rejection, skin changes syndrome, small bowel transplant rejection, solid tumors, specific arrhythmias, spinal ataxia, spinocerebellar degenerations, streptococcal myositis, structural lesions of the cerebellum, Subacute sclerosing panencephalitis, Syncope, syphilis of the cardiovascular system, systemic anaphalaxis, systemic inflammatory response syndrome, systemic onset juvenile rheumatoid arthritis, T-cell or FAB ALL, Telangiectasia, thromboangitis obliterans, thrombocytopenia, toxicity, transplants, trauma/hemorrhage, type III hypersensitivity reactions, type IV hypersensitivity, unstable angina, uremia, urosepsis, urticaria, valvular heart diseases, varicose veins, vasculitis, venous diseases, venous thrombosis, ventricular fibrillation, viral and fungal infections, vital encephalitis/aseptic meningitis, vital-associated hemaphagocytic syndrome, Wernicke-Korsakoff syndrome, Wilson's disease, xenograft rejection of any organ or tissue.

[0037] In another aspect the invention provides a method of treating a patient suffering from a disorder comprising the step of administering any one of the binding proteins disclosed above before, concurrent, or after the administration of a second agent, as discussed above. In a preferred embodiment the second agent is selected from the group consisting of budenoside, epidermal growth factor, corticosteroids, cyclosporin, sulfasalazine, aminosalicylates, 6-mercaptopurine, azathioprine, metronidazole, lipoxygenase inhibitors, mesalamine, olsalazine, balsalazide, antioxidants, thromboxane inhibitors, IL-1 receptor antagonists, anti-IL-1βmonoclonal antibodies, anti-IL-6 monoclonal antibodies, growth factors, elastase inhibitors, pyridinyl-imidazole compounds, antibodies or agonists of TNF, LT, IL-1, IL-2, IL-6, IL-7, IL-8, IL-12, IL-13, IL-15, IL-16, IL-18, IL-23, EMAP-II, GM-CSF, FGF, and PDGF, antibodies of CD2, CD3, CD4, CD8, CD-19, CD25, CD28, CD30, CD40, CD45, CD69, CD90 or their ligands, methotrexate, cyclosporin, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, ibuprofen, corticosteroids, prednisolone, phosphodiesterase inhibitors, adensosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, IRAK, NIK, IKK, p38, MAP kinase inhibitors, IL-1β converting enzyme inhibitors, TNFαconverting enzyme inhibitors, T-cell signalling inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors, soluble p55 TNF receptor, soluble p75 TNF receptor, sIL-1R1, sIL-1R11, sIL-6R, antiinflammatory cytokines, IL-4, IL-10, IL-11, IL-13 and TGFβ.

[0038] In a preferred embodiment the pharmaceutical compositions disclosed above are administered to the subject by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, and transdermal.

[0039] One aspect of the invention provides at least one anti-idiotype antibody to at least one binding protein of the present invention. The anti-idiotype antibody includes any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule such as, but not limited to, at least one complementarily determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework region, or; any portion thereof, that can be incorporated into a binding protein of the present invention.

[0040] In another embodiment the binding proteins of the invention are capable of binding one or more targets selected from the group consisting of ABCF1; ACVR1; ACVR1B; ACVR2; ACVR2B; ACVRL1; ADORA2A; Aggrecan; AGR2; AICDA; AIF1; AIG1; AKAP1; AKAP2; AMH; AMHR2; ANGPT1; ANGPT2; ANGPTL3; ANGPTL4; ANPEP; APC; APOC1; AR; AZGP1 (zinc-a-glycoprotein); B7.1; B7.2; BAD; BAFF; BAG1; BAI1; BCL2; BCL6; BDNF; BLNK; BLR1 (MDR15); BlyS; BMP1; BMP2; BMP3B (GDF10); BMP4; BMP6; BMP8; BMPR1A; BMPR1B; BMPR2; BPAG1 (plectin); BRCA1; C19orf10 (IL27w); C3; C4A; C5; C5R1; CANT1; CASP1; CASP4; CAV1; CCBP2 (D6/JAB61); CCL1 (1-309); CCL11 (eotaxin); CCL13 (MCP-4); CCL15 (MIP-1d); CCL16 (HCC-4); CCL17 (TARC); CCL18 (PARC); CCL19 (MIP-3b); CCL2 (MCP-1); MCAF; CCL20 (MIP-3a); CCL21 (MIP-2); SLC; exodus-2; CCL22 (MDC/STC-1); CCL23 (MPIF-1); CCL24 (MPIF-2/eotaxin-2); CCL25 (TECK); CCL26 (eotaxin-3); CCL27 (CTACK/ILC); CCL28; CCL3 (MIP-1a); CCL4 (MIP-1b); CCL5 (RANTES); CCL7 (MCP-3); CCL8 (mcp-2); CCNA1; CCNA2; CCND1; CCNE1; CCNE2; CCR1 (CKR1/HM145); CCR2 (mcp-1RB/RA); CCR3 (CKR3/CMKBR3); CCR4; CCR5 (CMKBR5/ChemR13); CCR6 (CMKBR6/CKR-L3/STRL22/DRY6); CCR7 (CKR7/EBI1); CCR8 (CMKBR8/TER1/CKR-L1); CCR9 (GPR-9-6); CCRL1 (VSHK1); CCRL2 (L-CCR); CD164; CD19; CD1C; CD20; CD200; CD-22; CD24; CD28; CD3; CD37; CD38; CD3E; CD3G; CD3Z; CD4; CD40; CD40L; CD44; CD45RB; CD52; CD69; CD72; CD74; CD79A; CD79B; CD8; CD80; CD81; CD83; CD86; CDH1(E-cadherin); CDH10; CDH12; CDH13; CDH18; CDH19; CDH20; CDH5; CDH7; CDH8; CDH9; CDK2; CDK3; CDK4; CDK5; CDK6; CDK7; CDK9; CDKN1A (p21 Wap1/Cip1); CDKN1B (p27Kip1); CDKN1C; CDKN2A (p16INK4a); CDKN2B; CDKN2C; CDKN3; CEBPB; CER1; CHGA; CHGB; Chitinase; CHST10; CKLFSF2; CKLFSF3; CKLFSF4; CKLFSF5; CKLFSF6; CKLFSF7; CKLFSF8; CLDN3; CLDN7 (claudin-7); CLN3; CLU (clusterin); CMKLR1; CMKOR1 (RDC1); CNR1; COL18A1; COL1A1; COL4A3; COL6A1; CR2; CRP; CSF1(M-CSF); CSF2 (GM-CSF); CSF3 (GCSF); CTLA4; CTNNB1 (b-catenin); CTSB (cathepsin B); CX3CL1 (SCYD1); CX3CR1 (V28); CXCL1 (GRO1); CXCL10 (IP-10); CXCL11(I-TAC/IP-9); CXCL12 (SDF1); CXCL13; CXCL14; CXCL16; CXCL2 (GRO2); CXCL3 (GRO3); CXCL5 (ENA-78/LIX); CXCL6 (GCP-2); CXCL9 (MIG); CXCR3 (GPR9/CKR-L2); CXCR4; CXCR6 (TYMSTR/STRL33/Bonzo); CYB5; CYC1; CYSLTR1; DAB21P; DES; DKFZp451J0118; DNCL1; DPP4; E2F1; ECGF1; EDG1; EFNA1; EFNA3; EFNB2; EGF; EGFR; ELAC2; ENG; ENO1; ENO2; ENO3; EPHB4; EPO; ERBB2 (Her-2); EREG; ERK8; ESR1; ESR2; F3 (TF); FADD; FasL; FASN; FCER1A; FCER2; FCGR3A; FGF;

[0166] In one aspect, DVD-Igs capable of binding target pairs such as NgR and RGM A; NogoA and RGM A; MAG and RGM A; OMGp and RGM A; RGM A and RGM B; CSPGs and RGM A; aggrecan, midkine, neurocan, versican, phosphacan, Te38 and TNF-a; Aβ globulomer-specific antibodies combined with antibodies promoting dendrite & axon sprouting are provided. Dendrite pathology is a very early sign of AD and it is known that NOGO A restricts dendrite growth. One can combine such type of ab with any of the SCI-candidate (myelin-proteins) Ab. Other DVD-Ig targets may include any combination of NgR-p75, NgR-Troy, NgR-Nogo66 (Nogo), NgR-Lingo, Lingo-Troy, Lingo-p75, MAG or Omgp. Additionally, targets may also include any mediator, soluble or cell surface, implicated in inhibition of neurite e.g Nogo, Ompg, MAG, RGM A, semaphorins, ephrins, soluble A-b, pro-inflammatory cytokines (e.g. IL-1), chemokines (e.g. MIP 1a), molecules that inhibit nerve regeneration. The efficacy of anti-nogo/anti-RGM A or similar DVD-Ig molecules can be validated in pre-clinical animal models of spinal cord injury. In addition, these DVD-Ig molecules can be constructed and tested for efficacy in the animal models and the best therapeutic DVD-Ig can be selected for testing in human patients. In addition, DVD-Ig molecules can be constructed that target two distinct ligand binding sites on a single receptor e.g. Nogo receptor which binds three ligand Nogo, Ompg, and MAG and RAGE that binds A-b and S100 A. Furthermore, neurite outgrowth inhibitors e.g. nogo and nogo receptor, also play a role in preventing nerve regeneration in immunological diseases like multiple sclerosis. Inhibition of nogo-nogo receptor interaction has been shown to enhance recovery in animal models of multiple sclerosis. Therefore, DVD-Ig molecules that can block the function of one immune mediator eg a cytokine like IL-12 and a neurite outgrowth inhibitor molecule eg nogo or RGM may offer faster and greater efficacy than blocking either an immune or an neurite outgrowth inhibitor molecule alone.

8. Oncological Disorders

[0167] Monoclonal antibody therapy has emerged as an important therapeutic modality for cancer (von Mehren M, et al 2003 Monoclonal antibody therapy for cancer. Annu Rev Med.; 54:343-69). Antibodies may exert antitumor effects by inducing apoptosis, redirected cytotoxicity, interfering with ligand-receptor interactions, or preventing the expression of proteins that are critical to the neoplastic phenotype. In addition, antibodies can target components of the tumor microenvironment, perturbing vital structures such as the formation of tumor-associated vasculature. Antibodies can also target receptors whose ligands are growth factors, such as the epidermal growth factor receptor. The antibody thus inhibits natural ligands that stimulate cell growth from binding to targeted tumor cells. Alternatively, antibodies may induce an anti-idiotype network, complement-mediated cytotoxicity, or antibody-dependent cellular cytotoxicity (ADCC). The use of dual-specific antibody that targets two separate tumor mediators will likely give additional benefit compared to a mono-specific therapy. DVD Igs capable of binding the following pairs of targets to treat oncological disease are also contemplated: IGF1 and IGF2; IGF1/2 and Erb2B; VEGFR and EGFR; CD20 and CD3, CD138 and CD20, CD38 and CD20, CD38 & CD138, CD40 and CD20, CD138 and CD40, CD38 and CD40. Other target combinations include one or more members of the EGF/erb-2/erb-3 family. Other targets (one or more) involved in oncological diseases that DVD Igs may bind include, but are not limited to those selected from the group consisting of: CD52, CD20, CD19, CD3, CD4, CD8, BMP6, IL12A, IL1A, IL1B, IL2, IL24, INHA, TNF, TNFSF10, BMP6, EGF, FGF1, FGF10, FGF11, FGF12, FGF13, FGF14, FGF16, FGF17, FGF18, FGF19, FGF2, FGF20, FGF21, FGF22, FGF23, FGF3, FGF4, FGF5, FGF6, FGF7, FGF8, FGF9, GRP, IGF1, IGF2, IL12A, IL1A, IL1B, IL2, INHA, TGFA, TGFB1, TGFB2, TGFB3, VEGF, CDK2, EGF, FGF10, FGF18, FGF2, FGF4, FGF7, IGF1, IGF1R, IL2, VEGF, BCL2, CD164, CDKN1A, CDKN1B, CDKN1C, CDKN2A, CDKN2B, CDKN2C, CDKN3, GNRH1, IGFBP6, IL1A, IL1B, ODZ1, PAWR, PLG, TGFB1I1, AR, BRCA1, CDK3, CDK4, CDK5, CDK6, CDK7, CDK9, E2F1, EGFR, ENO1, ERBB2, ESR1, ESR2, IGFBP3, IGFBP6, IL2, INSL4, MYC, NOX5, NR6A1, PAP, PCNA, PRKCQ, PRKD1, PRL, TP53, FGF22, FGF23, FGF9, IGFBP3, IL2, INHA, KLK6, TP53, CHGB, GNRH1, IGF1, IGF2, INHA, INSL3, INSL4, PRL, KLK6, SHBG, NR1D1, NR1H3, NR1I3, NR2F6, NR4A3, ESR1, ESR2, NR0B1, NR0B2, NR1D2, NR1H2, NR1H4, NR1I2, NR2C1, NR2C2, NR2E1, NR2E3, NR2F1, NR2F2, NR3C1, NR3C2, NR4A1, NR4A2, NR5A1, NR5A2, NR6A1, PGR, RARB, FGF1, FGF2, FGF6, KLK3, KRT1, APOC1, BRCA1, CHGA, CHGB, CLU, COL1A1, COL6A1, EGF, ERBB2, ERK8, FGF1, FGF10, FGF11, FGF13, FGF14, FGF16, FGF17, FGF18, FGF2, FGF20, FGF21, FGF22, FGF23, FGF3, FGF4, FGF5, FGF6, FGF7, FGF8, FGF9, GNRH1, IGF1, IGF2, IGFBP3, IGFBP6, IL12A, IL1A, IL1B, IL2, IL24, INHA, INSL3, INSL4, KLK10, KLK12, KLK13, KLK14, KLK15, KLK3, KLK4, KLK5, KLK6, KLK9, MMP2, MMP9, MSMB, NTN4, ODZ1, PAP, PLAU, PRL, PSAP, SERPINA3, SHBG, TGFA, TIMP3, CD44, CDH1, CDH10, CDH19, CDH20, CDH7, CDH9, CDH1, CDH10, CDH13, CDH18, CDH19, CDH20, CDH7, CDH8, CDH9, ROBO2, CD44, ILK, ITGA1, APC, CD164, COL6A1, MTSS1, PAP, TGFB1I1, AGR2, AIG1, AKAP1, AKAP2, CANT1, CAV1, CDH12, CLDN3, CLN3, CYB5, CYC1, DAB2IP, DES, DNCL1, ELAC2, ENO2, ENO3, FASN, FLJ12584, FLJ25530, GAGEB1, GAGEC1, GGT1, GSTP1, HIP1, HUMCYT2A, IL29, K6HF, KAI1, KRT2A, MIB1, PART1, PATE, PCA3, PIAS2, PIK3CG, PPID, PR1, PSCA, SLC2A2, SLC33A1, SLC43A1, STEAP, STEAP2, TPM1, TPM2, TRPC6, ANGPT1, ANGPT2, ANPEP, ECGF1, EREG, FGF1, FGF2, FIGF, FLT1, JAG1, KDR, LAMA5, NRP1, NRP2, PGF, PLXDC1, STAB1, VEGF, VEGFC, ANGPTL3, BAI1, COL4A3, IL8, LAMA5, NRP1, NRP2, STAB1, ANGPTL4, PECAM1, PF4, PROK2, SERPINF1, TNFAIP2, CCL11, CCL2, CXCL1, CXCL10, CXCL3, CXCL5, CXCL6, CXCL9, IFNA1, IFNB1, IFNG, IL1B, IL6, MDK, EDG1, EFNA1, EFNA3, EFNB2, EGF, EPHB4, FGFR3, HGF, IGF1, ITGB3, PDGFA, TEK, TGFA, TGFB1, TGFB2, TGFBR1, CCL2, CDH5, COL18A1, EDG1, ENG, ITGAV, ITGB3, THBS1, THBS2, BAD, BAG1, BCL2, CCNA1, CCNA2, CCND1, CCNE1, CCNE2, CDH1 (E-cadherin), CDKN1B (p27Kip1), CDKN2A (p16INK4a), COL6A1, CTNNB1 (b-catenin), CTSB (cathepsin B), ERBB2 (Her-2), ESR1, ESR2, F3 (TF), FOSL1 (FRA-1), GATA3, GSN (Gelsolin), IGFBP2, IL2RA, IL6, IL6R, IL6ST (glycoprotein 130), ITGA6 (a6 integrin), JUN, KLK5, KRT19, MAP2K7 (c-Jun), MKI67 (Ki-67), NGFB (NGF), NGFR, NME1 (NM23A), PGR, PLAU (uPA), PTEN, SERPINB5 (maspin), SERPINE1 (PAI-1), TGFA, THBS1 (thrombospondin-1), TIE (Tie-1),

TNFRSF6 (Fas), TNFSF6 (FasL), TOP2A (topoisomerase Iia), TP53, AZGP1 (zinc-a-glycoprotein), BPAG1 (plectin), CDKN1A (p21Wap1/Cip1), CLDN7 (claudin-7), CLU (clusterin), ERBB2 (Her-2), FGF1, FLRT1 (fibronectin), GABRP (GABAa), GNAS1, ID2, ITGA6 (a6 integrin), ITGB4 (b 4 integrin), KLF5 (GC Box BP), KRT19 (Keratin 19), KRTHB6 (hair-specific type II keratin), MACMARCKS, MT3 (metallothionectin-III), MUC1 (mucin), PTGS2 (COX-2), RAC2 (p21Rac2), S100A2, SCGB1D2 (lipophilin B), SCGB2A1 (mammaglobin 2), SCGB2A2 (mammaglobin 1), SPRR1B (Spr1), THBS1, THBS2, THBS4, and TNFAIP2 (B94).

IV. Pharmaceutical Composition

[0168] The invention also provides pharmaceutical compositions comprising a binding protein, of the invention and a pharmaceutically acceptable carrier. The pharmaceutical compositions comprising binding proteins of the invention are for use in, but not limited to, diagnosing, detecting, or monitoring a disorder, in preventing, treating, managing, or ameliorating of a disorder or one or more symptoms thereof, and/or in research. In a specific embodiment, a composition comprises one or more binding proteins of the invention. In another embodiment, the pharmaceutical composition comprises one or more binding proteins of the invention and one or more prophylactic or therapeutic agents other than binding proteins of the invention for treating a disorder. Preferably, the prophylactic or therapeutic agents known to be useful for or having been or currently being used in the prevention, treatment, management, or amelioration of a disorder or one or more symptoms thereof. In accordance with these embodiments, the composition may further comprise of a carrier, diluent or excipient.

[0169] The binding proteins of the invention can be incorporated into pharmaceutical compositions suitable for administration to a subject. Typically, the pharmaceutical composition comprises a binding protein of the invention and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Examples of pharmaceutically acceptable carriers include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Pharmaceutically acceptable carriers may further comprise minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the antibody or antibody portion.

[0170] Various delivery systems are known and can be used to administer one or more antibodies of the invention or the combination of one or more antibodies of the invention and a prophylactic agent or therapeutic agent useful for preventing, managing, treating, or ameliorating a disorder or one or more symptoms thereof, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the antibody or antibody fragment, receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of administering a prophylactic or therapeutic agent of the invention include, but are not limited to, parenteral administration (e.g., intradermal, intramuscular, intraperitoneal, intravenous and subcutaneous), epidurala administration, intratumoral administration, and mucosal administration (e.g., intranasal and oral routes). In addition, pulmonary administration can be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. See, e.g., U.S. Pat. Nos. 6,019,968, 5,985,320, 5,985,309, 5,934,272, 5,874,064, 5,855,913, 5,290,540, and 4,880,078; and PCT Publication Nos. WO 92/19244, WO 97/32572, WO 97/44013, WO 98/31346, and WO 99/66903, each of which is incorporated herein by reference their entireties. In one embodiment, a binding protein of the invention, combination therapy, or a composition of the invention is administered using Alkermes AIR® pulmonary drug delivery technology (Alkermes, Inc., Cambridge, Mass.). In a specific embodiment, prophylactic or therapeutic agents of the invention are administered intramuscularly, intravenously, intratumorally, orally, intranasally, pulmonary, or subcutaneously. The prophylactic or therapeutic agents may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local.

[0171] In a specific embodiment, it may be desirable to administer the prophylactic or therapeutic agents of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion, by injection, or by means of an implant, said implant being of a porous or non-porous material, including membranes and matrices, such as silastic membranes, polymers, fibrous matrices (e.g., Tissuel®), or collagen matrices. In one embodiment, an effective amount of one or more antibodies of the invention antagonists is administered locally to the affected area to a subject to prevent, treat, manage, and/or ameliorate a disorder or a symptom thereof. In another embodiment, an effective amount of one or more antibodies of the invention is administered locally to the affected area in combination with an effective amount of one or more therapies (e.g., one or more prophylactic or therapeutic agents) other than a binding protein of the invention of a subject to prevent, treat, manage, and/or ameliorate a disorder or one or more symptoms thereof.

[0172] In another embodiment, the prophylactic or therapeutic agent can be delivered in a controlled release or sustained release system. In one embodiment, a pump may be used to achieve controlled or sustained release (see Langer, supra; Sefton, 1987, CRC Crit. Ref. Biomed. Eng. 14:20; Buchwald et al., 1980, Surgery 88:507; Saudek et al., 1989, N. Engl. J. Med. 321:574). In another embodiment, polymeric materials can be used to achieve controlled or sustained release of the therapies of the invention (see e.g., Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Fla. (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, 1983, J., Macromol. Sci. Rev. Macromol. Chem. 23:61; see also Levy et al., 1985, Science 228:190; During et al., 1989, Ann. Neurol. 25:351; Howard et al., 1989, J. Neurosurg. 7 1:105); U.S. Pat. No. 5,679,377; U.S. Pat. No. 5,916,597; U.S. Pat. No. 5,912,015; U.S. Pat.

No. 5,989,463; U.S. Pat. No. 5,128,326; PCT Publication No. WO 99/15154; and PCT Publication No. WO 99/20253. Examples of polymers used in sustained release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N-vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. In a preferred embodiment, the polymer used in a sustained release formulation is inert, free of leachable impurities, stable on storage, sterile, and biodegradable. In yet another embodiment, a controlled or sustained release system can be placed in proximity of the prophylactic or therapeutic target, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)).

[0173] Controlled release systems are discussed in the review by Langer (1990, Science 249:1527-1533). Any technique known to one of skill in the art can be used to produce sustained release formulations comprising one or more therapeutic agents of the invention. See, e.g., U.S. Pat. No. 4,526,938, PCT publication WO 91/05548, PCT publication WO 96/20698, Ning et al., 1996, "Intratumoral Radioimmunotheraphy of a Human Colon Cancer Xenograft Using a Sustained-Release Gel," Radiotherapy &Oncology 39:179-189, Song et al., 1995, "Antibody Mediated Lung Targeting of Long-Circulating Emulsions," PDA Journal of Pharmaceutical Science &Technology 50:372-397, Cleek et al., 1997, "Biodegradable Polymeric Carriers for a bFGF Antibody for Cardiovascular Application," Pro. Int'l. Symp. Control. Rel. Bioact. Mater. 24:853-854, and Lam et al., 1997, "Microencapsulation of Recombinant Humanized Monoclonal Antibody for Local Delivery," Proc. Int'l. Symp. Control Rel. Bioact. Mater. 24:759-760, each of which is incorporated herein by reference in their entireties.

[0174] In a specific embodiment, where the composition of the invention is a nucleic acid encoding a prophylactic or therapeutic agent, the nucleic acid can be administered in vivo to promote expression of its encoded prophylactic or therapeutic agent, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Pat. No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see, e.g., Joliot et al., 1991, Proc. Natl. Acad. Sci. USA 88:1864-1868). Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression by homologous recombination.

[0175] A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include, but are not limited to, parenteral, e.g., intravenous, intradermal, subcutaneous, oral, intranasal (e.g., inhalation), transdermal (e.g., topical), transmucosal, and rectal administration. In a specific embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous, subcutaneous, intramuscular, oral, intranasal, or topical administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocamne to ease pain at the site of the injection.

[0176] If the compositions of the invention are to be administered topically, the compositions can be formulated in the form of an ointment, cream, transdermal patch, lotion, gel, shampoo, spray, aerosol, solution, emulsion, or other form well-known to one of skill in the art. See, e.g., Remington's Pharmaceutical Sciences and Introduction to Pharmaceutical Dosage Forms, 19th ed., Mack Pub. Co., Easton, Pa. (1995). For non-sprayable topical dosage forms, viscous to semi-solid or solid forms comprising a carrier or one or more excipients compatible with topical application and having a dynamic viscosity preferably greater than water are typically employed. Suitable formulations include, without limitation, solutions, suspensions, emulsions, creams, ointments, powders, liniments, salves, and the like, which are, if desired, sterilized or mixed with auxiliary agents (e.g., preservatives, stabilizers, wetting agents, buffers, or salts) for influencing various properties, such as, for example, osmotic pressure. Other suitable topical dosage forms include sprayable aerosol preparations wherein the active ingredient, preferably in combination with a solid or liquid inert carrier, is packaged in a mixture with a pressurized volatile (e.g., a gaseous propellant, such as freon) or in a squeeze bottle. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well-known in the art.

[0177] If the method of the invention comprises intranasal administration of a composition, the composition can be formulated in an aerosol form, spray, mist or in the form of drops. In particular, prophylactic or therapeutic agents for use according to the present invention can be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant (e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas). In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges (composed of, e.g., gelatin) for use in an inhaler or insulator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch. If the method of the invention comprises oral administration, compositions can be formulated orally in the form of tablets, capsules, cachets, gelcaps, solutions, suspensions, and the like. Tablets or capsules can be prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinised maize starch, polyvinylpyrrolidone, or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose, or calcium hydrogen phosphate) lubricants (e.g., magnesium stearate, talc, or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). The tablets may be coated by methods well-known in the art. Liquid preparations for oral administration may take the form of, but not limited to, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending

agents (e.g., sorbitol syrup, cellulose derivatives, or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol, or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring, and sweetening agents as appropriate. Preparations for oral administration may be suitably formulated for slow release, controlled release, or sustained release of a prophylactic or therapeutic agent(s).

[0178] The method of the invention may comprise pulmonary administration, e.g., by use of an inhaler or nebulizer, of a composition formulated with an aerosolizing agent. See, e.g., U.S. Pat. Nos. 6,019,968, 5,985,320, 5,985,309, 5,934,272, 5,874,064, 5,855,913, 5,290,540, and 4,880,078; and PCT Publication Nos. WO 92/19244, WO 97/32572, WO 97/44013, WO 98/31346, and WO 99/66903, each of which is incorporated herein by reference their entireties. In a specific embodiment, a binding protein of the invention, combination therapy, and/or composition of the invention is administered using Alkermes AIR® pulmonary drug delivery technology (Alkermes, Inc., Cambridge, Mass.).

[0179] The method of the invention may comprise administration of a composition formulated for parenteral administration by injection (e.g., by bolus injection or continuous infusion). Formulations for injection may be presented in unit dosage form (e.g., in ampoules or in multi-dose containers) with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle (e.g., sterile pyrogen-free water) before use.

[0180] The methods of the invention may additionally comprise of administration of compositions formulated as depot preparations. Such long acting formulations may be administered by implantation (e.g., subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compositions may be formulated with suitable polymeric or hydrophobic materials (e.g., as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives (e.g., as a sparingly soluble salt).

[0181] The methods of the invention encompasse administration of compositions formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

[0182] Generally, the ingredients of compositions are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the mode of administration is infusion, composition can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the mode of administration is by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0183] In particular, the invention also provides that one or more of the prophylactic or therapeutic agents, or pharmaceutical compositions of the invention is packaged in a hermetically sealed container such as an ampoule or sachette indicating the quantity of the agent. In one embodiment, one or more of the prophylactic or therapeutic agents, or pharmaceutical compositions of the invention is supplied as a dry sterilized lyophilized powder or water free concentrate in a hermetically sealed container and can be reconstituted (e.g., with water or saline) to the appropriate concentration for administration to a subject. Preferably, one or more of the prophylactic or therapeutic agents or pharmaceutical compositions of the invention is supplied as a dry sterile lyophilized powder in a hermetically sealed container at a unit dosage of at least 5 mg, more preferably at least 10 mg, at least 15 mg, at least 25 mg, at least 35 mg, at least 45 mg, at least 50 mg, at least 75 mg, or at least 100 mg. The lyophilized prophylactic or therapeutic agents or pharmaceutical compositions of the invention should be stored at between 2° C. and 8° C. in its original container and the prophylactic or therapeutic agents, or pharmaceutical compositions of the invention should be administered within 1 week, preferably within 5 days, within 72 hours, within 48 hours, within 24 hours, within 12 hours, within 6 hours, within 5 hours, within 3 hours, or within 1 hour after being reconstituted. In an alternative embodiment, one or more of the prophylactic or therapeutic agents or pharmaceutical compositions of the invention is supplied in liquid form in a hermetically sealed container indicating the quantity and concentration of the agent. Preferably, the liquid form of the administered composition is supplied in a hermetically sealed container at least 0.25 mg/ml, more preferably at least 0.5 mg/ml, at least 1 mg/ml, at least 2.5 mg/ml, at least 5 mg/ml, at least 8 mg/ml, at least 10 mg/ml, at least 15 mg/kg, at least 25 mg/ml, at least 50 mg/ml, at least 75 mg/ml or at least 100 mg/ml. The liquid form should be stored at between 2° C. and 8° C. in its original container.

[0184] The binding proteins of the invention can be incorporated into a pharmaceutical composition suitable for parenteral administration. Preferably, the antibody or antibody-portions will be prepared as an injectable solution containing 0.1-250 mg/ml binding protein. The injectable solution can be composed of either a liquid or lyophilized dosage form in a flint or amber vial, ampule or pre-filled syringe. The buffer can be L-histidine (1-50 mM), optimally 5-10 mM, at pH 5.0 to 7.0 (optimally pH 6.0). Other suitable buffers include but are not limited to, sodium succinate, sodium citrate, sodium phosphate or potassium phosphate. Sodium chloride can be used to modify the toxicity of the solution at a concentration of 0-300 mM (optimally 150 mM for a liquid dosage form). Cryoprotectants can be included for a lyophilized dosage form, principally 0-10% sucrose (optimally 0.5-1.0%). Other suitable cryoprotectants include trehalose and lactose. Bulking agents can be included for a lyophilized dosage form, principally 1-10% mannitol (optimally 2-4%). Stabilizers can be used in both liquid and lyophilized dosage forms, principally 1-50 mM L-Methionine (optimally 5-10 mM). Other suitable bulking agents include glycine, arginine, can be included as 0-0.05% polysorbate-80 (optimally 0.005-0.01%). Additional surfactants include but are not limited to polysorbate 20 and BRIJ surfactants. The pharmaceutical composition comprising the binding proteins of the invention prepared as an injectable solution for parenteral administration, can further comprise

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(54) **PROTEIN FORMULATION**

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(*) Notice: This patent issued on a continued prosecution application filed under 37 CFR 1.53(d), and is subject to the twenty year patent term provisions of 35 U.S.C. 154(a)(2).

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

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(58) **Field of Search** 514/2; 424/138.1, 424/130.1, 141.1, 155.1, 143.1, 145.1; 530/350, 387.7, 387.1, 388.1, 388.24, 388.8; 435/810

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(57) **ABSTRACT**

A stable lyophilized protein formulation is described which can be reconstituted with a suitable diluent to generate a high protein concentration reconstituted formulation which is suitable for subcutaneous administration. For example, anti-IgE and anti-HER2 antibody formulations have been prepared by lyophilizing these antibodies in the presence of a lyoprotectant. The lyophilized mixture thus formed is reconstituted to a high protein concentration without apparent loss of stability of the protein.

47 Claims, 10 Drawing Sheets

TABLE 9

Formulation	Polysorbate 20	Particles per mL ≧10 μm	Particles per mL ≧25 μM
Isotonic Sucrose	None	16,122	28
	0.005%	173	2
	0.01%	224	5
	0.02%	303	6
Hypertonic Sucrose	None	14,220	84
	0.005%	73	6
	0.01%	51	0
	0.02%		6
Isotonic Trehalose	None	33,407	24
	0.005%	569	4
	0.01%	991	16
	0.02%	605	9
Hypertonic Trehalose	None	24,967	28
	0.005%	310	11
	0.01%	209	6
	0.02%	344	6

One formulation developed for the anti-IgE antibody (i.e. 143 mg vial isotonic formulation of rhuMAb E25) which is considered to be useful for subcutaneous delivery of this antibody is shown in Table 10 below. A 10 cc vial is filled with 5.7 mL of rhuMAb E25 at a concentration of 25 mg/mL formulated in 5 mM histidine at pH 6.0 with 0.01% polysorbate 20. Sucrose is added as a lyoprotectant at a concentration of 85 mM which corresponds to a molar ratio of sugar to antibody of 500 to 1. The vial is lyophilized and reconstituted with 0.9% benzyl alcohol to one quarter of the volume of the fill or 1.2 mL. The final concentration of components in the formulation is increased four fold to 100 mg/mL rhuMAb E25 in 20 mM histidine at pH 6 with 0.04% polysorbate 20 and 340 mM sucrose (isotonic) and 0.9% benzyl alcohol. The formulation contains histidine buffer at pH 6 because of its demonstrated protective effect on antibody aggregation. Sucrose was added as the lyoprotectant because of previous use in the pharmaceutical industry. The concentration of sugar was chosen to result in an isotonic formulation upon reconstitution. Finally, polysorbate 20 is added to prevent the formation of insoluble aggregates.

TABLE 10

Pre-lyophilized Formulation (Fill 5.7 mL into 10 cc vial)	Reconstituted Formulation (1.2 mL 0.9% Benzyl Alcohol)
25 mg/mL rhuMAb E25	100 mg/mL rhuMAb E25
5 mM Histidine, pH 6.0	20 mM Histidine, pH 6.0
85 mM Sucrose	340 mM Sucrose
0.01% Polysorbate 20	0.04% Polysorbate 20
—	0.9% Benzyl Alcohol

What is claimed is:

1. A stable isotonic reconstituted formulation comprising an antibody in an amount of about 50 mg/mL to about 400 mg/mL and a diluent, which reconstituted formulation has been prepared from a lyophilized mixture of the antibody and a lyoprotectant which prevents or reduces chemical or physical instability of the antibody upon lyophilization and subsequent storage, wherein the molar ratio of lyoprotectant:antibody is about 100–510 mole lyoprotectant:1 mole of antibody, and wherein the antibody concentration in the reconstituted formulation is about 2–40 times greater than the antibody concentration in the mixture before lyophilization.

2. The formulation of claim 1 wherein the lyoprotectant is sucrose.

3. The formulation of claim 1 wherein the lyoprotectant is trehalose.

4. The formulation of claim 1 which further comprises a buffer.

5. The formulation of claim 4 wherein the buffer is histidine or succinate.

6. The formulation of claim 1 which farther comprises a surfactant.

7. The formulation of claim 1 which is sterile.

8. A stable reconstituted formulation comprising an antibody in an amount of about 50 mg/mL to about 400 mg/mL and a diluent, which reconstituted formulation has been prepared from a lyophilized mixture of an antibody and a lyoprotectant which prevents or reduces chemical or physical instability of the antibody upon lyophilization and subsequent storage, wherein the molar ratio of lyoprotectant:antibody is about 100–510 mole lyoprotectant:1 mole of antibody, and wherein the antibody concentration in the reconstituted formulation is about 2–40 times greater than the antibody concentration in the mixture before lyophilization.

9. The formulation of claim 8 wherein the antibody is an anti-IgE antibody.

10. The formulation of claim 8 wherein the antibody is an anti-HER2 antibody.

11. The formulation of claim 8 wherein the antibody is a full length humanized antibody.

12. The formulation of claim 8 which is isotonic.

13. A method for preparing a stable isotonic reconstituted formulation comprising reconstituting a lyophilized mixture of an antibody and a lyoprotectant which prevents or reduces chemical or physical instability of the antibody upon lyophilization and subsequent storage, wherein the molar ratio of lyoprotectant:antibody is about 100–510 mole lyoprotectant:1 mole of antibody in a diluent such that the antibody concentration in the reconstituted formulation is at least 50 mg/mL, wherein the antibody concentration in the reconstituted formulation is about 2–40 times greater than the antibody concentration in the mixture before lyophilization.

14. The method of claim 13 wherein the lyoprotectant is sucrose.

15. The method of claim 13 wherein the lyoprotectant is trehalose.

16. The method of claim 13 wherein the lyophilized mixture further comprises a bulking agent which is added in an amount which adds mass to the lyophilized mixture and contributes to the physical structure of the lyophilized cake.

17. A method for preparing a formulation comprising the steps of:

(a) lyophilizing a mixture of an antibody and a lyoprotecting amount of a lyoprotectant which prevents or reduces chemical or physical instability of the antibody upon lyophilization and subsequent storage, wherein the molar ratio of lyoprotectant:antibody is about 100–510 mole lyoprotectant:1 mole of antibody; and

(b) reconstituting the lyophilized mixture of step (a) in a diluent such that the reconstituted formulation is isotonic and stable and has an antibody concentration of about 80 mg/mL to about 400 mg/mL.

18. The method of claim 17 wherein the protein concentration in the reconstituted formulation is from about 80 mg/mL to about 300 mg/mL.

19. The method of claim 17 wherein the protein concentration in the reconstituted formulation is about 2–40 times greater than the protein concentration in the mixture before lyophilization.

20. The method of claim 17 wherein lyophilization is performed at a shelf temperature maintained at about 15–30° C. throughout the entire lyophilization process.

21. An article of manufacture comprising:

(a) a container which holds a lyophilized mixture of an antibody and a lyoprotectant which prevents or reduces

chemical or physical instability of the antibody upon lyophilization and subsequent storage, wherein the molar ratio of lyoprotectant: antibody is about 100–510 mole lyoprotectant:1 mole of antibody; and

(b) instructions for reconstituting the lyophilized mixture with a diluent to an antibody concentration in the reconstituted formulation of about 80 mg/mL to about 400 mg/mL.

22. The article of manufacture of claim **21** wherein the protein concentration in the reconstituted formulation is about 2–40 times greater than the protein concentration in the mixture before lyophilization.

23. The article of manufacture of claim **21** further comprising a second container which holds a diluent.

24. The article of manufacture of claim **23** wherein the diluent is bacteriostatic water for injection (BWFI) comprising an aromatic alcohol.

25. A formulation comprising a lyophilized mixture of an antibody and a lyoprotectant which prevents or reduces chemical or physical instability of the antibody upon lyophilization and subsequent storage, wherein the molar ratio of lyoprotectant:antibody is about 100–510 mole lyoprotectant:1 mole antibody.

26. A formulation comprising anti-HER2 antibody in amount from about 5–40 mg/mL, sucrose or trehalose in an amount from about 10–100 mM, a buffer and a surfactant.

27. The formulation of claim **26** further comprising a bulking agent which adds mass to the lyophilized mixture and contributes to the physical structure of the lyophilized cake.

28. The formulation of claim **27** wherein the bulking agent is mannitol or glycine.

29. The formulation of claim **26** which is lyophilized and stable at 30° C. for at least 6 months.

30. The formulation of claim **29** which is reconstituted with a diluent such that the anti-HER2 antibody concentration in the reconstituted formulation is from about 10–30 mg/mL, wherein the reconstituted formulation is stable at 2–8° C. for at least about 30 days.

31. The formulation of claim **30** wherein the diluent is bacteriostatic water for injection (BWFI) comprising an aromatic alcohol.

32. A formulation comprising anti-IgE antibody in amount from about 5–40 mg/ML, sucrose or trehalose in an amount from about 80–300 mM, a buffer and a surfactant.

33. The formulation of claim **32** which is lyophilized and stable at about 30° C. for at least 1 year.

34. The formulation of claim **1** wherein the antibody in the formulation is a monoclonal antibody.

35. The formulation of claim **1** wherein the reconstituted formulation fails to display significant or unacceptable levels of chemical or physical instability of the antibody upon storage at 2–8° C. for at least 30 days.

36. The formulation of claim **1** wherein less than about 10% of the antibody is present as an aggregate in the reconstituted formulation.

37. The formulation of claim **1** wherein less than about 5% of the antibody is present as an aggregate in the reconstituted formulation.

38. The formulation of claim **1** wherein the lyoprotectant is a non-reducing sugar.

39. The formulation of claim **1** wherein the lyoprotectant is a disaccharide.

40. The formulation of claim **39** wherein the lyoprotectant is a non-reducing disaccharide.

41. The formulation of claim **1** wherein the molar ratio of lyoprotectant:antibody is about 200–510 mole lyoprotectant:1 mole of antibody.

42. The article of manufacture of claim **23** wherein the diluent is sterile water.

43. The article of manufacture of claim **23** wherein the diluent is sterile saline solution.

44. The formulation of claim **25** wherein the antibody is a monoclonal antibody.

45. The formulation of claim **44** wherein the lyoprotectant is sucrose.

46. The formulation of claim **44** wherein the lyoprotectant is trehalose.

47. A stable reconstituted formulation comprising a monoclonal antibody in an amount of about 50 mg/mL to about 400 mg/mL and a diluent, which reconstituted formulation has been prepared from a lyophilized mixture of the monoclonal antibody and a sugar selected from the group consisting of sucrose and trehalose, wherein the molar ratio of sugar:monoclonal antibody is about 100–510 mole sugar:1 mole of monoclonal antibody, and wherein the monoclonal antibody concentration in the reconstituted formulation is about 2–40 times greater than the monoclonal antibody concentration in the mixture before lyophilization.

* * * * *

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HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use HYLENEX recombinant safely and effectively. See full prescribing information for HYLENEX recombinant.

HYLENEX recombinant (hyaluronidase human injection)
Initial U.S. Approval: 2005

INDICATIONS AND USAGE

HYLENEX recombinant is a tissue permeability modifier indicated as an adjuvant

- in subcutaneous fluid administration for achieving hydration (1.1)
- to increase the dispersion and absorption of other injected drugs (1.2)
- in subcutaneous urography for improving resorption of radiopaque agents (1.3)

DOSAGE AND ADMINISTRATION

- Subcutaneous fluid administration:
  Inject 150 U HYLENEX recombinant prior to subcutaneous fluid administration. It will facilitate absorption of 1,000 mL or more of solution. The dosage of subcutaneous fluids administered is dependent upon the age, weight, and clinical condition of the patient as well as laboratory determinations. The rate and volume of subcutaneous fluid administration should not exceed those employed for intravenous infusion (2.1)
- Increasing dispersion and absorption of injected drugs:
  Add 50-300 U (most typically 150 U) HYLENEX recombinant to the injection solution. (2.2)
- Subcutaneous Urography:
  Inject 75 U HYLENEX recombinant subcutaneously over each scapula, followed by injection of the contrast medium at the same sites (2.3)

DOSAGE FORMS AND STRENGTHS

- 150 USP units/mL single dose vials (3)

CONTRAINDICATIONS

- Hypersensitivity (4)

WARNINGS AND PRECAUTIONS

- Spread of Localized Infection. (5.1)
- Ocular Damage. (5.2)
- Enzyme Inactivation with Intravenous Administration. (5.3)

ADVERSE REACTIONS

- Allergic and anaphylactic-like reactions have been reported, rarely (6)

To report SUSPECTED ADVERSE REACTIONS, contact Halozyme Therapeutics, Inc. at 1-877-877-1679 or FDA at 1-800-FDA-1088 or *www.fda.gov/medwatch*.

DRUG INTERACTIONS

- Furosemide, the benzodiazepines and phenytoin are incompatible with hyaluronidase (7.1)
- Hyaluronidase should not be used to enhance the absorption and dispersion of dopamine and/or alpha agonist drugs (7.2)
- Local anesthetics: Hyaluronidase hastens onset and shortens duration of effect, increases incidence of systemic reactions (7.3)
- Large doses of salicylates, cortisone, ACTH, estrogens or antihistamines may require larger amounts of hyaluronidase for equivalent dispersing effect (7.4)

USE IN SPECIFIC POPULATIONS

- Pediatric Use: The dosage of subcutaneous fluids administered is dependent upon the age, weight, and clinical condition of the patient. For premature infants or during the neonatal period, the daily dosage should not exceed 25 mL/kg of body weight, and the rate of administration should not be greater than 2 mL per minute. Special care must be taken in pediatric patients to avoid over hydration by controlling the rate and total volume of the infusion (2.1, 8.4, 14).

See 17 for PATIENT COUNSELING INFORMATION

Revised: 1/2012

FULL PRESCRIBING INFORMATION: CONTENTS*

***Sections or subsections omitted from the full prescribing information are not listed.**

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

1.1 Subcutaneous Fluid Administration

HYLENEX recombinant is indicated as an adjuvant in subcutaneous fluid administration for achieving hydration.

1.2 Dispersion and Absorption of Injected Drugs

HYLENEX recombinant is indicated as an adjuvant to increase the dispersion and absorption of other injected drugs.

1.3 Subcutaneous Urography

HYLENEX recombinant is indicated as an adjunct in subcutaneous urography for improving resorption of radiopaque agents.

2 DOSAGE AND ADMINISTRATION

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit.

Always use aseptic precautions. Lightly pinch the skin up into a small mound and insert the needle/catheter into the subcutaneous space. Inject HYLENEX recombinant through the catheter hub or injection port closest to the needle/catheter. Begin administration of solution. Solution should start in readily.

2.1 Subcutaneous Fluid Administration

150 U of HYLENEX recombinant injected prior to start of subcutaneous fluid administration will facilitate absorption of 1,000 mL or more of solution. As with all parenteral fluid therapy, observe effect closely, with the same precautions for restoring fluid and electrolyte balance as in intravenous injections. The dose, the rate of injection, and the type of solution (saline, glucose, Ringer's, etc.) must be adjusted carefully to the individual patient. When solutions devoid of inorganic electrolytes are administered subcutaneously, hypovolemia may occur. This may be prevented by using solutions containing adequate amounts of inorganic electrolytes and/or controlling the volume and speed of administration.

HYLENEX recombinant may be added to small volumes of solution, such as fluid replacement solutions or solutions of drugs for subcutaneous injection. Subcutaneous fluids should be administered as directed by a physician. The dosage of subcutaneous fluids administered is dependent upon the age, weight, and clinical condition of the patient as well as laboratory determinations. The rate and volume of subcutaneous fluid administration should not exceed

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those employed for intravenous infusion. For premature infants or during the neonatal period, the daily dosage should not exceed 25 mL/kg of body weight, and the rate of administration should not be greater than 2 mL per minute.

2.2 Dispersion and Absorption of Injected Drugs

Dispersion and absorption of other injected drugs may be enhanced by adding 50-300 U, most typically 150 U hyaluronidase, to the injection solution.

2.3 Subcutaneous Urography

The subcutaneous route of administration of urographic contrast media is indicated when intravenous administration cannot be successfully accomplished, particularly in infants and small children. With the patient prone, 75 U of HYLENEX recombinant is injected subcutaneously over each scapula, followed by injection of the contrast medium at the same sites.

3 DOSAGE FORMS AND STRENGTHS

150 USP units/mL single dose vials

4 CONTRAINDICATIONS

HYLENEX recombinant is contraindicated in patients with known hypersensitivity to hyaluronidase or any of the excipients in HYLENEX recombinant. A preliminary skin test for hypersensitivity to HYLENEX recombinant can be performed. The skin test is made by an intradermal injection of approximately 0.02 mL (3 Units) of a 150 Unit/mL solution. A positive reaction consists of a wheal with pseudopods appearing within 5 minutes and persisting for 20 to 30 minutes and accompanied by localized itching. Transient vasodilation at the site of the test, i.e., erythema, is not a positive reaction. Discontinue HYLENEX recombinant if sensitization occurs.

5 WARNINGS AND PRECAUTIONS

5.1 Spread of Localized Infection

Hyaluronidase should not be injected into or around an infected or acutely inflamed area because of the danger of spreading a localized infection.

Hyaluronidase should not be used to reduce the swelling of bites or stings.

5.2 Ocular Damage

Hyaluronidase should not be applied directly to the cornea. It is not for topical use.

5.3 Enzyme Inactivation with Intravenous Administration

HYLENEX recombinant should not be administered intravenously. Its effects relative to dispersion and absorption of other drugs are not produced when it is administered intravenously because the enzyme is rapidly inactivated.

6 ADVERSE REACTIONS

The following adverse reactions have been identified during post-approval use of hyaluronidase products. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to drug exposure.

The most frequently reported adverse reactions have been mild local injection site reactions such as erythema and pain. Hyaluronidase has been reported to enhance the adverse reactions associated with co-administered drug products. Edema has been reported most frequently in association with subcutaneous fluid administration. Allergic reactions (urticaria or angioedema) have been reported in less than 0.1% of patients receiving hyaluronidase. Anaphylactic-like reactions following retrobulbar block or intravenous injections have occurred, rarely.

7 DRUG INTERACTIONS

It is recommended that appropriate references be consulted regarding physical or chemical incompatibilities before adding HYLENEX recombinant to a solution containing another drug.

7.1 Incompatibilities

Furosemide, the benzodiazepines and phenytoin have been found to be incompatible with hyaluronidase.

7.2 Drug-Specific Precautions

Hyaluronidase should not be used to enhance the dispersion and absorption of dopamine and/or alpha agonist drugs.

When considering the administration of any other drug with hyaluronidase, it is recommended that appropriate references first be consulted to determine the usual precautions for the use of the other drug.

7.3 Local Anesthetics

When hyaluronidase is added to a local anesthetic agent, it hastens the onset of analgesia and tends to reduce the swelling caused by local infiltration, but the wider spread of the local