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SUPERINTENDENCIA DL INDUSTRIA V COMLRCIO

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No. 06.101162- -00005-0000
Seæora Fecha: 2009-10-23 15:21:13 Dep. 2020 NUECREACIONE Ira. 11 PATENTEIYII Eve: 378 FASENACIONALI

Alix Carmenza CØspedes de Verge - Act. 593 IMPULSOPROCESAL Folios: I
Jefe de Division de Nuevas Creaciones
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
E. S. D.

Referencia: Expediente No. 06.101.162
Solicitud de Patente de Invención titulada: "AtenuaciOn sinergIstica de virus
de estomatitis vesicular, vectores de estos y composiciones inmunogØnicas
de estos"

Solicitante: Wyeth

DE AGILIZACION DEL TRAMITE

ALVARO CORREA ORDONEZ, mayor de edad.y vecino de Bogota D.C., identificado tat corno aparece at pie de mi firma, en iiii carÆcter de.apoderado de la sociedad en referencia, solicito respetuosamente que se agilice el trÆrnite para elexpediente de la referencia.

VARQY’CORREA ORDOEZ
79.143.366 de UsaquØn
T.P.20.28 I

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Industria y Comercio
SUPERNTENDENCIA

DIRECCION DE NUEVAS CREACIONES

RADICACION EXPEDIENTE No. 6-101162

EL EXTRACTO DE LA PRESENTE SOLICITUD DE PATENTE DE

FUE PUBLICADO EN LA GACETA DE PROPIEDAD INDUSTRIAL No 583 DE

FECHA 18 DE DICIEMBRE DE 2007 EL TERMINO HABIL SEIJALADO POR LA

LEY EXPIRA EL 14 DE MARZO DE 2008

NOTA: Dentro del plazo de los seis (6) meses siguientes a partir de Pa fecha de publicaciOn en Pa gaceta de Propiedad Industrial, deberÆ solicitar y cancelar el examen de patentabilidad so pena de que la solicitud caiga en abandono, de acuerdo con lo establecido en el ArtIculo 44 de la Decision 486/2000.

SE PRESENTARON OPOSICIONES POR PARTE DE TERCEROS.

SI NO

United States Patent tt

Con’ielmann

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Q4A.Ua 1RAN.
VIRUS

[76] Inventor: Karl Klaus Conzelmann. LAteusLrassc
9. 0-714[3 R Germany

21] Appi. Na.: 0108,130

27] Fated Feb. 28, 9887

Related US. Applkutiaau Data

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1301 Foreign Application Priority Data

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A/IN 39/155; 3011K 39/165
1521 U3.Cl-------------------------------------- 43511723; 4242051;
424211.1; 424212.1; 424/224.1; 424/93.6;
1 A51235. 1; 435/236; 43 l/t 72 1
1511 Field of Search.........-.................... 43.5/1723.775.1,
4351236, 122.1; 424/205.1, 211. 1, 212.1,
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[Ill Patent Number: 6,033,886

[41 Date of Patent: Mar. 7, 2(9)0

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Si lee giVcnitlriiteinhlsi proiruti Lag foil in tEat viral nrc nina tie
into th,, environment are lotowa to deterniinr the cell_
spitieitv, it rhcrelorc is post/rio to target the rensirmarn
iuitctious ra icsvin.s militant to specific cells relive than the
catural host cc its of rabies. by &osing the right conpie-
pie stiitit piyeopreiteitt
ihese glycoprote ins will further be called "gtvcoprotein
t analogs’. to indicate that they are involved in cell-spec//ic
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If ho. let be ml iced, that in some vi n. the "glvctipn_
ciii 0 analogs determining the ciett ’ipeciticrOv are not
glyiprntemmne ha: via glycrosy tated proteins. It is rican, that
ilacce pr.tt’iris au..itntr w than the scope I if the inventive.
therefore, in another preterred embodimentol the present
rivets ion, the insertion noel/or eleteticin in the open reading
fame encoding the rtycoprruteia U LS such that it rein lt in
o riutn-functional glvcoprotein Ci, or even in tile absence of
glycoprotein G. ihe Ci-] mutauit viruses with the non-
functional or absent gtycoproteiatG have robe grown in cells
that provide a glycoproteiti C analog in trans. in order to
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expressing uiulnwlaopremcLLs’c epitopea of i-tR.SV, gives a
very safe vaccine against this diveasic
Prreinthtenza viris vaccines have so far laced the same
in bEe mets as I1ICS V-vaccines. ’Fhcrriore. recombinant infec-
ions rabies virus wirh pare rnthteni.n glyeorelic in f analog
end additional, imnntnn.ugenic epitopere of rreiruiniiuetmza pro-
sides a gird anti safe vaccine against this disease.
tither import ant veterinary vaccines based on rccotnis
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1:1 ree,,niutsiaavt rabies virus of immonogenir determinants of
the tesroviraves; equine, bovine and peurcinte. toroviruve,
iii the contnaviruaesr bovine, canine, porcine and felivae
. nnavirus, cap ciatly the.spilvc-peoieins th reol
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lit an even wore preferred embodiment of the present - ’’" ICiIts C

in’tvation, the gtyenuproteiii analog used heir coriipleme.-ate_, i,I ti’i’ ’P’nit 1it i/n I itS Iv I p it tie ri,, r 1 u nr,,h(eirroLin Citself- ’gii?ftttra loaf Ia e g an kniceimlitinaint,ntmecric:ts rabies vinises with a glycopnintein nor

IL analog have sirverat iin1uirtnet advantages:
al they can he specific ally targeted to certain eel Is, organs
or hosts, depending inn the target of the gtycoanrvteia C
analog that was chosen.
This liar plies that rug speciflcai/s’ the respiratory tract or
the digestive tract can be targettxb Thus, e.g mutcrisal
responses can be obtained at a priae/oterniined site.
Alternatively, specific cells of the immune sysreinu can be
targeted-

b) they cart arlditien ally be carriers of trrraigti genetic
iinle’rrnat:on cnciitir.g epitopes frito: ar,a-ralsivs path’-
cups as explained above.
Alternatively, they ca_i be carrier,, of foreign genetic
information encoding toxic sir.)sta uces
A very irnparnaat applieatioc of viruses according to the
u:ivenrienn is obtained with viruses having both a elyctpm-
cia C analog according to a) and foreign genetic irtinn.i-
lion according to b)
Ilecoinbinant infections rabies viruses can be obtained

according to the present invention. that are targeted to at
specific cell type, normally attacked by a non -rabies virus,
white at the same time carrying an i.-etnunopnorective deter-
minant of that iron -rabies virus.

Such a visas i:arinces anmanity in he beat against the
non rabies vicar, whereas at the same:itrc it is fully sal%
elite in, the tack of genetic information sir tIne gtscripro iv in Ii
analog-
Allot be r nalortauioiher L algal taut crrtxntii men t of tile present invention
are nirases according tn inc present invention that are cut
targeted to (1)4-cells, that represent target oelis of HIV.
through genotypical eunmp]ernientatioa with lllVgp 120, and
let Iacultutively encode a cyti/oxic protein.
Such sirtises will selectively attack (’I 14-eel lv, eit:e/,rnee
inside these the cell,,, they will tail them.
Alternatively, recombinant infectious rabies viruses
according to the present invention can pnsts’letc very sate
vaccines against v:nratent,patbogenc viruses against svltith
at this moment no salt live vaccines exist- a recmonirinant
iai[ectirsrs rahies virus t;nrgctrd againsi e.g.the naturaL targut
ectts.f divine Respiratrrp Syncymiat ViriasiIhEt.SVI through
ilecientatue 1 wilt UICSV glyeiprreeia 6 natalIe and

lit diction anelidr ircser:iori mutants ace-oreling to the
invent ion can also he used to vaccinate a host in order to Inc

Sn able it, discriminate (serologically) between a hnot to which
a vaccine comp rising said RV rmnii taut is administered and a
heist infected with a parental RV. In this embodiment of the
invention the insert in the 1W iresertior, mutant may encode
a heterologeiis epittlite which is capable of eliciting a
is spect lie non-Rh irimane mtspoine in an inn cii lated heist, or
nay encode a gnttcin with enzymatic activity- such as (’Al
or iacZ ((onzetantnuir and Schnell, I994, supra). Apre"erred
region for tire incorporation of such inserts is the RV
asendogenc region As is demonstrated in the L/s:,ntples
I, iiiuieetnaas Sort deletions can be tutade in this region without
rEeuspt iris ensenriaL functions ofRV such as those necessary
tilt iaitcctir’nt or replicetion Ilai RV deletion mutant way
Lade an epiteipe of a RV proteIn against which as immune
response is normally raised by the vaccicntcs. in pin rinanlar
45 a RV rnatarit comprising a rlele[ion in the ()RFeacoding I he
G protein is suited for this ritrpensn. In the teese of a li/V
insertion mutant the iinser:irtn comprises a nacteic cciii
sequence ettcodtng a serological mother an:igen or cit
epita1te thereof.
su: In a farther embodiment el the invert ion a li/V aeuntar,i is
provided which is capehie ot expressing one or unripe lit-
trent tcterolengtnris epitiopes or poiypcptidles of a speeuic
pathogen
Stich a atirtant can be used to vaccitlate animals, bath
eittn:es:ic and urrn-uloauestic animals. e,gaiaas: esilellife rabies
mud siiull pathetgen.
Vacciaaivin with such a live vector vaccine is preferably
fol/itwed by replication if the li/V mn tint w:lhi.i the ieocu-
lated list, cspnessing in vis’ei the hmatcrol,rn,,ns eplarpe or
’a pent ypeptide along, with the li/V potyr.v1ntidcv. the potypep-
title, esprsi’a-,cd in the inoculated heist will then elicit an
tinl,nuue response against both [tb and the specific pthcrgen.
If the tieterentogcins polypeptide derived row hr specific
patheugen call stimulate a protective immune resjsnrlsc. then
he aitiaiial inoculated with Inc li/V inuta iii according to tlae
invention will he immune to snt’acqnent inilecti-.;n by thai
pailnoutco as well as to iufecrtioti liv ti/V Ttias a heft Lotognniu’e

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nucleic acid sequence incorporated into a sciabte reitioui of
the RV grnoarie meuy Inc eontinrtc,iu sly experiacel in vivo,
providing a solid, safe and loeglasting iamiiininuty to the
pattti in.
lan patti lit, tat prrseat ivratataetts peeiaJeu aIW vrcieae
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uncensratiea wi, suitable reiori id the li/V ge-lit, I
’]"he poishnility to manipniialir the n asL Ili,l_i -
negative-straaded RNAeenomc of on the DNA level by -
recomhiriaai DNA leeliniqucs was not possible until now,
because net iafceiieeas replicating s’irue cci iid be generati,rnt
however, a process is provided tnereie which allows the
engineering of a mutation into a coding region or inst
circling region of the viral geutennne on:he DNA level by -
means of recombinant DNA techniques followed by the
grimerainug of an infect/runs replicating ti/n harboring the
mutation in its genotnir
Ibis process according to the ieventieiie comprises the
stetne rnf

A) introducing into cells expressing a RNA pinlyrnternse;
I) one or more DNA molecules encoding the ti/V N. P
and I. proteins, aid
2) a DNA molecule comprising the RV eDNA genome
and

h)solo hug the viruses produced by the cells.
Normally, the eDNA of the rabies virus gemume is mmli-
fted by the incerrynearation of a rnatatie,n in the geuome
lire process inlay however also be used to e.g pacify
nie,rtaminmated pireuls In that c.aace, the original norm- to
mnn’a cr1 cl)NA Will be used.
In view of the fact that reescuc efficiency of a naoelel
urn ini-genome of RV comprising heternrlogous inserts snitti
plasmid encoding proteins is extremely low and moreover
crirretates with insect length (Coneelniann ned Sctieclh at
1,194, sapre) it old not he expected that untiatwo of a
productive infection from transfenried fell iengtir gencnnhic
RNA could be achieved by co-transtection with plasmiels
encoding zho RV N, P cad I proteir,s Ibis is ha more so as
large amounts of positive sense N, P and II spec ihic RNAs is
are prod-iced Itsirn the iransfecteid iseuiteiuereodinnptmuniehs
which were expected tnt layhririiire with simuttanertusly
expre-eastal itegutive-sleanded genonhie ti/Nab transcripts Pos
sihin hs’trridiz.alion, hatsever, which could attract note than
half if 1b, geiiorae was suspected to imircufere with the a.:
niernciot ciicapsiilletiiiiti step In addition, translation ot N. I’
anet L mRNA might be affected. Indeed it was triund thzl
OilS tine standard traustectioti prnatocol no lrrtectious s’ir’Jscs
could be. obtained. Etosvever, an dennnrnstrated in the
etc. iapies the application of en alternative teaeiafection pro- 55
toctit in eomlsinatican with the use of a RV eDNA genonne
generating positive stranded antigenomnie RNA traulsexiats,
gave rise no a replicating genetically etrgi nec red RV-
-the above-mentioned process allows the in vitro i,ionr-
paratinun of a mutation in th genome of a parental li/V by teu
means of recetmabinaut l)N,\ techniques followed by the
generation of an infc,ilion.-e replicati li/V inijtaat hartnour-
ing.s. mutation.

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1/V n I I n ti.,5

line engineering of a mttiatlon in a unrua-ncn.ueting iateri]cnic
region may influence the Ira ascription of a specific viral
reuse such thai the transcription if ik mali/NA -red the
subsequent traastation of the protein either all envelope
proteinn, such as the hi amid (I protein or a protein involved
in polyrnuenose activity, such as the N, 11 or I. protein, is
reduced resulting in a virus mnntant featuring attenuated
claoraeterislieis because tile muta Fit ’s capability of prod ueeiLIE
(infeetioesl progeny virus is reduced tin particular the
substItution of one or more nucleic aeriiI residues in this
intergenic region anchor transcriptional signalsequer,cescan
influence efficiency Of iraiuserriptioa.
hhrthormore, tine ia/i tit:uii itof in ci iniat/c waeteie Acid,

is i -- ml ei)’rnrith sn/rn I.,i 11,4i i lii i1Jm14.,iiid,pdI-ti
f4la ng41eel is,tcca e

›haul nil ira’ ir

In process according to lhn Invention the l)N’,a nrinl
ecule containing the li/V genetic information preferably
comprises a plasmid provided with alnpreipriate transcription
initiator and tnrnainate,r scn1ueinces ceeognizable by a peity-
me rose co-expressed by the tranisfee ted host cells
A preferred princes,’, a,nrevnreling to the in yen tin,n comprises
the use of host ccii,, tranislee’teel with ti/V DNA, said cells
being able to express bacteriophage 17 DNA-ciepeuident
li/NA polynrernse, expressed for example cytcnpta,srniically
frrnnn vaceinia virus rearomhimiant. In this case the ptasiaiietv
containing li/V DNA are provident with the Ti promoter ann
terminator sequences ((ouzelmann and Schnell, 1994,

supra)- For the preparation itt’ a live v-asrine the recombinant li/V
mutant according to the present invention can be grown lie
a cell culture derived for example fn.niu IIIHK, or human
eLipleniel ceLls. The sir-unsex thus grown cnn be harvested by
cerilectiung the tissue cell culture iluiels and.uor cells ’flue hive
vaccine maybe prepared in the for-nm of a saspen.sain or may
he lyophiized.
tin addition loan immunogenicatlY effective anaonnrt it the
recombinant RN’ the vaccine may evrnlai.n a Ill:armnuaceahically
acceptable carrier or diluent.
Examples nn’ pharmaeeutieafly aenc-eptable carriers or dilu-
errts useful in the present invention include stal,ili,’ers such
as SPUA, carintnhydrates (erg snirhitenl. r-iaunilent, snarchi.
sucrose, glucose, demnrara), proteins such as hrns’ine serum or
akitanued mile and hutheare (rug. phosphate buffer)
Optionally. unne or nxenre e-atinpoumls having -adjav,nt
activity maybe added in the vaccine. Sri liable adjuvants are
hoe example aLnminiiuunu hydroxide, phnrs1nhate or oxide,
oil-en utsitnns Ce.rt of tlayol I,"’or Macitoh 52itli sapriains
or vit,amiut Iv solutrilisale
The teretni dosage to be aelnminnistcred will vary depending
on the type of mammal lobe s’secinateei, r he age, we.ght and
mode of administatienn. -

Die dosage nra,,’ vary between ’auto ranges: hr ui 10’
jnfitiaunimal u-outd e g. he stuitalnle deusrs
A specific dosage can be for example about to’ pfei
animal.

A RV mu.tault according tin the invention ciii aLso be insert
in prepare all i.iaclivaied saccine.
For ad in in istr at ion to animaLs, the li/Vauant according to
the prcsenu insciatioir can be-giver, inter olin uurultvr
inhranasalls,ntntr;idermably, suheumaneecisls or tmilramusc a
burly.

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ft 10’ saixittc:iceorilii’vL to Inc i:iveEiIneirt as. i:rt,tnt- rl wflhn I.) call, serum Li *1% CL[[itlIiLtiniC, u.S LtiliccIliLi 41
11›dlvi [[l.[5 liii I iJS[Ill 5n rita ii Ci (5 i,eian_i.ill[it,. [11.0 i. at S wilh the recnirtiliniiai,t vacci [kill virus a 1-
skunksluinla and hrtes Itirthvrurvirc, also sac atiinnirn I sold (deities ci ii liriir Nall- Acad. Placi U.S A..117. 5122 hE2f..
[[.40 with Ii live RI vecirr capali.e ii expreng 1 icIer- i.ih[ti) ()rw boor post-trail sfecnon] cells were washed twice
I- is. iii L Ii as e.l watic,hart, medium Is. ag calf s,rum and i-artsfccccdl1]
tee e.r s.rae, i c ills ii ri ’LiCe]. Li picer 1 riii,iliire nuintimi rung S am p’l’S IN, 2 r rig p. 17. C P
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5 erleis SAD IllS liUcInrutieles I-InS arid 1I76(L-I1028_ silpik phisutive cells were oliserved In.t serIes nI Si] rails.
r pe it vi)) inrerl I h 1 0 1’ RNA p sn re pro I v epeI’un n S N lien en nit elI S rn... its
nhuler and the liepatilts delta vLriis (HI)V] aiti teenonie prvs.uisc of infectious 5ii05 were obtained ni here exjreni.
ribmecyinie sequence. In [rEeler i: Leirerate at lilasmid L. pro- mc:i[s, t:n addition, l’runnn cell ca tn ms whiIISis c in. erulated
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NV sequences siinlaicred in pSI)1 1 were firL nn1’]itied hy ni 1L’eilrins virus cnnumld he recovered two dave later.
INtl using iii 1 1 (’use printer IS’ At 6(daTA.i(hA ’F) ’l’hereiurue, in order to isolate a [resunacel errs low urnmhen’
(ishO 1.0 NO: I) which..lse In the s.OipkeIltrnIl.aO]p ’ RN? sri nrh’celcruv virus geuje[’aLid iwleeuafccicnl cells. lhe
genirune cmiii-, etrmraapinnds I. the 5 terinini 0] (’nIh spositive menial ir "edure was rjir’ndjlied l’inr is<ilatiei: nitransl’ec(ant
.,.,I negative sense viral liNAc,.qr:icr sulivequent partial viruses wIts and superuatants were hau’ees:ed 2 days pew
ncr Lit in if a synthetic Le’rnl{l/lrljin(meIaiterr 17./i).1 Inn tatni it r translecttrirt. Cells were susnrrr.eled in the supernatant by
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S/ni) ’C° al/el in erlie stair SAD a)"], rescue was ludicarcd by qoa 4,l plasroi, ce,- RV Is. Si.,, I: P,.,(,6, cite!

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pSAD-XCAI, ii p’iAD-VC’AT, r peetirelg, as dibed
or Example 1 After removal of vaccini, virus, the transcrip-
tint) pattern of the aecoinil laint RVs were analysed by
northern hvlaridizatbnn limb virioses transcribed CAI
niRNAs,,f the expected and eampnasition (FIG. 5). The
expression nil CAl cne’.yrr.a activity was determined in cells
infected witln thc two viruses, respectively, ira stand -at CAT
assays (Cannzelnnaiin and Setereil, 1994, snipra)- Death were
fond to express CAP elilcientiv Saccie’sive passages in cell
cii 11 ire,Its showed that rIte introduced foreign satin canes
are genetically stable, liven char 40 passages both viruses
expressed CAl’ etllcieurly ll(L 65 Additional experiments
were performed in order to cea reLic expression and inahav-
our of the ressambinna ant niraves in iotieered animals. Sit
week old mice (live each) were inapeciart intraceerebrntly with
I [fir of SAD VIAl, SA1) X(AF. and standard sealileece
RV SAD 1.11. respectively. Seven days alter iclection all
animals slaoweai typical rabies symptoms and died from
rabies within 11w [allowing week CAT activity was dem-
otnsaated in lxaias 01 nnie’e infected with SAl) VU/IT and
SAD XCAII. respectively. Both viruses could be reisolated
from mouse brains and expressed (’Al’ cell ceLlniree (bus, a
foreign gene can hr ir,trcnduced irntcn the genome of infectious
1W and he expressed stably and as well may serve as a
marker to ditfercutinte recombinant viruses.

EXAM P(ES
Expresseon of a lleternnin,go-is Vnral Antigen from Raanm-
bimnaat RV and Induction of an Immene Reap nose Agair,si
RV and the Heterologoais Virus
]be genorne of classical swine lever virus ((’Shy)
encodes three structural glycoproteinas (110, lii and E2f In
CSFV infected animals nautraiiring aa:iboalies are directed
against [2, whereas 110 inducesacellniar immune response,
cDNAeneoinpa,wsing the coding regina of the 112 protein and
the E0 protein oiCSFV sleatenAliort nespesitively were used
to replace the poendogene regain between the Asull and
IlindIll sitsfpSAD Vax described in Lvampie 4 Rnco Ri-
llintanrviruses(SAII-VEO and SAD -VE2, raspcctisely) were
recovered fa-oni transfectiana exeerinnents as detailed in
Example I In infected cells lie sEnses eepmssed CS FV LO
protein, and Its IV 112 protein, respectively (FIG. T)-
The

) -
reconilninan; viruses S/Il) VtfO and SAD Vli2 were
used to ini IT) u.riize pigs by the aura I mate. Standard fox baits
usually being ursa: tar oral imnuaneation of faces with the
attenuated RV SAD 1119 strain were loaded with IO iota of
SAI)-VLn{I, SAl)-VliZ and SAl) IilS,re.spcotively lisa nails
oleachprepeieationwere fed to tata pgrs each (pig #1 and #2;
SAD VEAl, 54 and 44, SAD Bill ifS and 116, SAD VE2).
Four weeks after immunization, the presence of neitralneing
antibodies riganust 1W and (s,I;(,r as analysed. Willi the
exception ml #5, alt pigs pensserwed [IV aeutraLia-.iung inutihod-
ies (litre a25I)) ennntirnnling aprake ci the vaccine bits. Pig
5 was tin eretdec riot farther considered. Pig #6 des ci raped
fiSt-V neulralncing antibodies at a litre of >l& As expected.
pigs SI to t did not develop (SI-V neuxalicing antibodies.
An inteanasel challenge with tIC pie of (’5EV strain AlErt
was perfornn,sd S weeks after inurnenization launancyte
monnbers enipigy and body teanpernrai. we’re monitored after
line challenge and shown in FIGS- II cad 9. respanclis’eln, All
pigs developed fever, hit pigs 111 and 92 as well as #6
rcsa.vcred more quricklan 11secnnntnsl;n.innal#4 died 15 days
post challenge with typ:czl 1:9Ev syrniatoms, ’c control #3
was killed on day 21 floe presence of (’5EV iwwralmrig

nnntil,odies in tIne fig leal with SAD VE2 areS the partial
protect run of the pigs than ’eccnvedeitin,n- SAL) VUirnr SAD
\’IL’ etennonstrate that laIn insunenral arri cellular immure
responses against two lien emil. ngcnnls vir-us Ins)’ inc induced
lay rec’nnnhinant [IV lic s’acciaes alan application by the
..iral relate.

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EXAMPLE 6

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) ’150505 Ot,Ll
un ’ a ln i.o a "eras propagating less efficicintly
o titan he stansiri,l snurins SAD B19, a reesanatbinant seas
prepared that possesses a mutated C proteire
Iunrnliis purl:›,, tic nen1nenace encoding the last 44,anlinno
acids cii the (I protean were delete,-]- First, the AL protein
canting piasmid, illlli-G (Conzeinriann and Schr,e]l, 1944,

rn sn-pea) was digested with Allill (posit non 4752 of the SAD
LII) sequence) and LernnllV (the latter site is present in 11ho
rni,]lit-te cloning site of the piasmid) and blunt er,ds were
generated by Kkause entoryrne. ligation of the risirltiang
.49111 and Ecrn,-inV ends resulted in the generation of a
t’anslatiam ternhinati(in csndon at the former AlItII,aeqaenea.
yin a kh 1)NAPpnMI-SMa1 frigmantnrcontaiRing the modi-
heal region was used to replace the aathe Tit ie PpuMl-lIstXI
frasinnent 4469 4995 of pSAD 11 6.

nenti)1c4 iui-jts. daeletio#, S. a1la -)72,i--4 iii(
lb cai cylerri i ii IS aaol the t 1n11ntei

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a - gin in ta slastisne of 1.), s’eetor-a)er,vŁ nucleotides
itnnnaucaliately efowuontee.am of the new G translation termina-
tion ccudon

’a Rec,nmbmaet RV(SAD [lCD) was recovered as duoscribsid
in Es ample C As expected, a truncated G mm tein was
expressed in cells infected santit SAD i)Cl) (lID. 105
Cannnpareel to standard sequence virus SAD 111,. 100 fold
tosser iitres were obtained with SAD Xl) virus after
infmarri,an of cells atan tooL of [a addition, a reduced rule
nnlspreed in cell cultures was observed (FItIR II), indicating
that the tnirncatiota of the C protein resulted in reduced
ussenjblyof -,-irionsor raalaicsxi cell infectivity nil s’iiions, Sin
analyse the behaviour (A SAD l)(’i) in infected animals, Sue
mice were injected iotracerebrally with i(t’ffu of SAD DC I)
- and S mice with the same dcsis of SAD 1,16-

EX,’IMI’LL 7

Gr000 lisa i I a.l4aIicgs i sits t pier-v (C) Mg.tanti by

eel noon in an In ne.lerll’1’fetL t c enti C protein coding region frsinnn
the RVgenoinna, the fall Lcogl:a clone pSAD CE (Exam ple 2)
was used. This clone differs from pSAl) 116 5y the presence
(if a unique Nhel site wititia the nontraoslaleal 3’ region of

the 11 gene (SAL) 819 pennitinin i3 l). By partial digestion
ax of pSAD U2 with P115(1 (SAD position 3170) and connaplele
die stioa xs’)h Nhel, voitascetaerl Iii ir by Klannow enawnne
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was enniuraest/ieted wilhl LiSAID dE to rowpiement the Ii
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RNA transcripts of SAl) d(l were aaalyrcel by N.nrnhaorn
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Moleola and VSV-G priatcuan end infection of celIa, with
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viruses. Compared to rabies virus C and Inc closely related
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VSV-G (10nnrnl in con trust to 104m1) After replacement of
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VI 8’ evils) hells were obraineil from tiaeAlDS Research and an:,
Itefere nv Reagent Pmeeanrnng). hIm pseudotype viieises
thus possess tine hmnst range and ccl specificity of lllvi

hEllo)’ l)LSCRIP lION OF 41111 DRAWINGS
lIft 1

Orgrinicatiman of tho [IV pseudogene region (4’) and eon-
etraoetirnr of recannni-,inant [IV gennemes (drawn to scale)
Numbers inndicata the nucleentide positions in the anti-

genome sequence or SAD 1119 On top, the entire liv genome with its five open reading frames is shown Mata
lions were nrri nut in pPsiXih containing part of the
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clone pSAD I 16 by etccliange of the Stul fragment
(44l14-tn)1n4k In the detall drawing, coding regir.na are
represented by erny boxes, non-coding sequences ns lutes.
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transcr,1ntioin snarl). [lie lion functional signal-like sequence
defining the stint of the T. regiaan is shown by the open bar.
Arrows indicate the posit ion of olignnadmantidc prinnerin (t3P
and 1-4.M nnsed for Rl-IX’R analysis of the qi regime In 5,41)
112, lilt-m (nI lliindlLl esocinsions resulted in inseniaon of 4
nuelv,:tdes and generation of a unique Nlnel-sinne In 5,41)
V*, a chIN’S, Irantmenl containing the. [IV NP cistr,.n bnirder
(SAD 1119 nacleotides t 423-IStl) -es-as inserted atm’ the
Styl a/tan; S/al) W9penssnneses a deletion ol the Stvl-’tIinnJItl
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iiimplilied scheme liar the conustnicli,in of rranescriptior
talasonith containing full length 1W cDN,’e Nuinlnere refer Ian
naclaotide positions he the SAD Dlii 1W antigcuouie
sea1eance (Cottzcinaann cI al, 1990). flne plasrnirl p,Sl)l.
I plus which servee, as a basis for rca-mn nslructioa of I-it
length [IV gentonnic DNA is a counterpart of p51)1-I
(Conenelrnann and Schnell, 11594) containing the 51)1-1 RV
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arid t176O-11928 in opposite direction with resonea’; tan 17
RNA pmulyniterase prcnneoter (17) and hepatitis delta virus
antigenonhic rein:eynne seqaance 111Th). Line ti-lanI lglII
fragment of p5171-1 pits was replaced with a I kh eDNA
construct that was assennolnlad from three SAt) 1119 cDNA
ctmannes as indicated Insertion of a 36 1mb Spht and a 72 lab
Aatll feagnnanl which were assembled from two eDNA
clones each resulted in the final plasmid pSAD 116 con-
taining full Length SAL) 1119 cI)NA Iransemnpuion of this
plasarnid by 17 [IDA polyineroise slieaiild yield positive
stranded (antigenomic) RNA possessing three extra non-
virai tIn residues at the 5’ and a precise)’ end after aatolysis
of the rmbanzytne (’F)) 257 promolcr; (T7T) TV transcription
tertninalor; (HDV) Ill)V enliganominu rihenesme scquencse
[1(13-
Demonstration of the genetic tag in the geaanme ml the
tranisfincrant sines SAD U2.

hixtal RNA from cells infected with standard [IV SAD
111’) (1119) mid translectaiat viruses SAD III, (lift) and
SAD U2(U2) was Isolated 2 days post infection and used for
[IT-I-SR anaplitleation of the respective If regionas with
primers GIP and [AM. The amplified DNA was separated in
a VIP agamse gel directly and after digestion with llindIlI
and NheI. respectively A Nihel restriction site is present only
in DNA derived from SAD U2M.DNIA size marker-

FIG- 4 arker101(14
PCR ataenlysisofSAD 819(819), SAD V’ V*), and SAD
WI- (W9) geanomes 111 1mClt, was performed as described in
PIG 3 with primers (131’ and 1-4M. Anetnliikutioii products
were s -pa rated in a 1:4 agarose gel
Fl(L 5:
Demonstration of (-Al mRNat)cs transcribed by recombi-
nant I1VS

A Nnnruhern blot of total RNA from cc [Is infected ss-ith
SAD lift IllS), SAD XCAF(X6) and SAD V(’Au’(VC 15)
was hybridized with probes specific for the C gene (ft).
pseedragcne-(Y), and CAT gene, res1nectivelv. Cu the left
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eateso While SAD XCAF transcribesmmm in mRNAc.’ni ng
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al RNA markers are given in kb
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na ’SAD VE2 (isolates a, I’, c), respectively ia-na clays pinst
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!rrilrLcs. Idler rneii!r:(iari si’s I l’,llhiClOF]:i 1 0iiik. alLey
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sc’:rslralr and C5]IC1SUIC to an X-ray flliii. As I CniIltliil, sed it II II times prrct iiitCClIOfl 6. Lrccl irliritIlo
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Expression of Human Immuniodefictency Type I Gag Protein Precursor

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’Ai,jh-Level Production of Virus -like Padcs Containing HIV Envelope

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show th it VSV car -a orumodate up 5c 5 41 -enorri size with rdy Id iuct:sn in SlilO. Wet.
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ii both V5 it HIV VLPs Such tog WV VI-Ps mW bee pr ci I:rr its
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INTRODUCTION

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vector L Jp ltLi

VSVgag5 iiL]III 1 G LJ/iii Xbal Swat
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VSVgagEnv LIJPIM_I caTG t EnvTI(j

VSVgagEnvG NLflIMJ_GgLc [ErivG [L//ij1

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1. Disgrarr of VSV and rceombina-is expressing Hit; poteirs. The gene orcer in the vector bSsed on mc bite VSV and VSV recombnanSs expressing thy I Pug prerit-son cr Gug arecujmslm and Lriv are Shown in a 3 to 5 orientatiOci on the negative ut-stOod viral RNA. The restriction endonuclexco CPu used for csistruC:ir.g ’,,"SV -ecombirostu in the olaumid -)NA repreuenSation of VSV are shown- The Mimi and XtnI sites are it the coning vector pVSV VXA2 and the VSoI sod NOel utes are a the VSV-5N2 vector {see under Mnte.iSIs and Methods). Constructs VSVgag4 and VSVpaps expressed the sag gene as the birth or SIth genes ci tin we recombinant VSV gene -,e. 1 mc XbaI and Sm-a. sets irWcsted above the VSVeag4 connttrrct ac The snip -its sites employed to siihc!one thy gag gene into the riu- or EnvG-contairdng VSV ecsci.Di, r sronucu Sly (Joh.so t it al- These constructs eup.essed Gag precursor prote.s it addition to the HIV-1 Env proteir IVSVgagEciv) or a chimeric Env protein with the cyhoplasmic dernin of the VSV ely oprOte:n IVSVagEnv3t

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AND METHODS

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ACKNOWLEDG

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO DIRECCIN DE NUEVAS CREACIONES

2020
Bogota, D.C.,

Abogado
ALVARO CORREA ORDO11EZ

ASUNTO Radicacióri 06-101162
TrÆmite 011
Evento 378
Actuaciôn 519

Oficlo . S 4.44 3 Folios 012
Patente de lnvenciôn Patente de Modelo de Utilidad II
Via Nacianal

PCT (fase nacional) Cap.l I Cap. II LII

No, Solicitud Internacional PCT/US2005/01 1499
Fecha de Presentación Internacional 05 Abril 2005

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331

Coma resultado del Examen de Fonda realizado, con fundamento en el ArtIculo 45 de a Decision 486 de la Comisiôn de la Cornunidad Andina, se comunica:

1. Documentos estudiados

Descripción:
Reivindicaciones

Di b ujas
Secuencias
Prioridad:

2. Objeto de la invención

Folios 99 a 198
Folios 199 a 231
Folios 236 a 242
Folios 232 a 234
Folio 235
US 60.561.214
US 60.644.902

06 Octubre 2006
06 Octubre 2006
06 Octubre 2006
06 Octubre 2006
06 Octubre 2006
09 Abril 2004
19 Enero 2005

TItulo de la solicitud: AtenuaciÆn sinergistica de virus de estomatitis vesicular, vectores de estos y compasicianes

El problema plariteado en esta solicitud consiste en la necesidad de obtener una atenuaciOn del virus de estomatitis vesicular con elfin de producir vectores para composiciones inmUnogenicas de usa en humanos que tengan un perfil alto de seg u rid ad.

Para resolver este problema tØcnico la solicitud en estudio proporciona un virus de estomatitis vesicular (VSV) modificado genOticamente que comprende al menos dos clases diferentes de mutaciones en su genoma, en donde las dos mutaciones VSV atenUen de manera sinpergica la patogenicidad.

El objeto de la presente Solicitud se relaciona con la Clasificaciôn Internacional de Patentes (CIP): C12N 5/86; 007K 14/145; 012N 7/04

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3. Excepción a la Patentabilidad (Art 20 P 486 literal (d))

El objeto de las reivindicaciones 22 y 23 que se refiere a un mØtodo de tratamiento (mØtodo para inmunizar un mamIfero), no es patentable de acuerdo con el Art citado.

4. De la Solicitud de Patente

Reivindicaciones (Art 30 0 486, Art 22 POT)

La reivindicaciôn independiente 1 no caracteriza adecuadamente el objeto reclamado (virus de estomatitis vesicular), ya que caracteriza el virus modificado por el tipo de posibles mutaciones y no por sus componentes, gen a genes mutados, la secuencia o secuencias modificadas, el nümero de mutaciones y las posiciones de dichas mutaciones presentes en el genoma. Una adecuada caracterización del objeto de la presente invención puede notarse en las reivindicaciones7, 12 y 14 que aunque definen tres tipos diferentes de combinaciôn de mutaciones especifican de manera concisa y clara lo que se pretende proteger.

La totalidad de la materia reclamada en la reivindicaciôn I y 15 nose encuentra soportada en (a descripciôn, ya que las mutaciones de vstago G, mutaciones de inserción de gen G, mutación de eliminaciôn de gen y sus combinaciones no se encuentran dentro del capitulo descriptivo como posibles mutaciones que se generan en el VSV. Se sugiere al solicitante eliminar dicha materia de (a reivindicaciOn principal.

La reivindicaciOn 2 no es clara puesto que no define las caracteristicas del VSV
sino par el contrario caracteriza el resultado a alcanzar.

Las reivindicaciones 3 a 5, 10, 11, 13 son muy generales y no especifican de
manera inequivocamente el tipo de mutaciones, posiciones y secuencia preferida
que presenta (a mutaciôn, Se sugiere al solicitante sustituir estas generalidades
01 secuencias concretas.

. - La reivindicaciÆn8 no se encuentra soportada en (a descripciÆndebido a que no

se establecen dentro de las realizaciones presentadas en la solicitud la combinaciOn de 3 tipos de mutaciones dentro del genoma del virus de estomatitis
vesicular genØticamente modificado. Debido a lo anterior se sugiere al solicitante
suprimir dicha reivindicaciÆri.

La reivindicaciôn 9 estÆcaracterizada por un resultado a alcanzar, puesto que
hacen referenda a la comparaciôn de un valor LD50. La anterior no define el producto (virus) ya que (a reIvindicación incluirla no solo la soluciOn propuesta por el solicitante, sino todas las alternativas presentes o futuras que Ileguen a ese resultado. Se sugiere reemplazarlo par las caracteristicas esenciales, estructurales a funcionales, que contribuyen a la obtenciOn de ese efecto.

La composición inmunogØnica reclamada en (a reivindicaciOn 21 no se encuentra adecuadamente caracterizada puesto que una composiciOn debe caracterizarse par todos y cada uno de sus componentes y proporciones, asi mismo definir nequivocamente dichos componentes, tal es e(caso del vector genØticamente modificado el cual debe definirse por las secuencias y el tipo de mutaciOn.

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La reivindicaciôn 21 contiene la expresión dosis inmunogenica la cual es imprecisa y no permite distinguir la invenciôn del estado de la tØcnica. Se sugiere sustituir dicha expresión par un tØrmino preciso o un rango concreto de concentración de dicho vector.

Las reivindicaciones 16 a 19 que hacen referenda a Ufl vector de VSV modificado genØticamente, no son concisas puesto que no definen el gen, las secuencias de ARN insertada de manera especifica, ni la secuencia de genes mutados del VSV. Asimismo, no presentan soporte en la descripciOn ya que las realizaciones preferidas involucran un solo tipo de gen (gag de HIV) y un grupo de secuencias mutadas del virus VSV. Se sugiere al solicitante modificar dichas reivindicaciones de tal manera que Iimite la materia reclamada.

La reivindicación 20 contiene todas las caracteristicas tØcnicas esenciales del vector VSV reclamado en la presente solicitud, par tal razôn se sugiere al solicitante modificar la reivindicaciôn independiente 16 agregÆndole dichas caracteristicas.

5. Unidad do inverición (Art 25 D 486)

La presente invención se refiere a un virus de estomatitis vesicular (VSV) genØticamente modificado que comprende par lo menos dos clases diferentes tipos de mutaciones en su genoma.

La solicitud de patente no tiene unidad de invención ya que comprende mÆsde una invención o grupos de invenciones descritos a continuaciOn:

Grupo 1: Reivindicaciones 1, 3 a 8 y 16 a 20 relativas a dos mutaciones que comprenden una mutaciôn del gen G truncado y una mutación del cambio de gen N y
los vectores obtenidos a partir de estas.
Grupo 2; Reivindicaciones 1, 10 a 12 y 16 a 19 relativas a dos mutaciones que comprenden una mutaciôri del gen G truncado y una mutaciÆn del gen M no
citopàtico y los vectores obtenidos a partir de estas.
Grupo 3: Reivindicaciones 1, 13 a 14 y 16 a 19 relativas a dos mutaciones que
. comprenden una mutaciôn N(ts) del gen N y una mutación L(ts) del gen L y los vectores obtenidos a partir de estas,

Las caracteristicas de las reivindicaciones 3, 10 y 13 definen materia sin ninguna
relaciôn tØcnica debido a que la combinaciôn de mutaciones para el VSV son diferentes y esto genera una respuesta a expresiôn en el genoma diferente. De igual
manera son conocidas en el estado de la tØcnica las diferentes modalidades de mutaciones de manera individual para este mismo virus.
- Mutación del gen G truncado (ver folio 131, linea 7 a 10; folio 132, linea 4 a 9; Dl)
- Mutaciôn del cambio de gen N (ver folio 129, linea 25 a 29; folio 130, linea 1 a 7)
- MutaciÆn del gen M no citoptico (ver folio 124, lInea 13 a 14; Dl)
- Mutaciôri N(ts) del gen N (ver folio 133, linea 11 a 21)
- Mutaciôn L(ts) del gen L (ver folio 133, linea 11 a 21)

TenIendo en cuenta lo anterior, ya que no pueden encontrarse elementos tØcnicos particulares correspondientes en las reivindicaciones, esas invenciones no estÆn vinculadas por un concepto inventivo Unico. En consecuencia, cada una de las reivindicaciones 3, 10 y 13 representa una invención diferente. For lo tanto, se considera que hay 3 invenciones en las reivindicaciones.

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6. DeterminaciOn del Estado de la TØcnica

El Estado de la TØcnica se determinO con base en la fecha de presentaciôn de la solicitud de prioridad (09 Abril 2004) y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Item Documento I Titulo Fecha de

Publicación

Dl US6033886 Recombinant infectious non -segmented 07 Marzo 2000

negve strand RNA virus
Expression of Human Immunodeficiency Virus
VIROLOGY, Type 1 Gag Protein Precursor and Envelope
D2 (2000): 268; Proteins from a Vesicular Stomatitis Virus 01 Marzo 2000
112-121 Recombinant: High-Level Production of Virus-
like Particles Containing HIV Envelope

Dl (folios 325 a 327) divulga virus RNA recombinantes y sus procesos de obtencidn en donde se introducen mutaciones en el genoma del virus mediante tØcnicas de ADN recombinante. URL:http:!/worldwide.espacenet.Com/pUbliCatiOflDetailS/biblio?DB=EP000C&I Iz0&adjacenttr ue&localeengb&FTD&date=20000307&CC=US&N R=6033886A&KC=A

D2 (folios 328 a 330) divulga la elaboración de vacunas para VIH que comprenden Ia atenuaciOn del virus VSV por modificación o mutaciones en su genoma.

7. Examen de Patentabilidad (Art 14 0486)

Novedad (Art 16 0486)

La reivindicaciOn I consiste en un virus de estomatitis vesicular genØticamente modificado (VSV) que comprende por lo menos dos clases diferentes de mutaciones en su genoma, las mutaciones son seleccionadas del grupo que consiste de una mutaciôn sensible a la temperatura (ts), una mutaciôn de punto, una mutaciôn de cambio de gen, una mutación de vÆstago G, una mutaciôn del gen M no citopÆtico, una mutaciôn de ARN ambisentido, una mutación del gen G truncado, una mutación de inserción de gen G y una mutación de eliminación de gen, en donde las dos mutaciones atenUan de manera sinØrgica la patogenicidad del VSV.

Comparaciôn de las caracteristicas tØcnicas esenciales, con las del Estado de la TØcnica:

Caracteristicas esenciales Dl
Virus de estomatitis vesicular genØticamente
modificado (VSV)
por Ic menos dos clases diferentes de
mutaciones en su genoma:
- mutaciOn sensible a la temperatura (ts)
- mutaciôn de punto
- mutación de cambio de gen
mutaciôn de vÆstagoG
mutaciÆndel gen M no citopätico
- mutaciOn de ARN ambisentido
- mutaciôn del gen G truncado
- mutción de inserciOn de gen G
mutaciOn de eliminaciOn de qen

Virus de estomatitis vesicular
genØticamente modificado(J

- mutación del gen G truncado
- mutación del gen M no citopªtico
- mutación de inserción de gen G

Las caracterIsticas esenciales de la composiciôn de la reivindicaciÆn1 habian sido divulgadas previamente en Dl, el cual enseæa virus mutantes genØticamente

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manipulados de la familia Rhabdoviridae dentro de los que se encuentran el virus de estornatitis vesicular (VSV) (folio 325, columna 1, linea 13 a 17; folio 327, columna 23, reivindicaciôn 1).
Dichos virus presentan dos tipos de mutaciones en su genoma que atenüan la patogenicidad del virus, dentro de dichas mutaciones se pueden encontrar la inserciôn, deleción y sustitución de nucleôtidos de zonas codificantes (genes principales) del virus (folio 325, columna 4, linea 54 a 67; folio 326, columna 9, linea 63 a 67); una de ellas corresponde a una mutaciôn de gen G truncado que modifica un nucleótido como arginina por isoleucina (folio 326, columna 10, linea 18 a 21; folio 327, columna 23, reivindicación 5; ejemplo 6) y la otra corresponde a una mutación del gen M que puede consistir en la inserción o deleción de nucleótidos (folio 325, columna 5, linea 45 a 50; folio 327, columna 23, reivindicaciôn 4; ejemplo 7y 89.

En consecuencia, la reivindicaciôn 1 no es nueva carece de novedad, segUn lo divulgado en el documento Dl.

Las reivindicaciones dependientes 2 y 10 no mencionan alguna caracteristica que haga nueva a la composición de la reivindicación 1, por lo cual se considera que no

40 son nuevas.

La reivindicación 16 consiste en un vector VSV modificado genØticamente que comprende el VSV de la reivindicaciôn 1, que comprende adicionalmente por Ia menos una secuencia de ARN extraæainsertada en o que reemplaza una region del genoma VSV no esencial para la replicaciOn.

Comparacion de las caracteristicas tØcnicas esenciales, con las del Estado de la TØcnica

Caracteristicas

Vector de VSV genØticamente modificado con por Vector de VSV genØticamente a menos par Ia menos dos clases diferentes de modificado

mutaciones-ensu genorna.......... adicionaimente una secuencia de ARN extraæa Secuencia de ARN extraæainsertada
insertada en a que reemplaza una region del en a que reemplaza una region del
genoma VSV no esencial para la replicaci6n L9efl ama VSVgag..

All Las caracterIsticas esericiales de la composiciOn de la reivindicación 16 habIan sido divulgadas previamente en D2, el cual enseæa vectores del virus de estomatitis
vesicular recombinante (rVSV) que contienen dos mutaciones en su genoma. Dichas mutaciones consisten en el rearreglo de la protelna G modificando su posiciOn 5en comparaciôn con la secuencia silvestre (folio 328, pagina 113, figura 1; pagina 14,
columna 1), la inserciOn de un gen adicional y la eliminaciOn de 29 arninoÆcidosdel
dominic citoplasmØtico de la protelna G del virus (mutación de gen G truncado) (folio 329, pagina 118, columna 2). Adicionalmente a estas modificaciones se introduce dentro del genoma el gen gag de HIV el cual se puede insertar entre los genes M y G 6 0 y L del genoma VSV (folio 328, pagina 113, figura 1; columna 2 (resultados)).

Por todo Ia anterior, la reivindicación 16 no es nueva, segün lo divulgado en el
documento D2.

Las reivindicaciones dependientes 17 a 19 y 21 no mencionan alguna caracteristica que haga nueva al vector reclamado en la reivindicaciOn 16, par Ia cual se considera
que no son nuevas.

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8. Conclusion

Los requisitos de Patentabilidad de la presente Solicitud estÆn afectados as!:

7Reividicaciones Item Documento

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LD2 1VUOLOGY.(2000);268;112-121 16a 19y21 Novedad

En cumplimiento del articulo 45 de la Decision 486 de la Comisiôn de la Comunidad Andina, se le indica que debe dar respuesta dentro del tØrmino de sesenta (60) dIas contados a partir de la fecha de notificación de la presente comunicaciôn. En caso de no dar respuesta al presente requerimiento, a si a pesar de la respuesta subsistieran los irnpedimentos para la concesiôn, se denegara la patente,

Notifiquese el presente oficio conforme a Ia dispuesto en el numeral 5.2, literal e), del capitulo quinto.deltitulorirode la Circuar Unica No. 10 de 2001.

JOSE LUIS SALAZAR LOPEZ

Director de Nuevas Creaciones (C)

El anterior requerimiento fue notificado par fijación en lista, de fecha

DIG. L
Flaborado por: Ana Delia PinzOn Garcia
Revisado For: Jacqueline Alfonso 1166
Aprobado por: Jose Luis Salazar Lopez

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