SUPERINTENENCJA DE!NDUSTIA V COMERCiO

.. Industriay Coniercio .No.06-038,331- -00003.0000

S U P E R I N T E N D E N C I A ... 29+92416:23: Dep. 2020 NUECRCIONt Ant. 329CTOINFORMACIO ENACIONALI N Folios- 28

FORMULARIO UNICO DE CORRECCIONES Y MODIFICACIONES

SOLICITUD DE:
I

CorreccIón de error material [] Modificacion a una solicituci

2 Nombre: Centocor Ortho Biotech Inc. IDENTIFICACIÔN

Dirección: 800/850 Ridgeview Drive, Horsham, C.C. NIT
SOLICITANTE Pennsylvania I 9O441Estados Unidos de America
Nacionalidad o Domicillo: Horsham, C.E. Otro
Pennsylvania, Estados Unidos de America.
Lugar de Constitución: Pennsylvania, Estados Cual
Unidos de America.

Nmero TelØfono:. Fax:.
E-mail: ..

.3 . Nombre: D1LIA MARIA RODRIGUEZ IDENTIFIcACION
D’ALEMAN

REPRESENTANTE. .. C.C. NIT

0 APODERADO Direcciôn: Crà. 11 No. 86-53 piso.6
C.E. łtro
TŒIØfono 6162051/61 Fax: 6161889 CuaI.______

E-mail: cIatkemodetcIarkemodetcom.co Nümero 41L6.882 T.P. 20824

4 NCimoro del expedients;
06-038.331

Descrlpclón do Ia corrocclón o modlflcaclón solicitada: Quo so anote

La FUSION gue hacela sociedad Centocor, Inc. Ortho Biotech Inc., con un cambio de nombre a Centocor Ortho Biotech Inc., domiciliada en 800/850 RidQeview Drive, Horsham, Pennsylvania 19044 Estados Unidos de America.

6 Comprobanto do pago No. 9 65,720 Fecha 3 do septiembre de 2009

I Anexos:

fl Cörnprobante do pago do Ia tasa
Li Poderes, Si fuere el caso Sustituciôn poder Centocor, Inc. a Dilia Rodriguez
J Documento que acredita Ia existencta yrepresentación legal de las pàrtes, cuanclo Sean personas

Juridicas. .... I, L Documentdque acredita Ia füSiôn do Ia sociedad Cerki, Inc. Ortho Biotech Inc.
8 (Th

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NOMBRE DILIA M. RODRIGUEZ. .

DLEMAN
._ "4.882

AFOSTILLE

(CONVENT/ONDE LA HAYE DU5 OCTOBRE 1961)

1 COUNTRY: UNITED SPATES OF AMERICA

2. THIS PUBLIC DOCUMENT HAS BEEN SIGNED BY.:
rAMANDA LIN BAYNES
3. ACTING IN THE CAPACITY OF:
NOTARY PUBLIC OF NEW JERSEY
4. THE SEAL/STAMP OF.:
AMANDA. LIN HAYES, NOTARY

’CERTIFIED

S 5 AT TRENTON, NEW JERSEY

6. THE. 24Th DAY OF JUNE 2009

7’.. "BY; R. David Rousseau, NEW JERSEY TREASURER

8 NO: A367051 .

9 SEAL/STAMP: 10 SIGNATURE

Ccni&ate Nnnber 11469920
Verify tius crticate at $,

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COLOMBIA
Cazrora 11 N° 86-53 Piso 6 Bogota Colombia Tel. (57 1) 6181088 Pax. (571) 6350824 Email ndo@claxkomodetcom.co

PODER

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y en actual elunpilnuento lie an objeto social, con domk,ilio enSOWSSU Rdgev1thDrlvo Horsham, Pennsylvania 19044, Es*adàs Unidos do
America -

porel Puente, inslruaonto confiero (conEmmos) podor ospoeW a.. do DILIA MAMA ROI)EIGU3Z D’AtBMAN. con Cddula do Cludadanfa No.. 41.654.882 do Bogota, y tp. 20824. y C1.,AUDIA LUCIA CARO RAMIRE4 can Cddula do Ciudadanfa No. 40.017.374 do Tunis, y tp. 36448, con domidilio on Bogota, Colombia, pam quo actuando on an onion en aerobic dot (do In) atorgante la (Is) reprascotan ante cua!quier onlidad, autoridad y10 persons, aspodalineft ante lea del orden Mministrativo, Contencroso Adnwustrativo Judicial, Tribunalea do Arbifrainonto yb cualquier elm on lorcFcrante a los sigulentea nanntos

1) Solicitor, framifar, obtener y/a rogistrarcualquiera do las modalidados do Propiedad Industrial, tales como: patents do iiweneidn, modulus do utilidad, disftos iodustrialea, vatiodados vegetates; nrarcasiemas, aerobics y ensoEas comoruiaks y donaminaciones do otiges, sal coma ens renovaeio-ma, pthxrops, tranatbioncias, cambics denonlbrey/o doinicillo, contictosy liccocias.

2) Solieltar, tramitar, obtenor yio registrar deronhos do autor, on cuniquicra do we prosontacionce sal conic Is proteccidu do secretes industrioles y know-bow.

3) Solicitar,.lrtuuitar, obtoner registrar registros saidlarios, permisos yb licencias do funciona*nianto; sat coma sus renovacianos o prdrrogas, frfamncias, cambios do nombro y/o donuciho coniratos y licencias
.4) Ejomer accionas do aposicidu, obsorvacibn, reclanme, IecOnSIdOraCida, reposicidn, apoiación, cancelacida administrativa a judicial, nulidad, protccoidn al Łonsumidor, coucesidu do liccocias, protaccidn do socretos industrialas y de*n6s acciones y recumos riacionadas con.dervclios dorivados do los asuntos aniba indicados y on contra do la competencia: desleel, accionea dciivàdas do In obtoniôn o osplotacion do licencias, accionos do indeinnización do poiuicios. Tanibidn podrâ(n) intervenir en jurcios civics, comecusalos, pennies contencioso-admnuatratn’os, tutelas y do policla; on accionra o juicios prornovidos per at (in) snandante a an contra ci (ella).
5) Registrar nombres do dominic atite Is ontidad coropetente.
6) En el ejomicia do rate mandato los mandatarios aniba snencionados quedan facuhados pain conitihar. caneclar, Iransigir comprometer on 4rbitros, desistir, recibir y toniar pososidn do In corn otjoto do litigio y renunciar o dosistir a derechos coixcodidos a on croso do cwtccsidn, adniitir I" bochos del procoso.
7) Ratiflear o no Is agenda oficiosa.
8) MI mismo faculto (facultamos) a los mencionados apoderados pain recibir notificaciones y dosiguar apoderados judicialea o extrajudiclales, sustibiir ci presento podoryrevocar Ins austihiciome quo hicierm.

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POWER 01? ATTORNEY

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leg lrepresontativc(s) of Centocor Ortho Bloteob Inc,

an organization existing under the laws of PENNSYLVANIA, USA

and inprmmtexecutson ofits business activity, domiciled 800/850 Rldgeview Drive, Horsham, Pennsylvania 191)44, United States ofAmerkm.

do hereby grant POWER OF.AITORNBY to DLIA MARiA RODRIGUEZ D’ALEMAN, identification card No. 41.654.882 ofBogota, and tp. 20824 and CLAUDIA LUCIA CARO RAIvffREZ, tp. 36448, identification card No. 40.017.374 of Tunis, residing in Colombia, to act in the order oftheir appointment in behalf of grantor before any entity, authority and/or natural or juridical person, specially before those Administrative, Administrative-Contentious, Judicial, arbitration courts, and/or any other, in all matters concerning the diltowingi

1) To apply, process, obtain and/or register any of the modalities ofIndustrial Property such as Patents ofInvention, Utility Models Designs, plant varieties, Mades, Slogans, TradeNames and emblems and appellations of as wall as their renewals, assignments, changes of name and/or domicile, agramments and

2) To apply, process, obtain and/or register copyrights, of any kind as well as protection ofindustrial secrets or know-how.
3) To apply, process, obtain and/or register sanitary registrations, operating authorizations and/or licenacs; as well as their renewals or assignments, changes ofnameand/ordomicile,agroeznentlandlicenses.
4) To bring actions ofopposition, observations, claims, reconsideration, appeal or legal temedies, cancellations, AUffity, protection to the consumer, granting of licenses, protection of trade socrets and other actions related with rights derived from the abovc- mentioned mailers and against the unfair competition, actions resulting from the obtaining or exploitation of licenses, recovering damages

actions. They can also intervene or participate in Civil, Commercial, Criminal or Contentious-Administrative Trials; the actions far writ mandamus, police
actions; in actions, lawsuits or litigations instituted by the grantor or against him.
5) To register domain names before the competent entity.
6) To the effect of this mandate the above-named proxy are empowered to: conciliate, cancel, settle, submit to arbitration, withdraw and to receive and take possession ofthe litigation related objet and waive or renounce the rights granted or in process ofgranting, adroit the facts ofthe case.
7) To ratiry or not the officious agency.
8)1 (we) hereby appoint the afore mentioned attorneys with powers to receive notifications and to designate judicial and extrajudicial attorneys, substitute this Power ofAttorney and to revoke such sustitut.ions.

GrautLd and signed at NEW H1WNSWICK, NJ USA

on this 27TH day of MAY ox, 2609

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CERTMCADO NOTARIAL . NOTA1UAL CERTIFICATE
(Cuwrdo elsolleitantees SOCIEDM)) . (When the applicant Is aCOEPORAflON)
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mayor do edad ydomiciliado . flegal age and domiciled in BRUN2W1(2(NJ_USA_set his
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ylotra eldacmnncutoqueantaneda : 2. The grantor is to him personafly known and that bthhe has legal capacity to
2. Comm al (a Is) otorgnnte y qua eats (a) c4 legahnento capacitado exectoflwdocwnut
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do. __. . ofthejuridical person çentocor Oz’tho Bioteda In
do leajuridica, Ceutoco1 Ortho Blłtech Jut.

4. Thementionedjuridical parson with place of 800I8SURldgevIewDrlvo
44a citada persona juridlea con sedeun 800/85aRldgevtewDrh’e, Horsham, PennsylvanIa 19044, United States ofMmsrlca, is duly organlard,
thrabasi, Pennsylvania 19044, Estadan Unldes deMnSrlca, catS his actual legal existence and th tthepuzposca for which the power ofattorney
logalmenta constituida, quo time aetuahuento uxistencia legal y quo ci is punted are within the scope Ofits corporate purposes.
nato pars ci owl so ha otorgado esta poder. catS comprendido cithu los. S. The grantor has in fact the tethered quality and that his/her repiasentation
quo constituyoiso objeto aoo4 Is legal. and that Ire/she isernpowered to giant this power ofaltonwy.

5. El.otorganlu time thcth,amcnte Is representacidn quo ostanta, quo cata 6.1 have based the abvc certifications 3,4 and 5, on the autitenfic documents aslegitinra y quo eatS facultado pars otorgar oft poder. which forsuchpuxpose were exhibited to me. 6. las antetiorca certificaeionas.de los puntos 3,4 y 5, mc coristan porque

be tenido a Is vista los doconrentos autdnticoa.

AMANDA UN HAYNES
NAM NotaryPublicN=pIrthr _--
NOTA. Then the Colombian Consul must legalize the signature of the Notary

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finns del Notario

do
Püblico.

lo anterior, ci Cdmel do. Colombia dobeS NOTARIAL CERTIFICATE (When the applicant Is an INDIVIDUALS)

CERTIFICADO NOTARIAL (Cumande ci sojicitante as INDIVIDUO)

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(nanrbro do is persona quo th,na.ol

mayor decdadydomieiliadoen________
an so _________________________ pr___ omocia
ci documonto quo antziocde, conoce al otorgante y quo esto catS legabnen-teeapacitadoPam Cl otorgamiento.

NotarioPSblico
NOTA A contfrmuacidn do Is anterior, el Côusul do Colombia dobe autenticar is finna del Notaxio P6blico.

Peru toilos los demds paisca ci Safer Cônsui do Colombia o ante qulen so otorguc, dehe expedir one certificaciSu dejando constancla 1) de Is eistencia legal do Is sociedad, 2) de que esta ejarcc actuilmente so objeto social, y3) do quo in persona o personas qua otorgan ci purist estSn facultadas pare hacerlo, dejando
constancla asi mismno do quo tuvo a I vista las pruehas qni acrediten tales clrcunstanclas..

LEGALIZACION POE EL CONSUL DE COLOMBIA
OPOR APOSTILLA

on this day of
the undersigned Notary.CERTErIES that

1.

(name ofthe person who signs the Power ofA&rney)

oflegal age and domiciled at___________________
________ signed in his presence the foregoing
donment the grantor is to him personally known and that ho/she has legal capacity to execute the document

Notary Public
NOTA Then the Colombian Consul must legalize the signature of the Notary Public.

For all other countries the Colombian Consul or before who It is granted must Issue a certification stating 1) the legal existence ofthe corporation, 2) that the corporate purposes are actually being accomplished, 3) that the person or perSons who grant this Power ofAttorney are empowered to do It. The Consul will certilS’ also that the documents evidencing those circumstances were czhihlted to him.

LEGALIZATION BY THE COLOMBIAN CONSUL OR BY APOSTILLK

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CERTIFICATION

I certify that Mark R. Warfield, Assistant Secretary. of Centocor Ortho Biotech Inc., a corporation existing, duly organized and complying with its corporate purposes in conformity with the laws of Pennsylvania, United States of America, whose home office is located in Horsham, Pennsylvania I further certify that said individual is duly authorized to sign any and all instruments in his capacity as officer of the company and that the purposes for which are within the scope of the objects or activities of said corporation.

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beforemethis

GRAI.DINE A. BAILEY
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CommlonPfr&/4/Zfl4

Esta certificación notarial debe ser ilenada ensu totalidad y legallzarse por Apostilia
This notarial certification must be filled in completely and be

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Traduccion of cial hecha pot Jimena Escobar Uribe, traductora e intrprete of cial segAn
reolución No 0369 del Mmisterio do Justicia, del documento legalizado mediante la APOSTILLA Na A-361051 do 24 de judo do 2009 expedi4a on Estados Urndos do A112-Amnca (Nueva Jersey) correspondiente a tin decumento do poder una certificación .J notarialenmglÆsPon sucorresponthentetraduceiônalespafiol(razónporlacualnosehace
su traducción) otorgadł a la socledad CENTOCOR ORTHO BIOTECH INC.,
domicibada en Horsham Pensilvania, Estados Umdos do America.

H
Doculnento de podor firmado el 27 de mayo do 2009 par Mark R Warfield, Secetario As.stente

Sello notarial Amanda. Lin Haynes, Notario Pub)S.coe va Jersey, comisión tennuna el 10/28/2013

. . . .

APOSTILL .

(Convención do la Haya de:5 do octubre de 18

1. Pals: Estados thnidos de mØria: .

2 Este documento publico ha sido firmado par AMANDA UN
BAES

3.. Actuaido en sü calidad de NotariQ Pth1ico de

4 Lieva el sello/estampilla de AMNJDA LIN HAYNEjiO

CERTI Fl CADO

5 En Trenton, Nueva Jersey

W 6 El 24 de jun10 do 2009...................................

7.. Por,R David Rousseau, Tescrerodia NuevsØjI

8 Certificado No, A367051

9 Se11o/Etampi11a 1 Gran Sello del

I

Traducción oficial hecha por Jiniena Escobar Uribe, traduetora e intØq,rete ôfieial segln resolución No, 0369 del Ministerio de Justicia, del documento legalizado mediaute la APOSTILLA No A-368604 de 13 de julio de 2009 expedida en Estados Unidos de AinØnca (Nueva Jersey) correspondiente a una certificación notanal relacionada con Is
sociedad CENTOCOR ORTIIO BIOTECH INC, domicihada on Horsham,
Pensilvania, Estados Unidos de America.

CERTIrICACIÔN

Yo certifico que Mark R Warfield, Secretario Asistente de Centocor Ortho Biotech Inc., Una sociedad existente, debidaxnente constituida y cumplierido con sus objetos corporativos de acuerdo con las leyes de Pensilvania, Estados Unidos de America., cuya casa matriz estª localizada en Hôrsham, Pensilvania. AdemÆs ce.rtifico que dicho individuo estÆdebidamente autorizadO para firmar cualquier y todos los instrumentos en su capacidad conio funcionario de la sociedad y que los propôsitos.para los cuales estÆn dentro del espectro de los objetivos o actividades de dicha sociedad

Firmado: Firma ilegible

Sello Juramentado y suscrito ante ml este dla 18 de (ilegible) de.2009.

Firmado Geraldine A Bailey, notario publico de Nueva Jersey, coinisión termina 1/14/2014.

APOSTILLA

(Convenciôn de la Haya de 5 de octubre

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I. Pals: EtadOS Unidos.cLe America
2. $te do.ctunento pübUco ha sido firmado pox’ GERLADINE A.
8AXLEY

3. Mtuando en su calidad de Notario Pfiblico 4 $tieva Jersey
4. Lieva el sello/estampilla de GERLADINE A. BAflEY, notario

e$RTIFIcAO -

5. n Trenton, Nueva Jersey -
6. 1 13 de.ju1i6 de 2.009
7. Par R. David -Rousseau, Tesorero de Nuva Jersey
8. Ceztif–cado No. b,368604
9. se1lo/stampi11a El Gran e110 del Estac1 de Nuea Jersey
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APOSTILLE

(CONVENTION DE L4 HAYE DU5 OCTOBRE 1961)

1. COUNTRY: UNITED STATES OF AMERICA

2. THIS PUBLIC DOCUMENT HAS BEEN SIGNED BY
CHRISTINA A CLICE:NER
3. ACTING IN THE CAPACITY OF:
NOTARY PUBLIC OF NEW JERSEY
4. BEARS THE SEAL/STAMP OF:
CHRISTINA A CLICKNER, NOTARY

CERTIFIED

5. AT TRENTONr NEW JERSEY

6. THE 7TH DAY OF AUGUST 2009

7. BY: R. David Rousseau, NEW JERSEY TREASURER

8. NO: A371436

9. SEAL/STAMP: 10. SIGNATURE

CerticateNumbe 115031562.
Vaity this ceitlficatc at

Ll

PENNSYLVANIA DEPARTMENT OF STATE CORPORATION BUREAU

Articles/Certificate ofMerger

Domestic Business Corporation (§ 1926)
Domestic Nonprofit Corporation (§ 5926)
J13 Limited Partnership (* 8547)

A4&T....

City. state ZipCode

H7/_cSL

Fes $150 plus $40 additional for each
Party in additional to two

Entity W. 675202
Date Filed: 12/19/2008
Effective Date: 12/29/2008
Pedro A. CortØs
Secretary of the Commonwealth

Document will be returned to the
name and address you enter to
the left.

Commonwealth of Pennsylvania
ARTICLES OF MERGER-BUSINESS 7 Page(s)

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T08354841.23

In compliance with the requirements ofthe applicable provisions (relating to articles ofmerger or consolidation), the
undersigned. desiring to effect a merger. hereby state that

1. The nexus ofthe corporation/limited oaiinershin urviv1n 4w. nr’4s’
Centocor Inc.

Check and 6omple4e one ofthefollQwing:
X The surviving corporation/limited partnership is a domestic businessfaonproflteOrporatiofl/thnfted partnership and the (a) address ofits current registered office in this Commonwealth or (b) name ofits commercial registered office provider and the county ofvenue is (the Dopartrrient is hereby authorized to correct the following Information to
to the records ofthe Department):
(a) Number and Street City State Zip County
\lJlu 1Jurr Pt.

(b) Name ofCommercial Registered Office Provider County

.DThe surviving corporation/limited partnership is a qualified foreign business/nonprofit corporation/limited partnership incorporated/formed under the laws of and the (a) address ofits current registered office in this Commonwealth or (b) name ofIts commercial registered office provider, and the county ofvenue Is (the Department is hereby authorized to correct the following information to conform to the records ofthe Department):
(a) Number and Street City State Zip County

(b) Name ofCommercial Registered Office Provider County
blo -

1The survivingcorporatlon/lirnited partnership is a nonqualifled foreign business/nonprofit corporation/limited partnership incorpomted(formed under the laws of . and the address ofits principal office under the
laws ofsuch domiciliary jurisdiction Is:
Number and Street City State Zip

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7. Check, and fapprepriaee complete, one ofthefollowing:

J. The plan of merger is set fbrth in full in Exhibit A aflac}ied hereto arid made a part hereof.

Pursuant to 15 Pa.C.S. § 19011* 8547(b) (relating to omission ofcertain provisions from filed plans) the provisions,
if any, of the plan ofmerger that amend or eonstirute the operative provisions ofthe Artichs of Incorporation/Certificate of Lªmited Partnership ofthe surviving oorpomtionflimited partnership as in effect subsequent to the ffectiva date ofthe plan arc set forth in full in Exhibit A attached hereto and mada a party hercot The full text ofthe plan of merger is on file at the principal place ofbusiness.ofthe surviving corporation/limited
partnership, the address ofwhich is.

Number and street CityState Zip County

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Centncer

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Joseph WcIk, Vice President, Piruuwc
Title

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DSCB: 15-1920926/8547-3

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day

- Centocor, Inc,
Name ctCooddP

Joseph J. WON vice Ptea%den; finance
Title

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Exhibit

PLAN OF MERGER

THIS PLAN OF MERGER dated as of December 200a, is entered into

pursuant to Section (iS Pa,C.5.) (1926) ofthePerinsylvania Business Entity Laws-and Section (14A 104. 1) ofthe New Jersey Business Entity Laws and-is among Centocor) Inc. ("Centocor"), a Pennsylvania corporation and the surviving corporation and Ortho Biotech Inc. (’ t>B-iotech’), aNew Jersey-corporation.

WHEREAS, Ortho Biotech desires to merge with and into Centocor and Centocor desires to merge with and into itself Ortho Biotech.

WHEREAS, the parties to this Plan, in consideration of the mutual covenants, agreements and provisions hereinafter contained, do hereby prescribe the terms and conditions ofsaid merger.

NOW, THEREFORE, the corporations, parties to this Plan by and between their respective boards ofdirectors, in consideration ofthe mutual covenants, agreements and provisions hereinafter contained -do hereby agree upon and prescribe the terms and conditions ofsaid merger, the mode of carrying them into effect and the manner and basis ofconverting the shares ofthe constituent corporation into shares ofthe corporation surviving the merger as follows-

(a) The Articles of Incorporation and: the By-laws of the surviving corporation as they exist on the effective date ofthe merger shall be and
remain the. Articles of Incorporation and By-laws of the corporation
surviving the merger, except that the name-of the surviving corporation on
the Articles ofIncorporation shall be amended in their entirety and read a. follows: 1. The name-of the Corporation is Centocor Ortho Biotech Inc

() The directors and ocers of the suing corporation shall continue in
office until the next annual meeting of stockholders and until their successors shall have been elected and qualified

(c) At the effective time of the merger, all property, rights, privileges and

other asset of every kind, and description of Ortho Biotech shall be transferred vested in and devolve upon Centocor without further act or

deecL

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Traducción oficidi hecha por Jimena Escobar Uribe, traductora e intØrprete oficial segün resolución No. 0369 del Ministerio de Jusficia, del documento legalizado mediante la APOSTILLA No. A371436 de 7 de agosto de 2009 expedida en Estados Unidos de America (Nueva Jersey) correspondiente a un certificado de fusiOn de la sociedad CENTOCOR ORTHO BIOTECH INC.

CERTIFICACION

For medio del presente certifico que el documento adjunto es una copia verdadera de los Articulos / Certificado de Fusion
de Centocor, Inc. y Ortho Biotech Inc. con un nombre de
carnbio a Centocor Ortho Biotech Inc.

Firmado: Christina A. Clickner, Notario. Pübiico
Mi comisiOn termina el 2 de rnarzo del 2010

Entidad No. 675202
Datos presentados: 12/19/2008
Fecha de vigencia: 12129/2008
Pedro A. CortØs, Secretario del Commonwealth

DEPART4ENTO DE ESTADO DE PENS ILVANIA
OFICINA DE SOCIEDADES

Articulos/ Certificado de FusiOn
(15 COdigo de PØnsilvania)
-  X Sociedad de Negoci.os DomØstica (1926)
- Sociedad Sin Animo de Lucro DomØstica (5926)
Sociedad Lirnitada (8547.) -

El documento Va a ser devuelto al noxubre y
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dos.

De acuerdo -con Los reqQerimientos de las thspszcione
aplicables (relacionadas con los articulos de fusion o
consoLidacjon), elsuscrito, deseoso de efetuar una fusiôn,
por ee(,Jio del prsentØ, declata’ qua:

i. El noinbre de La socjedad /sociedad limitada aobzeiv.iente a la fusiOn es. qeitocor, Inc..

2. Seale y complete trno de los si4uientest
La sociedad /ociedad ljmitacja sobreviviente es una sociedad /sociedad lirnitada domØstica de negocios /sin Ænimo de lucro la (a) direCción de su oficina - registrada actual as n e Coflowealth o (b) nombe del suministrador de, oficina de registro comercial y el eondado de aede-.es (el Departaento qeda por medio del presente autoizado pare corregir La siguie.nte informaci&ii para qua quede de acuerdo con los registbs del Departanento):

(a) Ntaro calle Ciutad Estado C6di Condado 200 Great Valley Parkway., Malvern Pnsilvania 19.5

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(b) Nombre del Suministrador de oficina de Registro Comercial Condado

La sociedad /sociedad limitada sobreviviente es una sociedad /sociedad limitada extranjera calificada de negocios /sin Ænimo de lucro constituida/ formada bajo las leyes de y la (a) direcciÆn de su oficina registrada actual es en
el Commowealth o (b) riombre del suininistrador de oficina de registro comercial y el condado de sede es (el Departamento queda por medio del presente autorizado para corregir la siguiente informaciôn para que quede de acuerdo con los registros del Departamento);
(a) Nntiero y calle Ciudad Estado Código Condado

(b) Nombre del Suministrador de. oficina de Registro Comercial Condado

0 La sociedad /sociedad limitada sobreviviente es una sociedad /sociedad limitada extranjera no calificada de negocios /sin Ænimo de lucro constituida/ formada bajo las leyes de y la dirección de su oficina principal bajo las leyes de dicha jurisdicciôn domiciliarias es
Nümero y calle Ciudad Estado Codigo

3 El noxubre y direcciôn de la oficina registrada en este Coxrimoawealth a el noxnbre de su suministrador de oficina de registro comercial y el condado de sede de cada una de las otras sociedades /sociedades lirnitadas domØsticas de negocios/ sin Ænimo de lucro y sociedades /sociedades limitadas extranjeras calificadas de ne

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siguientes:
Nombre Direcciónde Suministrador do OfjCina. Condado
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Registrada
Ortho Biotech Inc. No registrada en Pensilvania
Centocor., Inc. 116 Pine Street C’ Corporation Allegheny
Harrisburgh, System =
Pensilvania 1.71.01

4. $efale y, si. es apropiado, copletØ ’uno de los siquientes:

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5. La manera on is cual plan do. fusl(>n fue.adoptado por
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ombre Manera de AdopciOn
Centocor, Inc... Adoptada poracciAn d1a junta directiva y Accionistas do la sociedad, do a6uerdo con
. 15 COdigo de Pensilvania 1924(a)

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del plan estn incluidas en si totalidad en la Prueba A.
adjunta a la presentey hcen partede la misma. El texto
completo del plan do fusiónestª en Ids archivcs dollugar de ’.
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sobreviviente, cuya direcci-On es .
Ninbre y, calle Ciudad Estdo Cdigo Condado

EN T8TIMONI0 DE, LO CUAL la susita soci/ socieci.ad liniltada ha heho que so firmen estosxttculos/ certiflcado
de FusiOn por un funcionaric,debidamente autorizado del mismo
este dIalS do diciembro do, 2008.

Noinbre do la Sociedad/ SoctØdad Limitada: Centocor, Inc.
Firma: Firma ilegible
!itulo: Joseph. J.. Walk, Vicepresidente,’ Finanzas

Nombre do la Sociedad/ Sociedad Limitacia. Ortho Biotech Inc. Firma:

Titulo: flichelle L’Ryan, Vicepresidente, Finaizas

EN ThSIMOIO DE LO CUAL, la suscrita sociedad/ sociedd
jjajtada ha hetho quo so firmen estos: Articulo/ Certjficado do Fusin pr un funcionariö debi.damente autorizado del mismo ete dla 15 de diciembre do 2008.

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Titulo: Joseph J. Wolk, Videpresidente, Finanzas

Nombre de la Sociedad! Sociedad Limitada: Ortho Biotech Inc..
Firma: Firma ilegible
Titülo: Michelle R. Ryan, Vicepresidente, Finanzas
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Prueba A
PLAN DE FUSION

ESTE PLAN DE FUSION, fechado el 8 de dicieithre de 2008 es suscrito de acuerdo con la Sección (15 Côdigo De Pensilvania) (1926) de las Leyes de Entidades de Negocios de Pensilvania y Sección (14a:10_4.1) de las Leyes de Entidades de.Negocios de
Nueva Jersey y es entre Centocor, Inc. ("Centocor"), una
sociedad de Pensilvania y la sociedad sobreviviente y Ortho Biotech Inc. ("Ortho Biotech"), una sociedad de Nueva Jersey.

TENIENDO EN CUENTA QUE, Ortho Biotech desea fusionarse con y en Centocor y Centocor desea fusionarse con y en Ortho

Biotech.

TENIENDO EN CUENTA QUE, las partes de este Plan, en consideraciôn a los mutuos compromisos, acuerdos y disposiciones contenidas de acjui en adelante, per medio del presente indican los tØrminos y condiciones de dicha fusiOn.

AHORA, FOR LO TANTO, las sociedades, partes de este Plan, por
y entre sus respetivas Juntas Directivas, en consideraciOn a Jos mutuos compromises, acuerdos y disposiciones. contenidas de aqul en adelante, por medio del presente acuerdan e indican los tØrininos y condiciones de dicha
de lievarlos a cabo par que tenga efectos y

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bases de convertir las acciones de la sociedad constituyente en acciones de la sociedad sobreviviente a la fusion coma se indica acontinuación:

(a) Los Articulos de ConstituciOn y los Estatutos de la sociedad sobreviviente tal coma existen en la fecha de
vigencia de la fusiOn serÆn y permanecerÆn siendo los
Articulos de ConstituciOn y los Estatutos de la sociedad sobreviviente a la fusiOn, excepto que el nornbre de la sociedad sobreviviente en los Articulos de COnstituciOn serÆ modificaclo en su totalidad y serâ como se ’ndica a
continuaciOn: 1. El nonibre de la Sociedad es: Centocor Ortho
Biotech Inc.

(b) Los directores y funcionarios de la sociedad
sobreviviente contirxuarÆri en sus puestos hasta la reuniOn
anual de accionistas siguientes.y hasta que sus sucesores hayan sido el.egidos y calificados.

(c) Al momento efectivo de la fusiOn, todas las propiedades, derechos, privilegios y otros activos de cualquier tipo y descripciOn de Ortho Biotech serÆn transferidos a, otorgados a y delegados a Centocor sin un acto a hecho posterior.

(d) Todas las acciones expedidas y pendientes de Ortho
Biotech son de propiedad y serÆn de propiedad al momento
efectivo de la fusiOn de Johnson & Johnson, una sociedad de Nueva Jersey. Todas las acciones expedidas y pendientes de
Centocor son de propiedad y serÆn de propiedad al momento
efectivo de la fusiOn de Johnson & Johnson,
Nueva Jersey. Al momento efectivo de la fu
acciones expedidas y pendientes de. Ortho

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canceladas y ninguna acción del sobreviviente zerÆn por. 10 tanto epedidas e inter.aithiadas. .

(e) La fecha de vigericia de 1 uaiôn.serÆ el 29 dediciembre de2008.

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’APOSTILLA.

(Canveción de la I’Laya da 5 de octubre de 1961)

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3. Actuarido en sualidad: . 2 Ntario?üblico de Nueva Jersey .
4. Lleva el slo/es1arnpifla Ue: .
Christina A. Cliener, Notaxio .

C$RIFICAD0

5. En Trenton, Nueva Jersey .
El 7 de aosto de 2009 ....
7 PQr L David Rousseau, Tesorero dØNueva Jersey
13. NO. A371436 .

9. Sello/EstampilLa: Sello del,Estado de Nueva Jersey
10. firma: ilegible.

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Industfla y Cornercio
S U P E R I N T E N D E N C I A

DIVISION DE NUEVAS CREACIONES

RADICACION EXPEDIENTE No. 06-38331

EL EXTRACTO DE LA PRESENTE SOLICITUD DE PATENTE DE INVENCION

FUE PUBLICADO EN LA c3ACETA DE PROPIEDAD INDUSTRIAL No 569 DE

FECHA 31 DE OCTUBRE DE 2006 EL TERMINO HABIL SE1ALADO POR LA

LEY EXPIRA EL 01 DE FEBRERO DE 2007

NOTA: Dentro del plazo de los seis (6) meses siguientes a partir de la fecha de publlcación en (a gaceta de Propiedad Industrial, deberÆ solicitar y cancelar el examen de patentabilidad so pena de qua (a solicitud caiga en abandono, de acuerdo con lo estab(eciclo on el Articulo 44 de la Decision 486/2000.

SE PRESENTARON OPOSICIONES POR PARTE.DE TERCEROS.

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US006165476A

United States Patent [19].: iij Patent Number: 6,1659476 Strom et al.. [41 Date of Patent: Dec. 26, 2000

[54] FUSION PROTEINS WITH AN
IMMUNOGLOBULIN HINGE REGION
LINKER

[75] Inventors: Terry B. Strom; XIn Mao Zheu, both
of Brookline, Mass,

[.13] Assignee: Beth Israel Deaconess Medical
Center, Boston, Mass,

[21]. Appi. No.: 08/891,271

[22] Filed: Jul. 10, 1997

[51] Cl.7........................C070 21/00; CO7K 14/455; COIK. 19/00

[52] U.S. Cl...................................... 424/195.11; 424/85.2;
435/69.7; 435/325; 43/252.3; 435/3201-
530/351; 530/387.3; 530/399; 536/23.4

[58] Field of Search.................................. 530/351, 387.3,
530/399; 435/69.7,325,252,3,320.1; 424/83.1,
85.2, 192.1, 198.1,93,2; 536/23.4; 935/10

[56] References.Clted

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(List continued on next page.)

Pthflat’y Examiner-Lorraiue Spector
Attorney, Agent, or Firm-Hamilton, Brook, Smith & Reynolds, PC

The present invention relates to the production and use of fusion proteins with an immunoglobulin hinge region linker,

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1$,.lnterleulcin-16, Erythropoietin, Co1ony-Stinula ’og antigen rec gnitio4. 1 a C-tar md ort on of eec d
Factor-1, Granulocyte Colony-Stimulating Fac or, defines a c nstant (I region prir aril responsi le
Granulocyte-Macrophage Colony-Stimulating Factor, Lou- effector function.
ketnia Inhibitory Factor, Tumor Necrosis Fac or, Within thelight rid cavy oh ins, ills made up o al
Lymphotoxin, Platelet-Derived Growth Factor, Fibroblast 5 110 amino acids, form d screte d maii E oh domain is
Growth. Factors, Vascular Endothelial Cell Growth Fai or, together by single,,mt mel dis mdc on. The heav, ci
Epidermal Growth Factor, Transforming Growth Facto typically co taips 4 such dam aim, ’hi e the light ci
Transforming Growth Factor-a, Thrombopoietin, Stem ell typically co tains 2 d mains. he O terminus of
Factor, Oncostatin M Amphiregulin, Mullerian4nhiYoi lug ft N-term if the boa oh An, VH, in ci
Substance, B-Cell Growth Factor, Macrophage ’Migra ’on ic with the N termiral domain I the light chain, V,.
Inhibiting Factor,, Endostatin, and Angiostatiri. produce the inding rot ’on of th anti ad Moving t w
More specifically, the present invention relatesin a ho o- the C-termi us, th4 next three comai of the heav ci
fusion protein wherein two erythropoietin (EPO) molec lea are designat d C,,, C 2, and 3, sp ctively.

are linked by an amino acid sequence comprising an urn in-. Most baa chains have a hinge re ion between t e noglobulin hinge region sequence and wherein the ho o- and C12 domains con isting o a s: all tumber of an
fusion protein has increased biological activity, as dell ed acids, ’the h nge, is Lex le and low the binding me ’a
above, The present invention also relates to a hetero-fn on move freely dative to he rest o the al inle, At th h
protein wherein lL2 is linked via a hinge region sequenceto region are t e disulfide bridges whic ho d the two it
Fast,, and wherein the TL2-Fa$. fusion protein has hiol together, creating the t trainer a mdi rat nit.
cal activity. There are Lye classe ofimmtnogk,uli s (IgA, Ig
The present invention also relates ’to methods of prod to- IgG, and Ig 1) ii hgh ’vcrtebtates, LI a which co ta
ing a fusion protein comprising an irnmunoglobilin Ii’ ge hinge region. Odition.ly, scmt of ese classes of in
region which is used to join two or more protein molec las noglobulins have sbcl ases, e,., Ig hi s four sub la
that are identical, substantially similar, or different, as (IgfJ1, 1g02, 1g03, and gG4). (ALlbert B. t al., Chap ci
defined above. 25 The Immune System, I Mole* far B aLa of the C’11
The present invention also relates to methods of using he Edition, Garland Pubi’ hing, 14c., I’ w ’ork, N.Y.
fusion proteins described herein. The fusion proteins of he amino acid qucns o:’hinge r4gion fro these cia at
present invention can be used in the same manner as ie imninunogiOb lies and ubclasss, at we as class s
non-fused, wild type protein, especially where an increa., ad subclasses I am oher species,(are noompassed 1
biological activity Is desirable, For example, EPO 1w on 30 invention.

proteins of the present invention, which have a longer in The hinge regio is flee divided to broe regio a: viva half-life than non-fused wild type EPO, can be User to upper, middl and kw r hinge. The ppe hinge is 1

treat various anemic conditions, as described further belt w, as the numb r of amino acids twot nthe end of Ii e

As a result of the work described herein, home- nd 35 domain of t a heavy ham (C,,1) ind he first c St
hetero-fusion proteins with increased biological activity are forming an iterhevy chain diulfld bi dge. The
now available. These fusion proteins can be injctcd 1 as hinge is big in:prolinc and contains theInter-heavy e
frequently, or at the same frequency but in smaller doses, cysteine dis Ifide bridges.!’he lowe hinge connects
relative to the wild type protein, possibly with fewer si e- middle hinge to the.2 domain. See Sandlie,
effects, 40 Michaelson. Chapter 3: Engi eei-in tht Hinge Rej Ic Optimize C Dmplvinen -induce Cy:)ly is, In An it

DLJAILED DESC1UFflON OF THE Engineering: A Prantic il Guide W. 1. Freeman an INVENTION New York,!,Y. th es bings o whi h a herein in a
The present invention relates to biologically actfre 1w on rated by rc it erence in their entirom See also H ix
proteins comprising one or more proteins tinkd by an 45 Casterrnan,., NauraJ Occu tng nti odies Dcv A
amino acid sequence comprising the hinge region seque cc Light Chain, 363 Nac re 446 993 an Terskikh,
of an immunoglobulin. "Peptabody A Ne4 T pe of Hi Ii A dit Binding P o
The basic immunoglobulin, or Ig, structural unit is kno n 94 Proc, Nat Madt Sci USA 1 63 (197), the teachi
to comprise a totramer, each tetrarnem having two identi al both of whic are Iere’ a incurp rated by reference
pairs of polypeptide chains. These polypeptide chains me entirety.
designated as "light (L) and "heavy "(H) in reerence to Previ?usr search ha LdeAtiflr the ml a acid seq e
their molecular weight. The N-terminal portioi of each of these thi a hinge r4 gions iE sam h man and
chain defines a variable (V) region primarily responsible ror subclasses dicate4 in he Table.

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As Used herein, the term Ig "hinge" region refers to a erythropoietin can be measured as described in U.S. Pat.
.polypeptide comprising an amino acid sequence that shares Nos. 5,614,184 and 5,580,853, the teachings of which are
sequence identity, or similarity, with sportinG of a naturally- herein incorporated by reference in their entirety.

 occurring Ig hinge region sequence, which tUCl1.HICS the Fusion protein constructs are named by listing the respec- cysteine residues at which the disulfide bonds link the two 20 tive molecules. for example, EPO-L-EPO refersto a homa-

heavy chs of the irnmunoglabulin. Sequence similarity of fusion protein comprised of two EPO molecules joined by the hinge region linkers of: the present invention with an Ig binge region. Similarly, EPO-L-EPO-L-EPO can refer naturally-occurring immutioglobulia hinge region ammo to a homo-fusion protein comprised of three EPO molecules

acid sequences can range from. at least 50% to about. With, an Ig hinge region between each of the.three EPO

75-80, and typically greater than about 90% molecules. Other combinations, such as hetero.4usion pro- Derivatives and analogs of the hinge region can be teinsa.re possible, e.g.,.EP04-EPO-L.lL3 and EPO-L-1L3-
obtained by mutations. Aderivative or analog, as referred to L-IL3. Where there is more than one Ig hinge region, a herein, is a polypeptide comprising an amino acid sequence single Ig binge region can be attached to another Ig hinge

that shares sequence identity, or similarity, with the ta11 region in the fusion protein, e.g., EPO-L-L-EPO: length sequence of the wild type (or naturally occurring -(j It is important to tote that the protein, e.g.,erythropcietin, protein), except that it has one or tnore.amuio sequence an he attached (e.g.,jôirted or linked) to the hinge via either

dieronccs attributable, to a. deletion, insertion arid/or sub- its amino-or carboxyl-terminus. For example, as described

herein, a fusion protein has been produced, I1,2hinge-FasL, The present invention also encompasses fragments of the where the proteins are attached to the binge via the amino

hinge region. Such a. fragment need only be long enough to 35. terminus. Alternatively, the attachment is made to the hinge allow the proteins attached by the hinge region:fragment 10 via the carboxyl-terminus of the protein. attain a biologically active conformation,.: - The fusion proteins of the present invention typically are PRODUCTION OF FUSION PROTEINS..., ti, ç,,,,i-,,,,c tli. eif,,iri’,tth, ct the

 :ai;; hinge ’region The term "recombinant," as used herein, moans that a
N-terminal of a second protein to. the C-terminal of the protein is derived from recombinant (e.g., microbial, viral,
protein.’hinge Thus, the fusion proteins of the insect or mammalian) expression systems..: "MicrobiaV’
present- invention have a formula of R1-L-R2, where R.is a refers to recombinant proteins made in bacterial or fungal,
pmotein,ll.2 is an identical or substantially similar protein to e.g. yeast, expression systorrisi Proteins expressed in most
 R, and L (Linker) is an Ig hinge region sequence. bacterial cultures will be free of glycan. Proteins expressed
Alternatively, the fusion proteins of the present invention m yeast may have a pattern dilernt from that
have a formula of R -L-R21 where R1 is a protein, Rz is a of proteins expressed in mammalian cells.
different, or substantially different, protein from R,, and L As used herein, the term "nucleotide sequcnce or
.(Linker) is an Ig hinge region. The proteins are linked to one "nucleic acid sequence" refers to a heteropolymer of deox-
 anothcr in such a manner so as to produce a single protein so yribonuvleotides (DNA) or ribonueleotides (RNA). Nucleic
which retains the biological activity of each of the proteins acid sequences encoding the proteins provided in this inven-
in. the fusion protein. tion can be asernbled from DNA, either eDNA or
However, the level of biological activity of the naturally- -DNA,. or RNA and short oligornicleo.tide linkers to provide
occurring protein, or fragments, analogs, mutants, variants a synthetic nucleic acid sequence which is capable of being
or derivatives thereof, need not be identical to the activity of.ss eressed in a recombinant transcriptional unit
the naturally-occurring protein (also referred to herein as the The term "recombinant expression vector," as used
parent protein).. For example, a fragment of a cytokine herein, refers to a replicable DNA construct used either to
pmoteirt may exhibit only 50-801110 Of the activity of the amplify or to express DNA which encodes, the fusion
naturally-occurring cytokine, yet because two or more proteins of the present invention and which includes a
cytokines are linked to form a fusion protein, the fusion eo transcriptional unit comprising an assembly of (1) a genetic
-. protein will:still exhibit increased biological activity as element or elements having a regulatory role in gene.
compared -rn single molecule of the naturally-occurring expression, for example, promoters or enhancers; (2) a
cytokine. Tests to determine biological activity that are structural or coding sequence which is transcribed into
specific for each protein component.are well-known to those . mRNA and translated into protein; and (3) appropriate
of skill in the art and can include, for example, measuring as transcription and translation initiation and termination
increased hematopoiesis, platelet productiod receptor sequences. Structural elements intended for use in yeast
binding For example the biological activity of a mutant of expression systems preferably include leader sequence

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intravenous, intraarterial, intramuscular, subcutaneous ends encoding the binge reglo:
inhalation, including but not limited to, intrabronchigi, intra oliganuclootidq inserts a uniqu
nasal or oral inhalation, intranasal drops; to apical) and non nucleotides 5’ o the &a lationi
parenteral (e.g., oral, including but not limited to, ditar oligonueleotidc elininaI
rectal). 18 nucleic acid aequec
The carriers will be non-toxic to recipients at the dosage hinge. IY ng two
and concentrations employed. The formulation used nligonucleotids, 27
vary according to the route of administration selected (e.g, amino acid of iinge r4gi
solution, emulsion, capsule). For solutions or emulsion*and a unique BatnHl i suitable, carriers include, for example, aqueous or acobo1i / 0 binge. SyntheIc olignt
aqueous solutions, emulsions or suspensions, irtelud g of the EPO,)NA srai,
saline and buffered media. Parenteral vehicles can inclu e change the 11 xxldn
sodium chloride solution, Ringer’s dextrose, dextrose a d B5U}II Site spanning th
sodium chloride, lactated Ringer’s or fixed oils. lntraveno s. the mature prtein and vehicles can include various additives, preservatives, r is the termination code.
fluid, nutrient or electrolyte replenishers. See, general io A 4 B

Reminwn’s Pharmaceutical Science, 16th Edition, Ma Polymerase halo Rac

Ed. (1980). For initalation, the compound can be solubiliz d plasrnid, psv -EPO (C and loaded into a suitable dispenser for adininistraUoa (e. 202:225(199)) as temp an atomizer, nebulizer or pressurized aerosol dispense). 20 are as folio a: Fusion proteins can be administered individually, together or (SEQ ID N

in combination with other drugs or agents (e.g., ot er CAGGCJGA AcIIGAC
chemotherapeutic agents, immune.system enhancrs). 3’.ACCCGT CAC.tC
Fusion protein compositions may be used to enha cc GAGAGA A (S]
proliferation, differentiation and functional activation of 25 3’.AJ’CCFA ’GCCCGI
hematopuietie progenitor cells, such as bone marow cc Is. (SEQ ID N: 26). Pthi
Specifically, compositions containing the fusion protein ay follows: 5’ G(GGCI
he used to increase peripheral blood leulcocyte numbers nd CATCTG GACA

increase circulating granutocyte counts in myelosuppre ’ d 3’..CAGG’l’ GACACA 30 patients To achieve this result, a therapeutically effe ye 1 reac ions (0 u quantity of a fusion protein composition is administere to 0,5 izM of 5’ prime or a mammal, preferably a human, in association with a p At-
of dATP, d p; d(TP maceutical’carrier or diluent. 2 reM MgC The present invention is further illustrated by the foil w- X-100; 10 rdl rind

ing examples, which are not intended to be limiting in ny Polymerase (StratÆgcn
way. ’mineral oil (50’juli M
EXAMPLE 1 ’ subjected to 25 eyles
52° C. for 1mii

PIOI)UCT1ON OF AN ERYTHROPOIEIIN 40 (extension) in r Pr’

HOMO-FUSION PROTEIN Next, lb DNA seq a
One molecule having great therapeutic value but w th a mined. Ii.t, the ’PC
number of problems that might beneflt through attach cot agarose ge using th
to the hinge region of an immunoglobulin nolecuI, is 45 They are igaied to p
erythropoietin (EPO). Erythropoietin is typically ad mis- contain an sefl to V h
tered by intravenous or subcutaneous injection three mes reactions (0 l) con
weekly at a dose of approximately 25-400 U/kg, at a y any ag of PC -blunt,i lx Ii
cost of several thousand ’dollars. Moreover, when ing ligasc (0. BL

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injections must be made in order to maintain a’ theraceutic TOP l0 Con!petnt Cellsj (Inv level of the compound in the circulation is a problem according to proredtre estabisbe
CON’STRUCI’ION OF THE EPO-EPO DIMER 3-mercapethanl h4 added t the
gentle swiling with a pipett tip,
The nuelcotide sequence of wild type erythropcieti4i can gs ligation d scribe in be proc
he obtained from Jacobs, K., et at,, Nature 321:806(985), is tocubat d on ice Ifor 30
which is herein incorporated by reference in its entir+ty. seconds a 42 q. r vial is
An EPO-EPO fusion protein is constructed by linkig two 2Sf) 4u1 of retvame (310 C.
strands of EPO.cDNA with a DNA strand encodixg the tryptone,.5% yastfextract,
irnmunoglobulln hinge polypeplide: GAGCCCAA4TCT- sO mM Mg, 10 mM MgSO4
TGTGACAAAACTCACACATGCCCACCAC- added an the edus4bakent’c

CGTOCCCA (SEQ ID NO: 22). The initial preceding EPO dilution f tran$ft4ed cell

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DNA strand, referred to herein as IIPO A is generated y the moittcat on, Sigwa agar plates
Polymerase Chain Reaction (PCR) using EPO sped syn- mycin an X-ga. Tte plates tre it
thetic oligonucleotides. The linking DNA strand is quen- 55 Colonies are plick1 and 2.5 ml
tially lengthened to the proposed length by usingps -EPO kanarny are inom4lated wilh the
as template and 3’ primers with appropriately exte dccl 3’ are prep red:fr4m I cover ’girt

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WIZARD PLUS MINWREPSTh DNA Purification System Gibco).at 37° C. and 10% CO2. The cells are harvested by
Clones are analyzed by restriction digest fragment analysis. trypsinizing using 0.05% Tlypsin, 053 mM EDTA(Gibco)
The pCRBlunt-EPOAand pCRBlünt-EPO B DNA clones. and washing twice with phosphate buffered saline (PBS)/6
are digested with Bgfl, which would give uniquo4izod MM glucose solution, Colls am suspended in the above
fragmints for a correctly Inserted DNA and an insert on PBS(glucose buffer to a concentration of W05. cells/ml 03
ented in. the reverse dfrectioti. Clones with inserts in ml of cells are placed in electroporation cuvettes (0.4 cm
reverse direction are chosen and larger atnowfls (from 100 gap, Bin-Red) and 20ug of linearized pcDNAIEPO-EP
ml The of LB/150, g/ml kanamyciii) of DNA plasinids prepared plasmid DNA is added. cells are olectroporated under Promega’s WIZARD PLUS MAXIPREPSrM DNA
usingPtdfication

the following conditions: voltage-1.5 kV, field strength-

System. Clones with inserts, in the ’forward’ x 0.75 kVcpa. capacitor-3 AF, and resistor none (Pulse con-
direction produce the proposed EPO-EPO DNA, troller set atQ). Cells are plated in3O ml. ofpre-warmed

The EPO A DNA strand is linked to the EP9B DNA 0418 (geneticin, Gibco BRL) and incubated at 370 C. and strand using the procedure described as fo1los p(RBlunt..
LPO A(-) are digested with BareHi and Not I and the 677 ’

5% CO. After subcloning, high producing clones are

bp tragrocat gel’ puritied. pcgBlunt-EPO. B(-) i digested is selected by screening supernatants for EPO by ELISA
’Pa with BamBI and XbaI and the 557 bp fragment gel purified. (PharMingen, San Diego, Calif.), controls are 10ug of
The EPO’A 677 bp fragment isthan ligated to the EPO B 55.7 "DNAEPO . and 10 g of r

ncDNAI 1(-)

.bp fragment in a 1:1 molar ratio of EPO A 677 bp fragment EXAMPLE 2
to EPO ’B 557 bp fragment. Ligations are carried out
overnight at 16° C. The ligated EPO A-EPO B DNA 20 EMPLIFICATION: PRODUCTION OF AN IL-2-
fragments are purifled.using QIAQUICKTh (leI"Extraction FasL FUSION PROTEIN

Kit and ligated to pcDNA2.1(-) which have previously been Only certain leukemia or lymphoma, cells, or recently digested with NotI and Xbal and gel purified. The ligation activated T cells, bear the trirnolecular high-affinity IL-211

reaction contains a 5:1 molar ratio of DNA insert to.(IL-ZR’ is expressed -as high, intermediate or low-affinity
. .

. pcDNA2.1.(-). The incubation is carried out overnightat 1.6 binding sites). The high ’affinity’ IL-2R is a specific marker of

C. Clones are picked. from ampicillm-resistant colonies by. T cell activation, and is an inducible element responsible for restriction digest analysis, produced in microgram.enhancing the affinity of the IL-2R for IL-2 (Smith, 1989

quantities, and used to tran.sfect.COS I cells.: Strom et al., 1993; Strom ót al., 1992). The principle.ofusing
TRANSIENT EXPRESSION OF EPO DIMER IN 30 therapy directed agairisube high affinity IL-2R asamean:
Cos I CELLS of achieving selective immunosupprossion arid/or tolerance
is (Strom at al,, 1992; Waldmann, 1993).
’COS I cells are grown to 70% confluency in Dulbecco’s With a number of problems of immimogenlcity, suboptimal
Modified Eagle Medium, high glucose (4.5’ g/L Qibco),. affinity, short circulating half-life, and inability to direct host ’.

,

10%. fetal bovine serum (Hyclone) in the presence of.100 U 35 oytojytfc immune effector mechanisms against target cells ’

penicillin, 100 Mg streptomycin, 250 ngFungizone per nil of. the current high TL-2R targeting strategies, including mono- tissue culture medium (antibiotic-antimycotic cocktail from clonal antibodies directed against the p55 chain ofthe 1L211

Gibcn) at 370 C. and 10% CO2. The cells are harvested by and IL-2/toxin fusion proteins, may impose limits on their

trypsinizing using 0.0$%iypsin,0.53 mM EDTA(Gibco) clinical utility. These problems could be addressed by link- and washing twice with phosphate buffered saline (PBS)/640,’

mg the IL-2 poptide to the the FasL peptide via the unmu .

. . mM glucose solution. Cells arc suspended in the. above
PBS/glucose buffer to a-concentration of 2x166 cells/mi. 0.5 noglobin hinge region peptide, ’

:

ml of cells are placed in electroporation cuvettes (0.4 cm The structure similarities of FasLwith TNFa suggests that

the FasL also exists as a trirner. Moreover, the, anti-Faa

gap, Bio-Rad) and 10 g of pcDNAJEPQ-EPO added The antibody is an Ig M (Yonebara at al., 1989), a pentamer cells are electruporated under the following conditions: 4$ tending to not aggregate, whereas the anti-APO-1 antibody -.. voltage=0.3 kV, field etrength=0.75. kV/cm, capacitor250 is an 1g03 çFraiilh at ’al., 1989), which tends to aggregate. E, and resistor -none (Pulse controller set at:), Cells are The F(ab)2 fragment of the anti-APO-1 antibody and its
plated in 30 ml of pro-warmed DMEM, high glucose, 10% iaotvpes have little eytoto*ic activity (Takeshita etaL, 1992). FB& and incubated for.72 l at 37° C and 10%, CO2. The 11o’ever, these divalent anti-APO-1 antibodies induced - controls axe 10 ig of pcDNA-LPO and.10 g of pcDNA, apptosis when they are cross-linked by second antibodies.

2.1(). . ...

ThCse results indicate that dimerization of Faa is insufficient

The conditioned media is collected and centrifuged at to traosdue the apoptotic signal, and they are consistent
13 SOOxg for 10 mlii at 4° C 1 ml aliquots of each with a trimenc structure for the Faa hgand Therefore the
conditioned media are dialyzed against Minimum -Essential IL-2-FasL fusion protein requires target cells that express .

Medium a overnight with 3x changes.of medium. These ss both high affinity IL-ZR and Faa in order to induce epopto.. samples are assayed for.EPO activity by the method Of sis. Thisfusion protein would target only certain leukemia r Krystal...

lymphoma cells, or recently activaredT cells which bear

STABLE EXPRESSION OF EPO DIMER IN: CR0 both h. affinity IL?2R and Fas. Thus.. the target specificity
is ensured by the dimerized form o fusion pro The CELLS.

:

o FasL protein is a trausmembrane protein where the ecto ..

Chinese Hamster Ovary cells (CR0) and NS.1 myaloma cellular portion is the C terminus end of the protein. This
cells can be used for stable expression of EPO-EPO protein, was considered in making the fusion protein described
Cl-IC) cells, are grown to 70% cionfluency in Duibecco’s below, ...
. Modified Eagle Medium, high glucose (43 g/L Gibco,. CONSTRUCTION OF THE M1J1INE TL-2-FasL FUSION
10% fetal bovine serum (Hyclone) in the presence of 100 U 65 PROTEIN ..
. penicillin, 100 ug streptomycin, 250 rig Fungizonc per ml of The nucleotide sequences of wild type murine IL-2 and
tissue culture medium (antlbiotic-antimycotic cocktail from. FasL can be obtained from Kashiroa, N., at al., Nature

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United States Patent [19]

Young et al.

[54] ERYTI-IROPOIETIN RECEPTOR
ANTIBODIES

[76] inventors: Peter Ronald Young; Connie L.
Erkkson-Miller, both of SmithKline
Beecham Corporation Corporate
Intellectual Property

- UW2220’ P.O.
Box 1539, King Of Prussia, Pa..
19406-0939

Notice: This patent issued on

a continued pros-
ecution application filed under 37 CFR
1.53(4), and is subject to the twenty year
patent term provisions of

5 U.S.C.
154(aXZ).

[21] AppI. No.: 08/176,511

[22] PCT Filed: Jun. 7, 1996

[86] PCI’ No.: PCTIUS96/09613 § 371 Date: Jan. 28, 1997 §.102(e) Date: Jan. 28, 1997

[87].PCT.Pub. No.: W096/40231

PCT Pub. Date: Dec. 19, 1996

Related U.S. Application Data

[631 Continuation-in -part of application No. 08/474,673, Jun. 7, 1995, abandoned.

[Si] mt. C1.7..........................A61K 39/395; C12N 5/12;
C12P 21/08; C07K 16/28
[523 U.S. Cl,

.... 424/141.1; 435/325; 435/334;
530/388.1

[58] Field of Search..............................530/387.1, 387.3, 530/388.1, 388.23, 389.1, 3K2, 389.6;
424/141.1; 435/325, 334

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US006153190A

iij Patent Number: 691539190

[45] Date of Patent: *Nov. 28,2000

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Primary ExaminerGary L. ICunz
Assistant ExaminerStephen Gucker
Attorney. Agent, or FirmKirk Baumeister; William T. King

[57]

. ABSTRACT

Monoclonal ’antibodies to the arythropoietin receptor are disclosed.

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2 Claims, 16 Drawing Sheets

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ERYTHROPOIETIN RECEPTOR parable to th t ot ti
ANTIBODIES of the recept r

CROSS REFERENCE TO RELArED S MM.
APPLICATIONS s In one as Ct, flu

reliably gen rating. This is a national stage filing under 35 U.S.0 §371 of receptor. Thi moth PCT/US96/09613 flied Jun. 7, 1996, which is 8 a recombin limo

continuation-in-part of application Ser. No. 08/474,673 6 ad cellular dam in of
Jun. 7, 1995, now abandoned, io second extra th4a

FIELD OF THE INVENTION moiety. The

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The present invention relates generally to the generat on functional d main
of monoclonal antibodies by use of a selected antigen: and occurring in timei
more particularly to the generation of monoclonal antibo ins 15 an ac d spa
which are receptor agonists. portion of burnt
helix, for ox rnple.
BACKGROUND OF THE INVENTiON In anothe asec

Avast majority of receptors of the single transmembr ne duced by tb abo
class respond ligand binding by some form of aggr A.

20 generated a char

Lion. This aggregation can be between identicl ieee tar naturally urring
subunits (as in homodimerization, hotnotrimetization, biological a tivity
or between different receptor subunits (as is invention

antibodies,

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onocl heterodirnerization, heterotrirnerization, etc). This aggr
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unable to interact with the second subunit are still abl to sequence enoding the lextrt
bind, but no longer cause dimerization and.bloingical ati- molecule tu4Od In tram to a
vation of the receptor (P. R. Young, Carr. Opin Eioteh., a specific prteqlytc cijeaval
3:408-421 (1 992)1 I associated with a tridgng n
For example, there evidence in the literature hat Yet a furtier as
Suggests dimerization the erythropoietin receptor multimeric immtin
upon ligand binding Watowich at Additionail aspe
and Watowich et al., comprising a
Reports at out under the trol
have indicated that its second subunit inay directing re licalia
dimorize upon I1-6 binding Murkami et a’., sequence a
260:1808-1810 for receptors Still othe
homodirnerizaticin induced by ligand binding, nal reagents prism!
antibodies were discovered which had agonist of Lion,ai
erties. These include mAbs to and growth or- condition b admir
mane receptors Schrieber et the inveutio
258:846-853 Defize et al., Other asp cts au
5:1187-1192 (1986); Engelmann of al., described rther
265:14497-14505 (1990); and a!,, preferred bodin
256:1677-1680 (1992)]. In each case, these mAbs, by
their two antigen recognition sites, were able to b ing
together two receptors and thus activate them. Fab ag- a gra
mauls made from these mAbs were inactive. In some ses, fusion prot
the apparent affinity the antibody for receptor was M. on erythrop ietin
parable to that of the ligand, e.g., growth hormone et positive ntroV
al, cited above]. a gray
It also been discovered that antibodies fusion prot
tor have agonist properties [Suguwara at al., an erythro t,ietin
140.526-530 (1988)]. Previous, literature has describec the itive
production anti-erythropoietin receptor antibodies gray
D’Andrea.etat,Blood, 82:46-52(1993);A.D’Aidrea al., fusion
84:1982-1991 and Yet et 82:
See also, PCTApplication 438

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1996. While the Yet et al, refer nce
suggests the occurrence possible EPO-like activity one antibodies evelopq
the not characterized. Neither Yet al., no r the EpoRFe ion pro
other literature provides any reproducible manher and
ating agonist mAbs. fluoreseen’
There remains a’ need in the art for develapmc t a ba
additional have an affinity for receptors rn-

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plotting Epo maximum vsi dilutions ofsupernatants. The.. IGF2, and PDGF. PDGF includes ditheric ligand made of
supernatant designations are listed above the bars. two chains 4 and B and two receptor subunits a and.b.
HG. 6 is a bar graph demonstrating the ability of 4 mAbs and receptors can associate as horuo- or bet-
to EPORF to stimulate the prOlitbration and differentiation erodimers. Re1ain is also anticipated to be a. dunieric
of human boric marrowprogenitor cells to form red blood receptor prior to ligand binding based on its homology to
cell colonies (CFU-E), plOtting. Number of CFU..E vs,
positive and negative EPO controls and supernatant

insulin and IGF.

Receptors which are formed by beteroditners of two

tions The supernatant designations are list.edabOve the bars. different subunits may also be employed as contributors of
FIGS. IAIF is the DNA sequence [SEQ ID NO: 1] extracellular domain(s) of one or both of the subunits to the
in recombinant. Such receptors include, inter alia, GM-CSF, plastoid.tntalsEpoREc containing the sequence encoding the
EpoRFe fusion protein under control of a Drosophila S2 mto Tf- IL-5,.IL-6 ricostatin McN’F, i,,FGF,
promoter. Nuc1otides I. to 897: contain the promoter IL-13, a,.. an
Receptors, such as IL.3, which involvemore than one sequence. The EpoRFc fusion protein [SEQ ID NO: 2] is
encoded by the following: nucleotides 898 to 1647 encadó subunit may respond to this method, if dimerization of one
the EpoR extracellular domain protein, nucleotides 1648 to isof the subunits is required for signal transduction.
169 encode the Factor Xa cleavage sequence; nucleotides Receptors which form other aggregations, such as trzrncrs
of IL-2 receptor subunits, may also be used as sources of 1660 to 2361 encoding the human lgG1 Fe sequence. The.
remaind6r of the. sequence is derived from the plasmid extracellular domain(s) for the recombinant miniunogen of
See Exam Ia j.

this invention.

20 The extracellular domains of the selected receptor useful
FIGS, SA-8h is the DNA sequence [SEQ ID NO: 3] of in the recombinant immuuogen may be Isolated and/or

COSFCLInk vector from which a Kpnt/XbaT insert containing otherwise obtained by resort to the published and publicly the IgGi ic region was obtained, available receptor sequences. Methods conventional in the
DEFAILED DESCRIPTION OF art may be employed to isolate or synthesize the appropriate
INVENTION 25 nucleotide, sequences encoding these domains for furthr

manipulation in. the generation of the i.mmunogen of this
Thepresent invention provides reproducible and reliable invention.
composition and method for generating antibodies which By. the term "bridging moiety’ is meant a peptide or
demonstrate the agonist properties ofthe: naturally occurring non-peptide sequence that stably associates with itself and
ligand. of a receptor molecule. While, in principle, any in places the first extracellular domain and the second extra
purified preparation could be used to generate antibodies to cellular domain into functional proximity (La., a relationship
receptor, and from these some may be agonists, this between the two or more domains which mimics the three
:invention pinvides a.specific immunogep which corresponds dimensional functional proximity of the domains in the
to a znuiti.meric form ofa receptor in which the extracellular naturally occurring multimeric receptor, during or prior to
domains are in a similar disposition to that expected for 35:ligand binding. Preferably, the bridging moiety Is of a
receptors having two or more subunits the surface of the sufficient size to bring together the membrane proximal
cell regions (i.e., the regions closes to the transmenibrane
I. The Recombinant Iwmuxiogen region) of the extracellular domain. The precise dimensions
The recombinant-immunagen of the.prescnt:invóntion of the bridging region can be estimated from the relevant
consists of an extracellular domain of a selectOd receptor 40 crystal structures, such as those for growth hormone and its
molecule which is involved in ligand binding-via the inter receptor, or ’IV arid its receptor [see, e.g., DeVos et al.,
action of more than one extracellular domain. The extracel- Science, 255:306. (1992)].
lular domain is spaced apart from a second extracellular The suitability of a given bridging moiety can some
receptor domain by a bridging moiety. times be evaluated empirically. For example, a dimerie form
By. "extracellular domain" is meant that portion of a 45 of a receptor which dimerizes upon binding of ligand is
receptor protein which is localized on the extracellular expected to have a higher affinity for ligand than a mono-
surface of a cell and which contribOtes to interaction meric form of the extraceliular domain of the receptor, if the
binding with its native ligand. bridging group is appropriate.. This would be evidenced
The receptor subunit which contributes the extracellular either by direct binding studies of ligand to monomeric and
domain(s) of the.immunogen- may bO any receptor subunit so dimeric receptor, or by the relative ability of the two forms
which accomplishes ligand binding via a bopiodumer of the of receptor to neutralize the biological activity of the ligand.
extracellular dornain,.a heterodituer oftwo different subunits For example, FIG. 1 shows the ability of dimoric EpoRFe to
of the receptor’s- extracellular domain, or a. multirner of neutralize Epa activity about 100 times more effectively
subunits. Receptors which homodimerize upon ligand. hind than monomeric EpoR.
ing and thus may ontrihute extracellular domains to the An example of a bridging moiety is an amino acid spacer
recombinant immnunogeri ixiclude.those for, inter alia, eryth. sequence (e.g., between 1 to 10 amino acids in length and
ropomelin (CPO) and thromhopoietin (CMPL) (3 (’ST optionally encoding a cleavage site) fused to a dimerization
M-CSF, TGY-a, EGF, mice, growth hormone, prolactin, pin- or oligomerization domain which permits the formation of
centallactogen, c-kit.(stem cell factor receptor), p50 and p75 dimersoroligomers, respectively. In the-following example,
receptor. subunits of TNFa, and TNF. Other receptors 60 for instance, a spacer encoding the Factor Xa cleavage site
which are anticipated to dimerize based on homology to is part of the bridging moiety, and the dimerizatian domain
TNF and which can be included in this list are. Fast CD40 is the hinge CH2CH3 region of a human IgGi. This lgG
.CD27, CD30, 4-lOB and 0X40. component consists of the CH2, and (’113 domains and the
Similarly, receptors which are already in a hoinodumeric hinge region of IgGi including cysteine residues contribut-
form on the cell surface, prior to ligand binding may also es ing to inter-heavy chain disuMde banding, for example
contribute the extracellular domain(s) to the immunogen. residues 11 and 14 of the IgGi binge region [see, e.g., B.
;. These latter receptors include, inter alia, insulin,. IGF1 and Frangione, Nature, 216: 939441, (1967)]. Preferably the

,153,190

. 5 . 61.1
IgGi component consists of ammo acids corrospondin to domain This the first ridging oic cai be a CHI
residues 1-4 and 6-15 of the hinge, 1410 of CH2 and 1 01 of a light clain of!a acted i4unin g1ombn Its c ii
of CH’ of IgGi described by J Ellison at al NuclezcA 1d5 rnentary brilging &ioi ty is th CH region of the h
Res., 10: 4071-4079 (1982), Residue S of the bing is chain of thel same nog1obiUn o’ th entire?c
changed from ystelno in the published IgGi sequen to s including,th Cl-Il hi go,CII$ an Cl-fl regions
alamne by alteration of J Gi to (ICC in the iuc1eo dc heavy chau It is anticipted hat various b
sequence entities ma he erip1 yed in jrepa ng other mult c
The bridging moiety can also be an lc portion of ny imnunogen4 c g In era by asso atzeg three id
human inimunoglohuim with an intact hinge CR2 domains or three extra ellular 1oma s t cm one
region, including those derived from Ig(IgE, 1gM, IgA d in three subums o a sin e receptor. It p esently pm
lgG4. Still another suitable bridging moiety is an 4niiuo cid to use an F potk$n o an imuuoog ob in as a bttri
spacer fused to the C terminal domain of the TNF a moiety to asocIat th extracellular ccc tor coma
receptor of the shope fibroma poxvirus [C A Smith I, disclosed in Example 1 below.
BflRC, 176 335.-342 (1991)] The bridging moiet’ may iso II Constructori and Pm aration f Lb Re mbmant I

be an a dmienzation domain Like an anaphipathic helix, cli is nogeri I as a loucme zipper [see, e g, P Pack et al.,Bioc/ze,n., 31 6) The presnt invntz n also jirovi as he nucleic

1519-1584 (Feb 18 1992)) The bridging moiety may sequences ethoduig the ecombuant 1 m ogeus dc
be alkaline phosphatase. :. above. The niclaotile uenee enix ling the oxtrac I
Still other desirable embodiments of the bridging moi ty domains of e recepto useful the unogens in
are organic molecules which can functionally associate he 20 obtained frozi kuot’n r plor equo ces by conven
receptor extracellular domains as desired Sm.Lcb org ’c. means [see J Sambmo at al., Mplecu ar toning: A
molecules may he selected from among such rnolecu as rarory Main 1, ed Cold $prmn H rbor Laho
known to associate peptide sequences to each othefor ot or (1989)] For xap a uucleo des qua ces which
biological uses, e g hifuncuonal c’ross linkers such as the iv no of he nventio ma be lated by
carbodninide, glutaraldebyde and DSS BS3 and o cc is ventional use of polym case cii a rca tio or coriven
which may he obtained from several commercial scum a genetic engiàeripg lo ng tech ique Al emotively,
However, these associations may require combinatioü ’th sequences m y be con ructed sin oh mica] syn
specific target sequences for cross linking e.g., oil expo ed techniques
Cys or His for nickel chelate to achieve the appropri to Optionally a nucleoti e seque cc vInc encodes a
three dimensional disposition of receptor subunits. Cho ce in tide sequence which pro ides an azy atic leavage s
of an appropriate cross-linlccr can be determined by on which many e well,ho wn in t c art is d in fra
parison to known crystal structures of homologotis rec p the extracell lar do0al nuclec ide a quence prior
tors association w th the çlim rization r oh om rizatmon do
The suitability of a particular peptide or non-pep’ e of. the bridg g moiety This f ilita as leavage 01
entity as a bridging moiety may be functionally assessed in extracellular omaimi Fr m the bidgi g olety folio
a receptor ligand binding assay. The suitability of t e expression
bridging moiety may be determined if the recembin nt According to the linvil nIzon, tile nui We acid saqui
immunogen binds the receptor’s intended ligand w th encoding the extracellu ar domrins ay be modi–i
greater affinity than does the monomeric sequeoe of I e desired It is withmr Lb skill o the art to obtain
receptor extracellular domain Ligand binding assays for t a 411 polynucleotid seqiince encodt4g th a r captor dor
selected receptors are known to those of skill in the art a d useful in the invnton uch m4diü tin at the nu
maybe readily selected without undue experimentation S a acid level inc ude, fcr a pie, thodifi atuns to the ii
for example, the EpoR hgand assays described in (oma ii otxle sequenc wiuctli ai silent o win he ange the a
et al,, Blnexl, 82:456-464 (1993); Miura ci a],, ZktoL Ce!, acids, e.g. to mprosc a ression or Sc retibn. Such ii
Mot, 11:4895-4902 (1991) Witthuhn et al., Ce 1 4S fications mc1i a amino t rmmal, arbo trrminal or
74:227-436 (1995). . nal deletions 4f the imuel ethic selpien
id

dsired.

Mother bridging moiety or linker may be prejiared y Where the lridin in ’eLy is ax ’ sequerici
mutagenesis ofa receptor in the membrane proximal dom n nueleotide se4uence en cling it s a ained cor
to create unpaired Cys, which can diulfide bond to create a tionally and used to I e extra Hula domain seq
dimeric recepmor. Such mutations can be evaluated for appr so directly or a cleava a site oi via ddimonal seqt
priateness by observing whether the full length irecept r intended as spacr r xani le he the bric
containing such a mutation is able to conatmitively activa e moiety is an c portion F a hu an mumoglohulzn
the ligand s activity upon transfection into suitable targ t intact hinge Cl Hregi ii then cleot ciasiquence ci
cells (e.g., EpoR mutations) [see, e.g., Watowich eta! P ing the Fe grnri is hiaed m fm krlown anti
Nail Acad. Sci, USA, 89:2140-2144 (1992)] as sequences pr pared jy nventio a] t hnzues and I
Thus for example the bridging moiety of the tecomb in frame to e recepto seque ces r t the seqi
nant mmmunogen can associate two identical extrcellul r providing the nzymnitzc leavage site I
domains into an imniunogen that mimics a homoduma c To produce ecomlmn t imnnm gcw of tins invent:
receptor. Alternately, for example, the bridging moiety co a DNA sequenc of the invention a codi ig t extrace
associate two different extracellular domains of subunits of so receptor doin is fl,md in from to a i op ional dci
one receptor into an immnunogen that mimics a he site and furthe fused to nucleotide queice encod
erodimeric receptor. For example, for the htergdime peptide brid g moiety. Prepara$ion f e nucleic
association of different subunits from a hetercdumeri sequences ma be earned out cboricall It e zymatical
receptor, a different bridging moiety could be used foreec by a coinbina on of the 1 o inethds vit o or in vi
receptor subunit. The bridging moiety for a heterdimeri es appropriate, us, the W A seque ces ay prepan
receptor is preferably a domain that cannot associIc wit the en2ymauc ligatuop o appropr ate NA fragment
itself, but which preferentially associates with a secon conventional ethocls su h as th se d sen ad by E

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SUPERINTENDENCIA DE INDUSTRIA V COMERCIO DIRECCIÔN DE NUEVAS CREACIONES.

Abogada
Jimena Escobar Uribe

ASUNTO Radicaciô.n 06-38331
TrÆmite 011
Evento 001
ActuaciOn 430
Oficio

Folios f86428 o-;U

Patente de tnvenciôn fl Patente de Modelo de Utilidad

Via Nacional

Via PCT (fase nacional) Cap.l fl Cap. It

No. Sol. Internacional PCT/US2004/028976
Publicación Internacional W02005/032460

NO

1

Como resultado del examen de fondo, realizado con fundamento en el articulo45 do la Decision 486 de la ComisiOn de la Comunidad Andina, se le cornunica:

1.. Documentos estudiados

DescriDciÆn:

Reivindicaciones:

Secuencias:

Prioridad:

2. Objeto do la invención

Folios: 12 a 182 como se radicO inicialmente.

Folios: 183 a 197 como se radicO inicialmente.

Folios: 198 a 325 como se radicO inicialmente

30/09/2003, Estados Unidos, US 60/507,349

Titulo do la solicitud: "Mimeticuerpos humanos centrales do la region do bisagra, mimOticos de la erltropoyetiæa,coniposiciones, mØtodos y usos".

El objeto de la presente solicitud do patente de invenciOn consiste en suministrar cuerpos mimØticos de la region central de bisagra de la eritropoystina (EPO) de mamifero, porciones especificas y variantes para proteinas biolOgicamente activas, ligandos, Æcidosnucleicos cod ificantes, cØlutas hospederas y mØtodos para hacer y utilizar los mismos.

El problema planteado en esta soil itud es que los p6p idos Oirr Øti -,os disponibli s tienen una vida media yactividad.. aja respecto a la-E 0 rec.rn in ante, p rIo q e existe la necesidad de proporcion r versiones nejOra Jas yo n iocificada 3 de Jas prot&nas terapØuticas EPO que s peren uno a as d est$ pr bi mas

Para solucionar este problema tØcnico la solicitud en es ud o pro o cio a mimeticuerpos humanos centrales dela regi6nde, bisag a, mik co de la EP0,sils Æcidosnucieicos,mØtodó de preparaciÔn y corn osióio esqtie lo c ntiene

El objeto de la sohcitud definida an enormente s3 clasifco er la P A61

. 3. De la solicitud de Patente ,. .

.3.1. .Reivindicaciones:(ärtloulo 0 D 486

El capitulo reivtndicatorio presenta o carece de o6ncisi n y qlaridad por C41 to

1) En general el capitulo reivindic torto present do car ce d ci ri4ad y c cisi n puesto que la mayoria de reivin icaciones c ntehe tôrmiios ar ibiguot que o
permiten definir o limitar la mv nción, par e emplo se ua r i$ratmva nte E0
tØrmino "al.menos una", inclusa varias veôes n Ia rr isma eiv ncicaciOn, I o cu al

no permite definir el objeto al q e se refiere (eivmndm aciohes 1- 4, 26-34);

2) Existe un alto n.ümero.de reivindi aciones md pendi ntes 1de-40)dela isrra categoria, lo cual adŁmÆs des r una faltà d. conci IOn, pae ta una f Ita. e unidad de invenci.On de parte de la.solicitud;

3) El Æcido nucleico de un m meticuerpo entral al clue se refie e i le is
reivmndicaciones 1-4debeserde mnidoporsus aracte 1sticstØ niases n male incluido.la secuencia. nucleOti a especifica que I define. Si son niOItipl S
secuencias estas deben estar sustentadas n I esçriciO demo t ar u
sirnilitud estructural;.

:

4) De igual forma el polipØptido de. un mimeticu rpo a ntral al que se refi r n las reMndicaciones 5-20, dØbeser clefinido por s secu n

Icial specificaeinliearkis
posiciôæes qUe incluye en Østa secuencia, si es el casol Ad qrrAs defini rlo c Ł
. maæeraconcisa y no. a travØs de tantas reivi dicaci nes Inde p ndient s, Pu S hace que el capitulo carezca de concisiOn, I

5) Las reivindicaciones 2.1 a 26 quf se refieren un Aci o nuIei o, un antictierp
un vector 0 Una cØlula, no menc onan las can icterist,,as tcni as, esenc a es dØ
los productos a quo se refieren, es decir los quede men 6.0 St uctura, p ues o
que por ejemplo no se mencion n las EEO qL e oonforman1l el ci o nucl I o,. I s
SEQ CDR del anticuerpo las IS!-Q’y. tipoS do vector que e e np lea, las EQ 0 vectores que contienen las cØlu as; :

6). El mØtodo æiencionadoen lar ivindicaciOn 7 no esta d?fini do a parti e I’a $
etapas que lo conforman, sino quo se refie e äu a ünca t pa ge e al de
.f traducciOn de un Æcidonucleic (sin menclo ar: Ia EEO a la qw se ref ere) do manera que no parece un mØto o sino un pa ibleu a de a I v nción;

7) La composiciOnmencionada en las reivi.ndicacion’ee 28 a 31 no es definida partir de loselementos fÆrmacolOgicos.(SEQpoliØ tido) $1 Ia p oporci n S d
los mismos, de manera qUe no es posible Jefinirla. Ad4m s Iguna e las

605

formas propuestas de esta composlctón carecen de sustento (reivindicacón 30 y 31);

8) Un dispositivo que comprende at meno.s un polipØptido, anticuerpo 0 Øcido nucleico (reivindicaciones 36-38), no hace parte del cuerpo cierto de la invenciOn, puesto que este dispositivo tiºne sus propias caracteristicas que no tienen que ser compartidas con las caracteristicas esenciales del mimeticuerpo de la
invención

9) La reivindicación 38 presenta una falsa dependencia;

De esta manera, el capItulo reivindicatorio presentado no cumple con los requisitos establecidos en el art 30 de la Decision 486.

4. Excepción a la patentabilldad (articulo 20, literal c) y d))

Las reivindicaciones 32 a 35 se refieren a un mØtodo de tratamiento que puede ser aplicado a humanos y animales. De igual forma las reivindicaciones 39 a 40 que se refieren a un mØtodo que produce cØlulas, plantas o animates es considerado materia no patentable.

Teniendo en cuenta que segUn la norma comunitaria no se consideran patentables: "C) las plantas, los enimales y los procedimientos esencialmente biolOgicos pare Ia producción de. plantas o ahimales quo no sean procedimientos no biolOgicos o microblob gicos; d) los mØtodos terapeuticos o quirürgicos pars el tratamiento humano o animal, asf como los mØtodos de diagnOstico apilcados a los seres humanos o a an/males", la maeria de (as reivindicaciones mencionadas deberÆ excluirse.

5 No es una invenciôn (ärticulO 15 D486 literal b)y c)

La cØlula tal como esta definida en la reivindicación 25-26 no hace parte.del cuerpo clerto de la invenciOn pues no posee sus caracteristicas esenciales definidas. Teniendo en cuenta que segün la norma comunitaria "No se considerarØn invenciones: b) el todo o parte do los seres.vivos tal como so encuentran en ía. naturaleza, los procesos biolOgicos naturales, el material biolOgico oxistente en la naturaleza o aquel quo pueda ser a/s/ado, inclusive genoma o germoplasma do cualquier ser vivo natural", la materia reclamada en estas clÆusutasno podra ser patentada.

De esta manera y de acuerdo a Jo establecido en et articulo 15 de la DecisiOn 486 el contenido de las citadas reivindicaciones deberÆ excluirse.

6 Determlnación del Estado do la TØcnica

La fecha que se ha tenido en cuenta para determinar el Estado de la TØcnica es: Fecha de presentaciOn de las solicitudes prioritarias: 30/09/2003.

Teniendo en cuenta la fecha indicada se realizO (a büsqueda en el estado de la tØcnica de documentos retactonados con (a solicitud encontrØndose los sguientes documentos mÆsrelevantes:

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regjon’lihke 26/12/ Doohinge I
D2 US 6,15190 poietin re e r ai itibodis’ _28/11/ 000

Resumen de los clocumentos citac os

Dl la invenctOn se relaciona con protelnas d fusiO i cor ac
mØtodos pare hacerlas a travØs Ide uniOn Co alent de dos
pohpØptidos, o derivados de est s La union covale ite e fc
secuencia aminoÆcidade Ia idir bisagra de I inmur oglobulir

D2 la invenciOn provee un mØtodo para la gener iciOæ da un ntic antagonista de un receptor. Este inditodo empi a COMD antger inmunOgeno recombinante el cual i onsiste de ui primei domin,o molecule receptora espactado de tin segundo d rntnio xtracelu por una fracciOn de puente

7. AnÆlisisde patenbilidad (a Iculo 14 D4 6)

a Nivel inventivo (ArtIculo 18 D4815)

La solicitud se refiere a Æcidosnuc Øicos de un r inheiici ierpo cer bisagra, mimetico de la EPO, qL e comprend al m nos in codifica al menos una secuencia de aminoªcid)S de I s SE) I polinucleotido complementario del mismo

Dada Ia ambiguedad de la invenci(n reivindicad bus iued se cuenta que multiples secuencias drian estar ii cluida en el Æci mimØtico de EPO

Tal como manifestO Is autonc ad internaci inal del exam patentabilidad, esta oficina esta d acuerdo en ue b doci me (DI) US 6,153,190 (D2) cort prometen el nivel inven,vo Especificamente, el documento Dl enseæa lapr duci n de rot una region bisagra de inmunoglob line ligada (column 13 I1na
52) En sus ejempbos esta Ufl co istructor de uslón ornpen (columna 6, linea 15-36) protein de homofu iOn de EPO (col columna 15, lInea3O) TambiØrien enametodosparh icerp otE qua ía protelna de fusiOn puedi ser usada ara, tr itar ne composiciones farmacØuticas que contienen la f irotelnede fu 16.r 45- columna 13, linea 37)

La diferencia entre Dl la solicitud en estudio e qua el docuiner anticuerpos especificos que conte igan una reg On con las cra en la solicitud Por tanto, el prot lema resuelt’ pot I invncl protemna heterodimØrica con propi Wad de 6,geno.

Sin embargo, tab desarrollo ya ha fa sido divul ad e D2, en mØtodos para generar anticuerp s de diverso tipos (hurrani policlonales monoctonales), se divulge un mm inOge o mutmm (columna 2, linea 5-45), fraccione moviles de ti o CH2 CH 3 (c 65columria 6, lmnea 66) Tambie se enseæa c moh4 cerum in

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c ilumna 12, bin I

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II?Isticasfiflka

To

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Os, qu eric

heterodimØrico (columna 8, linea 37-55) y sus diferentes usos como mØtodo de diagnóstico y de investigaciôn (columna 12, linea 57 - columna 13, llnea 50).
tn

Por tanto, a partir de los documentos Dl y 02, un tØcnico versado on la materia habria podido realizar la invenciôn tal coma es reclamada en la solicitud, par tanto no es evidente el aporte que hace La invenciOn al campo tØcnico, hacienda que Pa solicitud incumpla carezca de nivel inventivo e incumpla el articulo 18 de la 0486.

En cumplimiento del articulo 45 de La DecisiOn 486 de la ComisiOn de la Comunidad Andina, se le indica que dØbe dar respuesta dentro del tØrmino de 60 dlas contados a partir de la fecha de notificaciOn de la presente comunicaciôn. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentospara la concesiôn, se denegarÆla patente.

NotifIquese el presente oficlo conformea Ia dispuesto en el numeral 5.2, literal e), del capitulo quinto del titulo prLmero de Pa Circular Unica No 10 de 2001.

JOSE LUIS SALAZA LOPEZ
Director de Nuevas Creaciones (E)

El anterior, requerimiento fue notificado por fijaciOn en lista, de fecha 2Ot

CONCEPTO TECNJCO No. EXAMINADOR TECNICO
4595 Codigo No.202052