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OLARTERAISBECK
OLARTE RAISBECK MOURE & CASTRO LTDA.

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
No. 07-058654- -00003-0000
Fecha: 2008-07-14 17:18:53 Dep. 2020 NUECREACIONI
Tra. 11 PATENTEIYII Eve: 379 FASENACIONALII
Act. 538 PETICION Folios: 2

Señores
SUPERINTENDENCIA DE INDUSTRIA Y COM
DIVISION DE NUEVAS CREACIONES
E. S. D.

Ref.: Expediente No. 07.058.654
Solicitud de Patente de Invención titulada: "VIRUS RECOMBINANTE DE LA ENFERMEDAD DE NEWCASTLE".
Solicitante: BAYER SCHERING PHARMA AKTIENGESELLSCHAFT
Nuestra Ref.: P2007/000081

SOLICITUD DE EXAMEN DE PATENTABILIDAD SEGÚN EL ARTÍCULO 44 DE LA DECISION 486, GACETA No. 587

J. IAN RAISBECK, mayor de edad y vecino de Bogotá, D.C., identificado tal como aparece al pie de mi firma, en mi carácter de apoderado de la sociedad solicitante, respetuosamente me permito solicitar que se examine si la invención reúne los requisitos de patentabilidad previstos en la misma, dando cumplimiento a lo establecido en el articulo 44 de la Decisión 486 de la comisión de la Comunidad Andina. Adjunto recibo por el monto de $210.000 pesos para cubrir la tasa correspondiente, según la Resolución No. 44372 del 26 de Diciembre de 2007.

Atentamente,

J. IAN RAISBECK
C.C. 79.376.996 de Bogotá
T.P. 135.799

Anexo: Recibo por $210.000 pesos

LMB/P2007/000081 080619 Sol Exam

228

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 08 - 55,764
FECHA : JULIO 11 DE 2008

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

No. 07-058654- -00003-0000

Fecha: 2008-07-14 17:18:53 Dep. 2020 NUECREACIONE
Tra. 11 PATENTEIYII Eve: 379 FASENACIONALII
Act. 538 PETICION Folios: 2

***** C O N S I G N A C I O N *****

[redacted]SITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
OLARTE RAISBEK	CONSIGNACION	BANCO DE BOGOTA	062754387	4801882	11/07/2008	8,852,000.00

***** C O N C E P T O *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-01 SOLICITUDES	114 PTC CAP-II EXAMEN DE PATENTIBILID	210,000.00
		TOTAL : $	210,000.00

SON: DOSCIENTOS DIEZ MIL PESOS

[redacted]

RESPONSABLE : _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No._____

Industria y Comercio
SUPERINTENDENCIA

DIRECCIÓN DE NUEVAS CREACIONES

RADICACIÓN EXPEDIENTE No. PCT 07-58654

EL EXTRACTO DE LA PRESENTE SOLICITUD DE **PATENTE DE INVENCIÓN**

FUE PUBLICADO EN LA GACETA DE PROPIEDAD INDUSTRIAL No **587** DE

FECHA **18 DE ENERO DE 2008** EL TÉRMINO HÁBIL SEÑALADO POR LA LEY

EXPIRA EL **16 DE ABRIL DE 2008**

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

SE PRESENTARON OPOSICIONES POR PARTE DE TERCEROS.

SI _____ NO ____x____

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado
J. IAN RAISBECK

ASUNTO	Radicación	07-58654
	Trámite	011
	Evento	378
	Actuación	519
	Oficio	No. 10210
	Folios	005

Patente de Invención	■	Patente de Modelo de Utilidad		☐
Vía Nacional				
Vía PCT (fase nacional)	■	Cap. I	☐	Cap. II ■

No. Solicitud Internacional: PCT/EP2005/012186
Fecha de Presentación Internacional: Noviembre 10 de 2005

Como resultado del Examen de Fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Folios 50 a 132	08/Junio/2007
Reivindicaciones:	Folios 133 a 140	08/Junio/2007
	Folios 170 a 176	08/Junio/2007
	Folios 183 a 185	08/Junio/2007
Dibujos:	Folios 141 a 151	08/Junio/2007
Secuencias:	Folios 152 a 154	08/Junio/2007
Prioridad:	EP 04090432.8	12/Noviembre/2004

2. Análisis de la Prioridad

Se acepta la reivindicación de prioridad porque esta solicitud fue presentada dentro del plazo establecido (art. 9 d 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Modificaciones (Art 34 D 486 y Art 19, 22 y 34 PCT)

Las modificaciones del capitulo reivindicatorio, folios 183 a 185, no son aceptadas porque no se encuentran en idioma español.

Se aceptan las modificaciones a las reivindicaciones en los folios 170 a 176, puesto que no amplían la materia, y sobre estas se hará el estudio de patentabilidad.

4. Objeto de la invención

Título de la solicitud: "Virus recombinante de la enfermedad de Newcastle"

El problema planteado en esta solicitud consiste en la necesidad de suministrar virus

recombinantes útiles en el tratamiento de cáncer.

Para resolver este problema técnico la solicitud en estudio proporciona virus recombinante de Newcastle, que contiene secuencias de ADR o ARN que codifican para proteínas de unión, enzimas covertidora pro-droga o proteasas.

El objeto de la presente Solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): A01K67/027B; A61K35/76; A61K8/34; A61K8/35; A61K8/37; A61K8/49F2; A61K8/92C; A61K8/97; A61K8/99; A61Q19/08; C07K14/125

5. No es una invención (Art 15 D 486 literales (b))

El objeto de la reivindicación 5 no se considera una invención, de acuerdo con el Art citado, ya que se refiere a proteínas de unión de origen animal.

6. Excepción a la Patentabilidad (Art 20 D 486 literal (d))

El objeto de las reivindicaciones 17 a 19, 24, 25, 27 y 28 no es patentable de acuerdo con el Art citado, ya que se refiere a métodos de tratamiento.

7. De la Solicitud de Patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

El capitulo reivindicactorio no es claro porque:

- Las reivindicaciones 1, 20 y 21 que tratan sobre un paramixovirus oncolítico recombinante que comprende un ácido nucleico con al menos un transgen no son claras, ya que dicho paramixovirus oncolítico y transgen son caracterizados por el producto que codifican. Por otra parte, para el caso de la reivindicación de virus, se recuerda al solicitante que este debe ser caracterizado por su cepa debidamente identificada con un Certificado de Depósito, y el transgen debe ser caracterizado por su secuencia nucleotida completa, que debe estar en el listado de secuencias adjunto con su respectivo SEQ ID NO.

- El objeto de las reivindicaciones 4, 6, 7, 8, 9, 10 que se refieren a un virus oncolítico recombinante no es claro ya que caracterizan el virus por la proteína de unión que es capaz de expresar, se solicita que se aclare si se pretende reivindicar el virus o la proteína que codifica el virus. Si las reivindicaciones se refieren a las proteínas de unión, estas deben ser caracterizadas por su secuencia de aminoácidos completa y deben estar en el listado de secuencias adjunto con su respectivo SEQ ID NO, y no caracterizadas por su función o por su estructura tridimensional. Si las reivindicaciones se refieren al virus, este debe ser caracterizado por su cepa debidamente identificada con un Certificado de Depósito, y el transgen debe ser caracterizado por su secuencia nucleotida completa, que debe estar en el listado de secuencias adjunto con su respectivo SEQ ID NO.

- La reivindicación 11 que se refiere al nucleocápside de un paramixovirus de acuerdo a las reivindicaciones 1 a 10 no es clara ya que no refiere a la estructura de la misma, que debe ser caracterizada por la secuencia de las proteínas que lo componen.

- Las reivindicaciones 12, 13 y 14 que se refieren al genoma de paramixovirus de acuerdo a las reivindicaciones 1 a 10 no son claras ya que no caracterizan

correctamente el genoma ni la secuencia de control reclamados. Se recuerda al solicitante que un ácido nucleico debe ser caracterizado por su secuencia completa y debe estar en el listado de secuencias adjunto con su respectivo SEQ ID NO.

- Las reivindicaciones 15 y 16 no son claras, al tener dependencia de las reivindicaciones 10 a 14, que no se encuentran correctamente caracterizadas, tal como ya se menciono.

- Las reivindicaciones 22 y 23 presentan una falsa dependencia de las reivindicaciones 1 a 10, las cuales se refieren a virus donde la proteína es una proteína de unión, mientras que la reivindicación 22 habla de que dicho transgen codifica para una enzima convertidora de pro-droga.

- La reivindicación 26 presenta una falsa dependencia de las reivindicaciones 1 a 10, las cuales se refieren a virus donde la proteína es una proteína de unión, mientras que la reivindicación 22 habla de que dicho transgen codifica para una proteasa.

- La reivindicación 23 no se encuentra sustentada en la descripción, donde se menciona el posible uso de virus recombinantes que expresan proteasas (folios 68 a 70), pero no aporta pruebas que demuestren que dichos virus fueron obtenidos, o que cumplen con la actividad para la cual fueron diseñados

8. Unidad de invención (Art 25 D 486)

La solicitud no cumple con el requisito de unidad de invención porque se refiere a 3, grupos inventivos, definidos por las siguientes características esenciales:

- Grupo 1° Reivindicaciones 1-16, que se refieren a un virus oncológico recombinante que comprende un ácido nucléico con un transgen que codifica para una proteína de unión con actividad terapéutica cuando es expresada por la célula tumoral con virus, la nucleocápside, el genoma y el ADN que codifica el genoma y/o antigenoma del virus, la célula y la composición farmacéutica que contienen el virus.
- Grupo 2° Reivindicaciones 20, 22 y 23 que se refieren a un virus oncológico recombinante que comprende un ácido nucléico con un transgen que codifica para una enzima convertidora prodroga con actividad terapéutica cuando es expresada por la célula tumoral con virus
- Grupo 3° Reivindicaciones 21 y 26 que se refieren a un virus oncológico recombinante que comprende un ácido nucléico con un transgen que codifica para una proteasa con actividad terapéutica cuando es expresada por la célula tumoral con virus

Se sugiere presentar las solicitudes divisionales que considere necesarias, ó limitar la solicitud inicial a una sola invención, eliminando de ella las otras invenciones.

9. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de prioridad: Noviembre 12 de 2004, y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación*
D1	J. Virol. 2004, 78(18):10054-10063.	A Recombinant Newcastle Disease Virus (NDV) Expressing VP2 Protein of Infectious Bursal Disease Virus (IBDV) Protects against NDV and IBDV	Septiembre 2004
D2	Drug Discovery Today, Vol. 9, No. 17, 759-768	Oncolytic viruses for the treatment of cancer: current strategies and clinical trials	Septiembre 2004

D1 difunde el desarrollo de una vacuna alternatica para IBDV que usa un el enfoque de la genética reversa, diseñaron un NDV que expresa VP2 una proteína inmunógeno protectora (p10055 columna izquierda párrafo 4 y pagina 10062, columna derecha, ultimo párrafo) http://jvi.asm.org/content/78/18/10054.abstract

D2 divulga virus que se replican selectivamente en tumores que tienen ventajas sobre las terapias convencionales para cáncer, como la expresión de proteínas útiles en tratamiento de cáncer tales como proteínas anti-angiogénicas o inmunomoduladoras (Página 765, columna izquierda párrafo 4, y página 766 columna derecha párrafo 2). http://www.sciencedirect.com/science/article/pii/S1359644604032210

10. Examen de Patentabilidad (Art 14 D486)

Nivel Inventivo (Art18 D486)

La reivindicación 1 consiste en "Un paramixovirus oncolítico recombinante que comprende un ácido nucleico con al menos un transgen, en donde el ácido nucleico de este transgen(es) codifica para una proteína de unión que tiene una actividad terapéutica cuando es expresada por la célula tumoral infectada con virus."

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características **esenciales**	D1	D2
Paramixovirus oncolítico	Virus de Newcastle, que es un virus oncolítico	Virus oncolítico
Ácido nucleico de transgen(es) codifica para una proteína de unión	NDV (virus de Newcastle) recombinante que expresa una proteína VP2 (proteína de unión)	Transgen terapeutico
Proteina de unión que tiene una actividad terapéutica cuando es expresada por la célula tumoral infectada con virus	VP2, que es una proteína de unión, y que la vacunación con el virus recombinante produce una respuesta inmune para IBDV	Proteínas anti-angiogénicas o inmunomoduladoras

Los documentos D1 y D2 se consideran el estado de la técnica cercano a la invención definida en la reivindicación 1.

D1 describe un NDV (virus de Newcastle) recombinante que expresa una proteína VP2, que es una proteína de unión, y que la vacunación con dicho virus produce una respuesta inmune para IBDV (enfermedad de bursitis infecciosa) (p10055 columna izquierda párrafo 4). Por otra parte el mismo documento considera el tratamiento de cáncer y la terapia génica usando NDV (pagina 10062, columna derecha, ultimo párrafo).

D2 describe el uso de virus oncolíticos entre los cuales se encuentra el virus de Newcastle, que tienen transgenes capaces de expresar proteínas de interés terapéutico en cáncer. Dichas proteínas pueden ser profármaco como enzimas o proteínas anti-angiogénicas o inmunomoduladoras (Página 765, columna izquierda

párrafo 4, y página 766 columna derecha párrafo 2).

La diferencia entre la invención, definida en la reivindicación 1 y D1 consiste en que la invención no identifica la proteína de fusión reclamada.

La diferencia entre la invención, definida en la reivindicación 1 y D2 consiste en que la proteína expresada en el caso de la invención es usada en el tratamiento de cáncer.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: "modificar el virus de Newcastle conocido en D1 para obtener un virus capaz de expresar proteínas con efecto terapéutico contra el cáncer".

Sin embargo, la utilización del virus de Newcastle recombinante para el tratamiento de enfermedades, ya está divulgada en D1. En consecuencia, la persona del oficio normalmente versada en la materia, incluiría un transgen que codifique para una proteína con actividad terapéutica de D2 en el procedimiento de D1 para llegar así al objeto de la reivindicación 1 de la solicitud. Por lo cual se considera obvia.

Las reivindicaciones dependientes 2, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 20, 21 no mencionan alguna característica que haga nueva a la reivindicación 1, por lo cual se considera que no son nuevas.

11. Conclusión

Los requisitos de patentabilidad de la presente solicitud están afectados así:

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	J. Virol. 2004, 78(18):10054-10063.	2, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 20, 21	Nivel Inventivo
D2	Drug Discovery Today, Vol. 9, No. 17, 759-768	2, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 20, 21	Nivel Inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante y presentarla por escrito

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.

JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (C)

El anterior requerimiento fue notificado por fijación en lista, de fecha 13 JUN

Elaborado por: Sandra Carolina Crespo Cárdenas
Revisado Por: Consuelo Leguizamon Leguizamon
Aprobado por: José Luis Salazar López

Expediente 07 58654 Primer Artículo 45

07-58654

235
285

Oncolytic viruses for the treatment of cancer: current strategies and clinical trials

Stefan J. Ries and Christian H. Brandts

Tumor-selective replicating viruses offer appealing advantages over conventional cancer therapy and are a promising new approach for the treatment of human cancer. The development of virotherapeutics is based on several strategies that each provides a different foundation for tumor-selective targeting and replication. Results emerging from clinical trials with oncolytic viruses demonstrate the safety and feasibility of a virotherapeutic approach and provide early indications of efficacy. Strategies to overcome potential obstacles and challenges to virotherapy are currently being explored and are discussed here. Importantly, the successful development of systemic administration of oncolytic viruses will extend the range of tumors that can be treated using this novel treatment modality.

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The goal in developing new therapies for the treatment of cancer is to design agents that have a large therapeutic index (i.e. high potency against malignant cells), but only limited pathogenicity to normal tissue. Naturally occurring lytic viruses have evolved to infect, replicate in and lyse human cells. It is evident that the replication cycle of many viruses exploits the same cellular pathways that are altered in cancer cells [1,2]. Recent advances in our understanding of the molecular biology of cancer, as well as the availability of technologies to genetically engineer viruses, have led to the concept of oncolytic viruses. These biological agents are thought to hold the toxic and discriminatory power that is expected from an efficacious therapy against human cancer and they have several appealing properties. Whereas conventional chemotherapeutic drugs distribute relatively uniformly throughout the human body and follow the typical log kinetics of cell killing, the local replication of administered oncolytic viruses amplifies the input dose and creates a high concentration of therapeutic agent at the target site. This accumulation helps to spread the agent to adjacent tumor cells and limits potentially toxic side effects within normal tissue. Because direct cell killing caused by viruses is an active and highly complex process that involves many cellular pathways, the occurrence of drug resistance appears unlikely. Furthermore, additional mechanisms, such as the stimulation of the humoral and cellular immune response of the host [3], could potentially enhance virus-induced tumor regression. Finally, by arming the oncolytic viruses with therapeutic genes, their antitumor toxicity could be increased [4].

Strategies to generate tumor-selective viruses

There are several strategies that achieve tumor-selectivity of replication-competent viruses, some of which are discussed here (Table 1 and Figure 1).

Inherent tumor-selectivity

One approach to achieve viral tumor-selectivity is to use viruses that possess inherent tumor-selectivity. Several RNA virus species are tumor-tropic, which is partly the result of their ability to grow exclusively in cells with defective antiviral response systems [e.g. Newcastle-disease virus (NDV) and vesicular stomatitis virus (VSV)] [5,6]. In the case of NDV and VSV, these RNA viruses are sensitive to inhibition by interferon, and thus normal cells are almost completely protected from infection and replication while tumor cells that lack a functional interferon response are rapidly lysed. Replication of reovirus, which is another RNA virus with inherent tumor-selectivity, is restricted by activation of the double-stranded

1359-6446/04/$ – see front matter PII: S1359-6446(04)03221-0

INFORME DE PRODUCCION DE SERVICIOS NUEVAS CREACIONES

Industria y Comercio
SUPERINTENDENCIA

Informe		**Entidad***	
Nombre*	AQUÍ EN MAYUSCULAS	**Año***	2012
CC*		**Mes***	Abril
Sector*		**Hoy**	Jueves , 10 Mayo 2012

	EXPEDIENTE						ENTREGADO		
							Para Revisión	Para Firma/notificación	
#	PI / MU*	VIA PCT / NAL*	Año*	N°.*	País*	Tipo actuación*	Fecha entrega dd/mm/aaaa*	Aprobado por*	Fecha aprobación dd/mm/aaaa
1	PI	PCT	2008	62862	SU	1er. Art. 45	09/Abr/2012	GJAR	
2	PI	PCT	2008	97279	AL	1er. Art. 45	09/Abr/2012	GJAR	
3	PI	PCT	2008	62146	SE	1er. Art. 45	09/Abr/2012	GJAR	
4	PI	PCT	2008	64068	JP	1er. Art. 45	09/Abr/2012	GJAR	
5	PI	PCT	2008	92595	SU	1er. Art. 45	13/Abr/2012	GJAR	
6	PI	PCT	2008	136727	US	1er. Art. 45	13/Abr/2012	GJAR	
7	PI	PCT	2008	135290	KR	1er. Art. 45	13/Abr/2012	GJAR	
8	PI	PCT	2008	95668	JP	1er. Art. 45	19/Abr/2012	GJAR	
9	PI	PCT	2008	131485	KR	1er. Art. 45	19/Abr/2012	GJAR	
10									
11									
12									
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14									
15									
16									
17									
18									
19									
20									

PI	9
MU	0
TOTAL	9

Art. 45	9
Concesión	0
Negación	0
Recurso	0
Total	9

Art. 46	0

Observaciones, comentarios y otros:

Firma entrega

Firma recibido

Firma Director de Nuevas Creaciones

Table 1. Strategies to achieve tumor-selectivity

Strategy to achieve tumor-selectivity	Advantages	Disadvantages	Virus species	Stage of development	Refs or source
Inherent tumor-selectivity	Viral modifications are not required No foreign DNA elements required	Specificity can not easily be modified Depends on natural lytic strength of virus Targeted modifications are technically challenging	Reovirus	Clinical	[7], Oncolytics Biotech[a]
			Newcastle-disease virus	Clinical	[66,67]
			Vesicular stomatitis virus	Preclinical	[5,6]
			Autonomous parvovirus	Preclinical	[10]
Attenuation by deletion of viral genes or gene fragments	Attenuation based on general tumor biology Broad mechanism of selectivity (targets signaling pathways) No foreign DNA elements or genes required	Risk of revertants to wild-type Multiple functions of viral genes	Adenovirus	Clinical	[19,29]
			Herpes simplex virus	Clinical	[11], MediGene AG[b]
			Vaccinia virus	Preclinical	[13]
			Poliovirus	Preclinical	[14]
			Measles virus	Preclinical	[5]
Transcriptional targeting	Technology well established Targeting based on known tumor biology	Insertion of foreign DNA elements Tumor escape by promoter shut-off	Adenovirus	Clinical	[58], Cell Genesys[c]
			Herpes simplex virus	Preclinical	[41]
Cellular targeting	Specific infection of tumor cells Reduced attenuation required Potential reduction in toxicity Potential reduction in dose size required	Technically challenging Detargeting and targeting required Tumor escape by mutation of receptor Insertion of foreign DNA elements	Adenovirus	Preclinical	[43]
			Herpes simplex virus	Preclinical	[44]

[a]http://www.oncolyticsbiotech.com; [b]http://www.medigene.com; [c]http://www.cellgenesys.com.

RNA activated protein kinase (PKR) by early viral transcripts [7]. However, increased levels of Ras activity, as is frequently observed in a wide variety of human tumors, counteract this inhibition by activating a phosphatase that antagonizes the PKR effects, which consequently enables virus replication [8]. Autonomously replicating parvoviruses (e.g. B19 or H1) that efficiently lyse transformed fibroblasts while sparing normal cells have been reported [9,10]; the mechanism of this process of differentiation has yet to be elucidated.

Attenuation of wild-type viruses through the targeted deletion of viral genes

Another strategy is based on restricting virus replication to malignant cells, which involves deletions of viral gene regions or entire genes that are crucial for efficient replication in normal tissue but that are dispensable in tumor cells ('attenuation') (Figure 1a). Genetically modified, tumor-selective mutants have been described for a variety of virus species, including herpes simplex viruses (HSV), adenoviruses, vaccinia viruses and polioviruses [11–14]. Tumor cells and cells that have been infected by viruses exhibit significant similarities in their abilities to interfere with signal transduction pathways, for example, promoting the transition from the prereplication (G1) to replication (S) stage of the cell cycle [1,2]. In particular, p53- and retinoblastoma protein (Rb)-governed checkpoints must be passed before cellular proliferation can be initiated. Several viral gene products that interact with cellular

CONSULTA DE EXPEDIENTES

PARAMETROS: MODULO CONSULTA DE EXPEDIENTES

26	09 003209	0	2009-01-16	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Harvey Alonso Jaramillo	2012-05-08 09:24	Juan Carlos Chaparro Velez	FONDO
27	09 009959	0	2009-02-03	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Harvey Alonso Jaramillo	2012-05-23 16:00	Clara Ines Barrera Garzon	Fondo
28	09 010461	0	2009-02-04	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Harvey Alonso Jaramillo	2012-05-23 16:00	Clara Ines Barrera Garzon	Fondo
29	09 010508	0	2009-02-04	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Harvey Alonso Jaramillo	2012-05-23 16:00	Clara Ines Barrera Garzon	Fondo
30	09 010511	0	2009-02-04	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Harvey Alonso Jaramillo	2012-05-23 16:00	Clara Ines Barrera Garzon	Fondo
31	09 010534	0	2009-02-04	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Harvey Alonso Jaramillo	2012-05-23 16:00	Clara Ines Barrera Garzon	Fondo
32	09 010539	0	2009-02-04	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Harvey Alonso Jaramillo	2012-05-23 16:00	Clara Ines Barrera Garzon	Fondo
33	09 010549	0	2009-02-04	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Harvey Alonso Jaramillo	2012-05-23 16:00	Clara Ines Barrera Garzon	Fondo
34	09 010565	0	2009-02-04	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Harvey Alonso Jaramillo	2012-05-23 16:00	Clara Ines Barrera Garzon	Fondo
35	09 011550	0	2009-02-06	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Harvey Alonso Jaramillo	2012-05-23 16:00	Clara Ines Barrera Garzon	Fondo
36	09 013387	0	2009-02-11	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Harvey Alonso Jaramillo	2012-05-23 16:00	Clara Ines Barrera Garzon	Fondo
37	09 014393	0	2009-02-13	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Harvey Alonso Jaramillo	2012-05-23 16:00	Clara Ines Barrera Garzon	Fondo
38	09 022192	0	2009-03-04	11 Pct-patente de invencion capitulos i y ii	379 Fase nacional incluido capitulo ii	1	Pub	Reasignacion	Harvey Alonso Jaramillo	2012-05-23 16:00	Clara Ines Barrera Garzon	Fondo
39	09 022307	0	2009-03-04	11 Pct-patente de invencion capitulos i y ii	379 Fase nacional incluido capitulo ii	1	Pub	Reasignacion	Harvey Alonso Jaramillo	2012-05-23 16:04	Clara Ines Barrera Garzon	Fondo

components, and thereby influence the cell cycle and cell survival, have been identified [1,2].

To target HSV replication to malignant cells, a variety of mutants have been designed with functional inactivation of the viral genes that encode for thymidine kinase, ribonucleotide reductase and infected cell protein 34.5 (ICP34.5) (e.g. HSV1716, dlsptk and hrR3) [11]. The cellular forms of viral ribonucleotide reductase and thymidine kinase are not expressed in quiescent cells, but are upregulated during the G1 and S phases of the cell cycle because they generate deoxynucleoside triphosphates (dNTPs), which are needed for DNA synthesis. This limits replication of HSV that is defective in such gene functions to rapidly proliferating cells, such as tumor cells [15]. The neurovirulence factor ICP34.5 has been characterized as an inhibitor of PKR. Therefore, PKR-induced shutoff of cellular protein synthesis following infection with HSV is circumvented by ICP34.5 [16]. However, Ras activity directly inhibits PKR-mediated effects, and thus the action of ICP34.5 is not required in cell lines that have a constitutively activated Ras signaling pathway [17]. To reduce the probability of the occurrence of wild-type revertants, and to increase the safety level, some viruses contain multiple mutations within their genomes (e.g. G207) [18]. In addition to HSV, other tumor-selective adenoviruses generated using this approach have been reported [19].

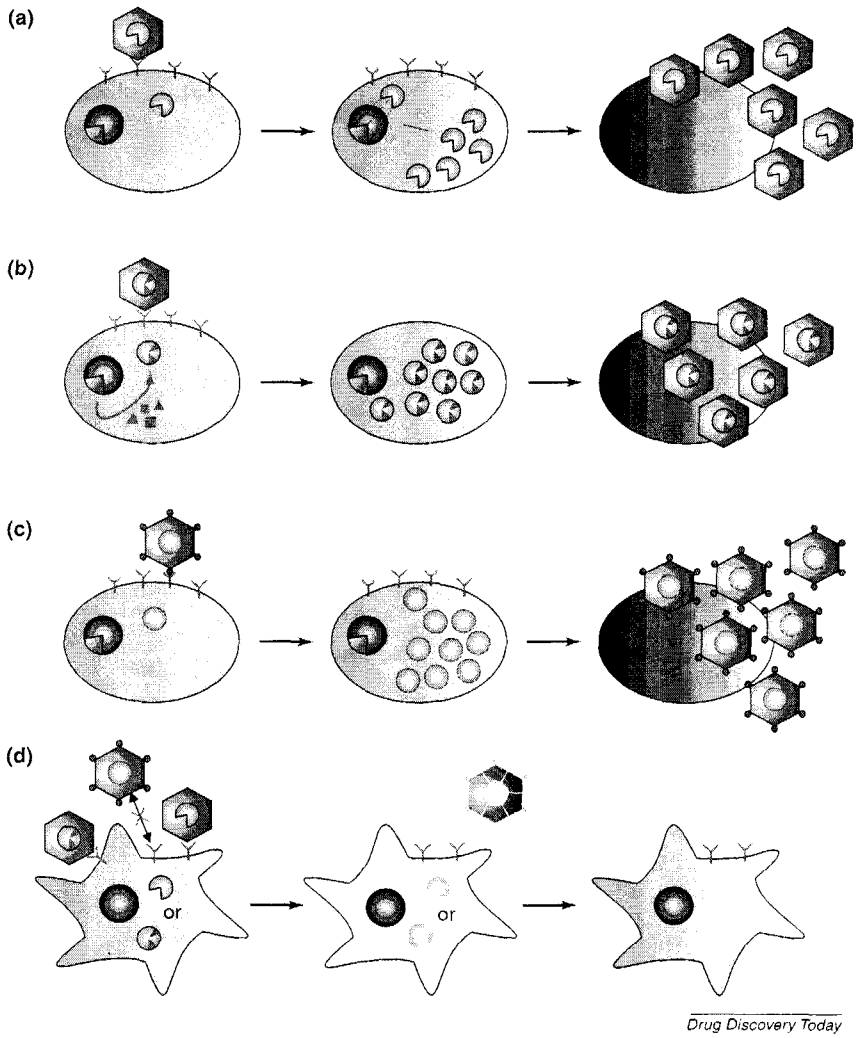

Figure 1. Strategies to achieve tumor-selectivity of oncolytic viruses. **(a)** Attenuation. Viral gene regions or entire genes that are crucial for the efficient replication of the virus in normal tissue are deleted from the viral genome. The genetic setting of tumor cells can complement those missing functions. **(b)** Transcriptional targeting. The expression of specific viral genes that are essential for viral replication is restricted to tumor cells through the use of tumor-specific promoters that are incorporated into the viral genome. **(c)** Cellular targeting. The natural cell tropism of the virus has been changed through genetic modification of the viral attachment proteins in such a way that only tumor-specific surface proteins are recognized as viral binding receptors. This strategy limits the entry of oncolytic viruses to tumor cells. **(d)** Effect of oncolytic viruses on normal cells. Although cellular-targeted oncolytic viruses (c) are unable to enter normal cells, the infection of normal cells with oncolytic viruses produced using strategies (a) and (b) leads to abortive infection.

The adenoviral early gene 1 A (E1A) proteins are potent transactivators that induce the entry of quiescent cells into the cell cycle and direct their progression to the S-phase; this sequence is predominantly initiated by the binding of the E1A proteins to the Rb protein, which triggers the release of the E2F transcription factor that is important for regulation of expression of cellular genes that control cellular DNA synthesis and proliferation [20]. However, the uncontrolled release of E2F and entry of quiescent cells into the cell cycle induce the accumulation of active p53 in the nucleoplasm, which causes growth arrest or apoptosis before the virus can replicate productively [21]. Therefore, adenoviruses encode another set of proteins, the E1B proteins (E1B55K and E1B19K), that counteract the p53-mediated effects triggered by E1A [22–24]. The E1B55K-deficient adenovirus dl1520 (Onyx-015) was the first replication-selective adenovirus to undergo rigorous

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

DEPENDENCIA: DIRECCION DE NUEVAS CREACIONES USUARIO: GLORIA JACQUELINE ALFONSO ROZO FECHA: 2012-05-31 15:44:17

CONSULTA DE EXPEDIENTES

PARAMETROS: MODULO CONSULTA DE EXPEDIENTES

EXPEDIENTE:

FECHA:

DEPENDENCIA:

FUNCIONARIO: CC: 80220549 - JUAN CARLOS CORTES GARCIA

ESTADO DEL PRESTAMO: PRESTAMO

INFORMACION DEL OPERADOR: DEPENDENCIA: OPERADOR:

No.	Radicación	Ctl	SecEve	FechRadi	Trámite	Evento	Fold	Tipo	Estado	Persona	FechEstado	Operador	Observaciones
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2	08 042425		0	2008-04-25	11 Pct-patente de invencion capitulos i y ii	379 Fase nacional incluido capitulo ii	1	Pub	Reasignacion	Juan Carlos Cortes Garcia	2012-03-08 12:02	Clara Ines Barrera Garzon	Fondo
3	08 042731		0	2008-04-25	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Juan Carlos Cortes Garcia	2012-03-08 12:02	Clara Ines Barrera Garzon	Fondo
4	08 063917		0	2008-06-20	11 Pct-patente de invencion capitulos i y ii	379 Fase nacional incluido capitulo ii	1	Pub	Reasignacion	Juan Carlos Cortes Garcia	2012-03-22 15:55	Clara Ines Barrera Garzon	Fondo
5	08 068127		0	2008-07-03	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Juan Carlos Cortes Garcia	2012-03-22 15:59	Clara Ines Barrera Garzon	Fondo
6	08 083709		0	2008-08-12	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Juan Carlos Cortes Garcia	2012-05-09 14:50	Juan Carlos Chaparro Velez	FONDO
7	09 001017		0	2009-01-07	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Juan Carlos Cortes Garcia	2012-05-02 11:26	Clara Ines Barrera Garzon	Fondo
8	09 002749		0	2009-01-15	11 Pct-patente de invencion capitulos i y ii	379 Fase nacional incluido capitulo ii	1	Pub	Reasignacion	Juan Carlos Cortes Garcia	2012-05-09 14:07	Juan Carlos Chaparro Velez	FONDO
9	09 003360		0	2009-01-16	11 Pct-patente de invencion capitulos i y ii	379 Fase nacional incluido capitulo ii	1	Pub	Reasignacion	Juan Carlos Cortes Garcia	2012-05-09 14:08	Juan Carlos Chaparro Velez	FONDO
10	09 003380		0	2009-01-16	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Juan Carlos Cortes Garcia	2012-05-09 14:08	Juan Carlos Chaparro Velez	FONDO
11	09 003502		0	2009-01-16	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Juan Carlos Cortes Garcia	2012-05-09 14:09	Juan Carlos Chaparro Velez	FONDO
12	09 003840		0	2009-01-19	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Juan Carlos Cortes Garcia	2012-05-09 14:09	Juan Carlos Chaparro Velez	FONDO
13	09 005501		0	2009-01-22	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Juan Carlos Cortes Garcia	2012-05-09 14:09	Juan Carlos Chaparro Velez	FONDO
14	09 006714		0	2009-01-26	11 Pct-patente de invencion capitulos i y ii	379 Fase nacional incluido capitulo ii	1	Pub	Reasignacion	Juan Carlos Cortes Garcia	2012-05-09 14:09	Juan Carlos Chaparro Velez	FONDO
15	09 007273		0	2009-01-27	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Juan Carlos Cortes Garcia	2012-05-09 14:09	Juan Carlos Chaparro Velez	FONDO
16	09 007344		0	2009-01-27	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Juan Carlos Cortes Garcia	2012-05-09 14:10	Juan Carlos Chaparro Velez	FONDO

Este reporte es genera[redacted]on base en información de la base datos de la SIC, la cual podrá ser ve[redacted]cada cuando se requiera.

clinical trials as a cancer treatment [25]. Although the exact mechanism of action of Onyx-O15 is not completely understood [26–29], results from animal models and clinical trials indicate that this compound is a promising anticancer agent.

Another mutation approach is based on the deletion of the E1A conserved region 2; this deletion prevents interaction of E1A with the Rb protein. Several deletion mutants, including some mutants in combination with cell cycle-dependent promoters (e.g. Onyx-411, Δ24 and dl922-947), have been reported [30–32]. Based on the attenuation approach, several oncolytic versions of vaccinia viruses that comprise mutations in the genes encoding thymidine kinase and/or vaccinia growth factor (VGF), which render the viruses highly tumor-selective, have been described [13]. Although preclinical results with these mutants are promising, the clinical utility could be limited by premature immunologic clearance of the virus and inadequate bystander effects. Poliovirus mutants are also available that selectively replicate in cell lines that are derived from human glioblastomas; this selectivity is the result of an altered internal ribosomal entry site (IRES) [33].

Transcriptional targeting

A third approach to restricting viral replication to malignant cells involves the engineering of tumor- or cell type-specific promoters and enhancers into viruses to limit the expression of the genes that are essential for viral replication in tumors ('transcriptional targeting') (Figure 1b). Replication-competent adenoviruses that have restricted expression of the *E1A* and *E1B* genes have been produced for prostate carcinomas using prostate-specific promoters, such as the prostate-specific antigen (PSA) promoter, the probasin promoter and combinations of both (e.g. CV706 and CG0787) [34,35]. Selective expression of E1A has been attempted in specific carcinomas, such as hepatocellular carcinoma (α-fetoprotein promoter) and breast carcinoma (mucin-1 promoter and estrogen-receptor promoter) [36–40]. In addition, the general characteristics of tumor cells (e.g. telomerase promoter and hypoxia-inducible factor responsive elements) have been used to design oncolytic adenoviruses [36–40]. As well as adenoviruses, replication-selective HSVs have been designed and investigated. The expression of the essential immediate-early *ICP4* gene, which is under control of the albumin-enhancer-promoter, principally restricts replication of HSV to the liver and to hepatocellular carcinoma [41]. In a similar approach, the calponin promoter has been used to generate HSV mutants that replicate selectively in malignant human soft tissue and bone tumors.

Cellular targeting

Tumor-selective uptake of replication-competent viruses can be achieved by modifications of the viral coat in a process that is known as 'cellular targeting' (Figure 1c). In theory, this strategy offers the advantage that more virulent viruses can be used: because these virus mutants would not infect normal cells, there should be a reduction in toxicity. Adenoviruses have been extensively studied using the cellular targeting approach, and there have been several attempts to modify the fiber proteins to redirect the natural vector tropism away from normal cells towards malignant cells [42,43]. More recently, HSV vectors with modified vector tropism have been produced. For example, engineered HSV-1 vectors have been designed that can only enter cells that express the interleukin-13 (IL-13) receptor α2, such as malignant brain tumors [44]. In these mutants, the natural binding sites for sulfated proteoglycans in the viral glycoproteins B and C have been ablated, and IL-13 has been inserted [44]. Interestingly, blood-borne Sindbis vectors that specifically target and kill tumor cells after systemic administration have been recently reported [45]. Tumor regression could be achieved in a variety of different tumor models, including syngeneic and spontaneous tumors. Although the exact mechanism of tumor-selective infection has yet to be elucidated, it seems possible that Sindbis vectors infect tumor cells via interaction with both heparan sulfate and the 67 kDa high-affinity laminin-receptor (LAMR), which is substantially upregulated in numerous human cancers and is related to increasing invasiveness and malignancy [45]. Furthermore, in contrast to normal cells, the majority of LAMRs on malignant cells are not occupied by laminin, which appears to render Sindbis vectors able to infect tumor cells specifically [45].

Escape from the immune system

In a recent study, Yu *et al.* [46] systemically administered vaccinia virus mutants to mice and demonstrated that these mutants selectively enrich and amplify in gliomas, as well as prostate, bladder and metastatic mammary carcinomas. The authors proposed that a small number of systemically applied microorganisms could enter tumors through leaky vasculature. Because the immune system is largely suppressed in tumors, microorganisms that are distributed to a tumor escape the immunosurveillance system of the host. By contrast, the immune system of the host clears the remaining circulating viral particles shortly after intravenous delivery.

Clinical trials with oncolytic viruses

As replication of human viruses is typically restricted to human cells, issues of tumor specificity, replicative efficacy

CONSULTA DE EXPEDIENTES

PARAMETROS: MODULO CONSULTA DE EXPEDIENTES

26	09 022189		0	2009-03-04	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Laura Carolina Pardo Perez	2012-05-28 17:26	Clara Ines Barrera Garzon	Fondo
27	09 023356		0	2009-03-06	2 Patentes de invencion	1 Registro/deposito/concesion/deposito	1	Pub	Reasignacion	Laura Carolina Pardo Perez	2012-05-28 17:27	Clara Ines Barrera Garzon	Fondo

Table 2. Clinical trials with oncolytic viruses

Virus species	Virus	Administration	Cancer type	Stage of clinical development	Status	Refs or source
Adenovirus	Onyx-015	Intratumoral injection	Head and neck cancer	Phase II	Completed	[49]
		Intratumoral injection	Pancreatic cancer	Phase II	Completed	[51]
		Mouthwash	Oral dysplasia	Phase I	Completed	[52]
		Intraperitoneal injection	Ovarian cancer	Phase I	Completed	[53]
		Hepatic intraarterial infusion	Liver metastases of colorectal	Combined Phase I and Phase II	Completed	[54,55]
		Intravenous	Metastatic colorectal cancer	Phase II	Completed	[57]
	CV706	Intraprostatic injection	Prostate cancer	Phase I	Completed	[58]
	CG7870	Intraprostatic injection	Prostate cancer	Combined Phase I and Phase II	Ongoing	Cell Genesys[a]
	Ad5-CD/TKrep	Intraprostatic injection	Prostate cancer	Phase I	Completed	[59,60]
Herpes simplex virus	G207	Intratumoral injection	Glioma	Phase I	Completed	[61]
	NV1020	Hepatic intraarterial infusion	Liver metastases of colorectal cancer	Combined Phase I and Phase II	Ongoing	[62], MediGene AG[b]
	HSV1716	Intratumoral injection	Glioma	Phase I	Completed	[64]
		Intratumoral injection	Metastatic melanoma	Pilot study	Completed	[65]
	OncoVEX	Injection into skin metastases	Skin metastases of solid cancers	Phase I	Ongoing	BioVex[c]
Newcastle disease virus	MTH-68	Intravenous	Glioma	Pilot study	Completed	[66]
	PV701	Intravenous	Advanced solid cancers	Phase I	Completed	[67]
Reovirus	Reolysin	Intratumoral	Skin metastases of solid cancers	Phase I	Completed	Oncolytics Biotech[d]
		Intratumoral	Prostate cancer	Phase I	Completed	
		Intratumoral	Glioma	Phase I	Ongoing	
		Intravenous	Advanced solid cancers	Phase I	Ongoing	

[a]http://www.cellgenesys.com; [b]http://www.medigene.com; [c]http://www.biovex.com; [d]http://www.oncolyticsbiotech.com.

and viral distribution cannot be fully addressed in animal models of cancer. Therefore, several research groups and companies have rapidly moved into clinical testing. Initial testing began with intratumoral injection and proceeded to intracavitary (such as intraperitoneal) and intravascular administration (such as hepatic artery infusion). More recently, systemic (i.e. intravenous) applications have been studied. Several clinical trials on the use of replication-competent viruses have now been published (Table 2).

Adenovirus

Of all the viruses that are undergoing clinical development, the adenovirus Onyx-015 (also known as dl1520 and CI-1042) has made the most progress, and has proven to be a safe single agent in Phase I and II trials for the treatment of patients with squamous cell carcinoma of the head and neck (SCCHN) [47,48]. SCCHN patients were chosen for initial trials because head and neck tumors harbor p53 mutations, the tumors can be injected directly, the clinical response can be assessed and biopsies can be obtained. Onyx-015 was injected intratumorally and treatment was well tolerated, with the main toxicity being mild flu-like symptoms (in particular, fever and chills). Viral replication was detected in 20% of patients and, although an antitumor response was seen in 14% of patients, a clinical benefit was not seen in the majority of the patients. However, when combined with chemotherapy, intratumoral injection of Onyx-015 demonstrated an impressive clinical

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

DEPENDENCIA: DIRECCION DE NUEVAS CREACIONES USUARIO: GLORIA JACQUELINE ALFONSO ROZO FECHA: 2012-05-31 15:37:26

CONSULTA DE EXPEDIENTES

PARAMETROS: MODULO CONSULTA DE EXPEDIENTES

EXPEDIENTE:

FECHA:

DEPENDENCIA: 2026-GRUPO DE TRABAJO DE CIENCIAS QUIMICAS

FUNCIONARIO: CC: 80503882 - ANDRES GIL HELFFHRITTZ

ESTADO DEL PRESTAMO: PRESTAMO

INFORMACION DEL OPERADOR: DEPENDENCIA: OPERADOR:

No.	Radicación	Ctl	SecEve	FechRadi	Trámite	Evento	Fold	Tipo	Estado	Persona	FechEstado	Operador	Observaciones
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2	07 074026		0	2007-07-19	11 Pct-patente de invencion capitulos i y ii	379 Fase nacional incluido capitulo ii	1	Pub	Reasignacion	Andres Gil Helffhrittz	2012-05-30 14:30	Clara Ines Barrera Garzon	Fondo
3	07 101336		0	2007-09-28	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Andres Gil Helffhrittz	2012-05-11 16:43	Juan Carlos Chaparro Velez	RTA ART 45
4	07 132023		0	2007-12-13	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Andres Gil Helffhrittz	2012-05-02 10:53	Juan Carlos Chaparro Velez	RTA ART 45
5	08 033859		0	2008-04-04	11 Pct-patente de invencion capitulos i y ii	379 Fase nacional incluido capitulo ii	1	Pub	Reasignacion	Andres Gil Helffhrittz	2012-05-23 10:56	Clara Ines Barrera Garzon	Rta 45
6	08 038372		0	2008-04-16	11 Pct-patente de invencion capitulos i y ii	379 Fase nacional incluido capitulo ii	1	Pub	Reasignacion	Andres Gil Helffhrittz	2012-05-11 16:43	Juan Carlos Chaparro Velez	RTA ART 45
7	08 040719		0	2008-04-22	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Andres Gil Helffhrittz	2012-05-11 16:43	Juan Carlos Chaparro Velez	RTA ART 45
8	08 043821		0	2008-04-29	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Andres Gil Helffhrittz	2012-05-23 10:57	Clara Ines Barrera Garzon	Rta 45
9	08 044733		0	2008-05-02	11 Pct-patente de invencion capitulos i y ii	379 Fase nacional incluido capitulo ii	1	Pub	Reasignacion	Andres Gil Helffhrittz	2012-05-30 14:31	Clara Ines Barrera Garzon	Fondo

response rate in 63% of the evaluated patients, with 27% (eight patients) demonstrating full response to therapy (i.e. complete disappearance of all tumor manifestations) and 36% indicating partial responses (i.e. tumor shrinkage by over 50% of initial tumor volume) [49]. This response rate is far in excess of the expected response rates of patients that were heavily pretreated with chemotherapy alone [49]. Six months after the end of the study, none of the responding tumors had progressed, whereas all non-injected tumors that were treated with chemotherapy alone had advanced. Tumor biopsy specimens obtained after treatment showed tumor-selective viral replication and necrosis induction [49]. Based on these encouraging results, Onyx Pharmaceuticals (http://www.onyx-pharm.com) initiated a randomized Phase III clinical trial with Onyx-015 for recurrent SCCHN. These trials have currently been stalled and their progression has yet to be sanctioned.

Onyx-015 was also administered intratumorally to patients with unresectable pancreatic cancer through computed tomography (CT)-guided injection [50] or endoscopic ultrasound injection in combination with intravenous gemcitabine [51] in a Phase I and a combined Phase I and Phase II clinical trial, respectively. Two patients incurred duodenal perforations from the rigid endoscope tip, but the treatment was generally well tolerated. In the combination trial, two partial regressions and two minor responses were observed, but these were not clearly attributable to Onyx-015 administration [51]. Another tested indication of Onyx-015 includes local mouthwash therapy for premalignant oral dysplasia [52]. Histological resolution was seen in seven (37%) out of 19 patients, but these effects were transient in the majority of patients. To test intracavitary applications of Onyx-015, a Phase I trial of intraperitoneal injection of Onyx-015 was undertaken in patients with recurrent and/or refractory ovarian cancer [53]. No significant toxicity was observed at the maximum dose of 10^{11} plaque-forming units (pfu). However, no clinical or radiographic evidence of a tumor response was observed in any of the patients [53].

Intravascular applications have proved particularly promising. Hepatic arterial infusion of Onyx-015 in combination with 5-fluorouracil (5-FU) and leukovorin for liver metastases of gastrointestinal (GI) tract tumors was tested in a combined Phase I and Phase II clinical trial [54,55]. Although increased levels of liver enzymes and hyperbilirubinemia were transiently observed in a subset of patients, the regimen proved to be safe and feasible. Delayed secondary peaks of viremia at 72 h post-inoculation were detected and interpreted as an indication of viral replication. Out of 27 patients, three (11%) had a partial response, while 13 (48%) had a minor response or stable disease. However, an antitumoral activity that could be directly attributed to Onyx-015 was difficult to assess because most patients were not refractory to 5-FU and leukovorin chemotherapy [54,55].

To test systemic administration, Onyx-015 was given by intravenous infusion to patients with metastatic solid tumors (Phase I) [56], and in a Phase II clinical trial to patients with metastatic colorectal cancer [57]. Toxicity was manageable and consisted primarily of flu-like symptoms (including chills, rigors and fever); however, one patient out of 18 was hospitalized for severe lethargy. No liver toxicity was observed and viral DNA was detectable for as long as 72 h in 36% of the patients, all of which developed neutralizing antibodies. Three out of the 18 treated patients had minor reductions of carcinoembryonic antigen (CEA) levels and seven patients were assessed as having stable disease for 11 to 18 weeks. However, a progression in disease was ultimately observed in all patients [57].

Early clinical trials with adenoviruses that are transcriptionally targeted to replicate in prostate cancer cells have demonstrated promising results. A Phase I clinical trial was carried out in patients suffering from locally recurrent prostate cancer with adenovirus CV706 (PSA-restricted expression of E1A and/or E1B) [58]. Intraprostatic injection of this virus caused mild flu-like symptoms and hematuria. However, 13 out of 20 patients (65%) experienced a reduction in serum PSA of ≥30% from pre-treatment levels [58]. Patients receiving the highest intraprostatic dose of CV706 showed a reduction of PSA ≥50%, which underlines the biological activity of this compound, and post-CV706 treatment prostate biopsies revealed viral replication in individual patients [58].

In two Phase I clinical trials, the efficacy of the 'armed' adenovirus Ad5-CD/TKrep as a treatment for prostate cancer was tested as an intraprostatic injection, either alone [59] or in combination with radiation therapy [60]. Two days after adenovirus injection, the prodrugs 5-fluorocytosine and valganciclovir were administered and no dose-limiting toxicity was observed. Seven out of 16 (44%) patients demonstrated a ≥25% decrease in PSA levels and 19% showed a ≥50% decrease in PSA [54]. As expected, the response rates were increased when the course of treatment was supplemented with radiation [60].

Herpes simplex virus

The HSV-1 variant G207 was tested in a Phase I dose-escalation study that was designed to determine the safety of stereotactic inoculation for the treatment of recurrent malignant glioma [61]. Twenty-one patients received between 10^6 and 3×10^9 pfu. There were no serious side effects and, importantly, no patient developed HSV encephalitis.

CONSULTA DE EXPEDIENTES

PARAMETROS: MODULO CONSULTA DE EXPEDIENTES
EXPEDIENTE:
FECHA:
DEPENDENCIA: 2026-GRUPO DE TRABAJO DE CIENCIAS QUIMICAS
FUNCIONARIO: CC: 1075654222 - RUBIELA PACANCHIQUE VARGAS
ESTADO DEL PRESTAMO: PRESTAMO
INFORMACION DEL OPERADOR: DEPENDENCIA: OPERADOR:

No.	Radicación	Ctl	SecEve	FechRadi	Trámite	Evento	Fold	Tipo	Estado	Persona	FechEstado	Operador	Observaciones
1	07 129191		0	2007-12-06	11 Pct-patente de invencion capitulos i y ii	379 Fase nacional incluido capitulo ii	1	Pub	Reasignacion	Rubiela Pacanchique Vargas	2012-05-30 10:16	Clara Ines Barrera Garzon	Fondo
2	08 003741		0	2008-01-16	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Rubiela Pacanchique Vargas	2012-05-08 07:54	Juan Carlos Chaparro Velez	FONDO
3	08 024615		0	2008-03-07	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Rubiela Pacanchique Vargas	2012-05-08 07:53	Juan Carlos Chaparro Velez	FONDO
4	08 054915		0	2008-05-29	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Rubiela Pacanchique Vargas	2012-05-30 10:17	Clara Ines Barrera Garzon	Fondo
5	08 062741		0	2008-06-18	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Rubiela Pacanchique Vargas	2012-05-30 10:17	Clara Ines Barrera Garzon	Fondo
6	08 102836		0	2008-09-26	11 Pct-patente de invencion capitulos i y ii	379 Fase nacional incluido capitulo ii	1	Pub	Reasignacion	Rubiela Pacanchique Vargas	2012-05-30 10:17	Clara Ines Barrera Garzon	Fondo
7	08 130957		0	2008-12-10	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Rubiela Pacanchique Vargas	2012-05-30 12:16	Clara Ines Barrera Garzon	Fondo
8	08 131390		0	2008-12-11	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Rubiela Pacanchique Vargas	2012-05-08 07:54	Juan Carlos Chaparro Velez	FONDO
9	08 131986		0	2008-12-12	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Rubiela Pacanchique Vargas	2012-05-30 10:17	Clara Ines Barrera Garzon	Fondo
10	09 035814		0	2009-04-07	11 Pct-patente de invencion capitulos i y ii	379 Fase nacional incluido capitulo ii	1	Pub	Reasignacion	Rubiela Pacanchique Vargas	2012-05-30 10:16	Clara Ines Barrera Garzon	Fondo
11	09 035915		0	2009-04-07	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Rubiela Pacanchique Vargas	2012-05-30 10:16	Clara Ines Barrera Garzon	Fondo
12	09 035919		0	2009-04-07	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Rubiela Pacanchique Vargas	2012-05-30 10:15	Clara Ines Barrera Garzon	Fondo
13	09 036257		0	2009-04-08	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Rubiela Pacanchique Vargas	2012-05-30 10:15	Clara Ines Barrera Garzon	Fondo
14	09 037411		0	2009-04-14	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Rubiela Pacanchique Vargas	2012-05-30 10:15	Clara Ines Barrera Garzon	Fondo
15	09 037597		0	2009-04-14	11 Pct-patente de invencion capitulos i y ii	378 Fase nacional incluido capitulo i	1	Pub	Reasignacion	Rubiela Pacanchique Vargas	2012-05-30 10:15	Clara Ines Barrera Garzon	Fondo
16	09 038945		0	2009-04-17	11 Pct-patente de invencion capitulos i y ii	379 Fase nacional incluido capitulo ii	1	Pub	Reasignacion	Rubiela Pacanchique Vargas	2012-05-30 10:09	Clara Ines Barrera Garzon	Fondo

In several individual patients, radiographic imaging suggested an antitumor response [61].

Early clinical data suggested a safe toxicity profile for the virus NV1020, which is in ongoing combined Phase I and Phase II trials as a therapy for liver metastases of colorectal cancer [62]; NV1020 is currently being developed by MediGene AG (http://www.medigene.com). OncoVex® (BioVex; http://www.biovex.com/index.html), which expresses the immunostimulatory granulocyte–macrophage colony-stimulating factor (GM-CSF), is currently in Phase I clinical testing for skin metastases of solid tumors, but no clinical data has as yet been reported.

HSV1716 was administered by intratumoral injection to a small group of nine patients suffering from relapsed malignant glioma [63]. The study demonstrated the therapeutic feasibility of HSV1716 with viral doses up to 10^5 pfu, with no signs of encephalitis. These findings were confirmed in another trial in which 12 glioma patients underwent intratumoral injection with 10^5 pfu, followed by surgery [64]. The data documented intratumoral replication within high-grade gliomas without causing toxicity in both HSV-seropositive and HSV-seronegative patients [64]. In addition, HSV1716 was injected intralesionally into subcutaneous nodules of metastatic melanoma of five patients [65]. As an internal control, a second nodule was injected with sterile saline. A flattening of previously palpable tumor nodules and microscopic evidence of tumor necrosis were observed. Although patient numbers are too small to draw definite conclusions, the observed effects do warrant further investigation [65].

Newcastle disease virus

MTH-68 and PV701 are two attenuated, non-recombinant strains of NDV, which is an avian paramyxovirus that causes flu-like symptoms in humans. Csatary *et al.* [66] examined the oncolytic potential of NDV by treating four cases of advanced high-grade glioma with daily intravenous injections of live attenuated NDV MTH-68 [66]. Two of the four patients had near complete disappearance of their gliomas, while the remaining two patients experienced stabilization of their disease. Although these four cases were treated with different dosing regimens, these data are nevertheless encouraging and provide grounds for further development [66]. Intravenous administration of PV701 was tested in a large Phase I clinical trial as a single agent in 79 patients with advanced cancers [67]. Flu-like symptoms, fever and hypotension were recorded, but no serious side effects were observed. Interestingly, with repeated cycles, the occurrence and intensity of flu-like symptoms decreased. The majority of patients developed antibodies to NDV. After intravenous treatment, 14 patients had stable disease for four to >30 months, seven demonstrated minor responses, two had a partial response and one patient with chemotherapy-refractory tonillar (squamous cell) carcinoma had a complete response but then relapsed seven months after treatment began [67].

Reovirus and vaccinia virus

No clinical data on the use of reovirus or vaccinia virus (expressing the immunostimulatory GM-CSF protein) have been published. As summarized in Table 2, several Phase I trials have been completed or are ongoing.

Challenges of oncolytic virotherapy

Oncolytic viruses can rapidly replicate in and spread through 2D cell cultures that are derived from a variety of different tumor types. However, there are several factors that could hamper the efficient spread of oncolytic viruses within a solid tumor mass [68]. Physical barriers such as necrotic areas within the infected tumor, normal stroma cells and extracellular matrix, or the presence of the basal membrane, could limit the distribution and infection of the diffuse virus. Mathematical models of viral replication have underlined the importance of diffuse tumor inoculation for the control of tumor growth and for the initiation of a self-perpetuating process of intratumoral viral replication [69,70]. In addition, the physical size of the administered virus particles and their interaction with the receptors that are present on normal cells could be crucial. New delivery technologies, such as convection-enhanced delivery (CED) of drugs to the brain, will need to be explored for oncolytic viral therapy of brain tumors to achieve an even virus distribution. CED enables potent drugs, which would otherwise be too toxic to the body, or drugs that are not capable of passing through the blood–brain barrier, to be slowly and continuously infused into particular brain tumors through small plastic catheters. After surgery, the drug is administered via the catheters using an infusion pump over a period of several days, and then the catheters are removed. Tools have been developed that facilitate enhanced virus administration within the tumor using several simultaneous needle injections. Importantly, the immune system of the host could limit ongoing viral replication within the tumor, and rising antibody titers could neutralize repeatedly administered viruses before the tumor has been successfully eradicated [12]. Although these neutralizing antibodies do not appear to be a major limitation for the intratumoral injection of oncolytic viruses that spread through direct cell–cell contact (e.g. HSV and measles virus), they could become a crucial factor for systemic therapy that uses intravenous administration [68]. The complement system might be another impediment

sólidas como son los pseudopolimorfos, ni su forma de obtención, para el caso particular de la sal cristalina aquí reivindicada el Examinador afirma que el desarrollo de la forma reivindicada carece de actividad inventiva alguna.

En conclusión, no existe motivación alguna en D1 para que la persona medianamente versada en la materia buscara obtener una forma cristalina del compuesto, y menos aún en D3, que es un documento de conocimiento general. El método desarrollado en la presente invención establece de manera clara y precisa las condiciones que se deben tener en cuenta para obtener los polimorfos de la sal de ácido málico del compuesto N-[2 (dietilamino)etil]-5-[(5-fluoro-2oxo-3H-indol-3-ilideno) metil]-2,4-dimetil-1H-pirrol-3-carboxamida, ninguna de las cuales se encontraba enseñada en D1 y menos aún en el documento general D3.

Se le aclara al solicitante, que si bien es cierto que la cantidad de experimentación requerida para verificar y entender la formación de las formas cristalinas de un compuesto puede ser sustancial y extensa, no implican un nivel inventivo puesto además de ser rutinarios, la generación de un nuevo ordenamiento en el estado cristalino no se genera por la habilidad del investigador para inducir las características del estado sólido de la molécula, debido a que el polimorfismo es una propiedad "inherente" de las moléculas, es la "capacidad que presenta un compuesto" para cristalizar en más de un sistema cristalino.

Por lo tanto se reitera que tal como se ha explicado en los puntos anteriores, el estado de la técnica (D1 y D3) afectan el nivel inventivo, porque la persona del oficio medianamente versada en la materia dentro de los estudios de preformulación debe determinar e investigar por pruebas rutinarias de ensayo y error los polimorfos, pseudopolimorfos o hidratos que se encuentren en los principios activos como la sal del ácido maleíco del compuesto N-[2 (dietilamino)etil]-5-[(5-fluoro-2oxo-3H-indol-3-ilideno) metil]-2,4-dimetil-1H-pirrol-3-carboxamida conocida en D1 porque las diversas formas polimórficas encontradas en un principio activo suelen presentar comportamientos fisicoquímicos diferentes en propiedades de interés en las formulaciones farmacéuticas tales como la densidad, la dureza, higroscopicidad, velocidad de solubilización, estabilidad térmica, etc..., hecho que el formulador o diseñador de productos farmacéuticos debe conocer con el fin de poderlos adecuar a la forma posológica de interés y así garantizar su uniformidad, biodisponibilidad y estabilidad según lo establecido en el documento D3 (Ver folio 254, página 2227).

9. *iii) Por último, me permito presentar nuevamente los siguientes datos, que el Examinador desestima, en donde se evidencia las características superiores de las formas cristalinas reivindicadas:*
   . el cristal de Formula 1 presenta una pureza del 98% con menos de un 2% de impurezas y que no muestra degradación significativa en estudios de estabilidad;
   . la higroscopicidad de la Forma 1 es muy baja, absorbiendo menos del 0.5% de agua en un rango de 0-90% de humedad relativa. En contraste la Forma II es muy higroscópica, absorbiendo un 1 5% de humedad en el mismo rango de humedad relativa;
   . la Forma 1 se funde a aproximadamente a 1 96°C, mientras que la Formula II se funde a 181°C;
   . los datos TGA para la forma cristalina 1 no muestra una perdida significativa de peso al alcanzar el punto de fusión, lo que es indicativo de la ausencia de solvente residual y/o agua retenida en los cristales.

   De acuerdo con lo anterior, y teniendo en cuenta las disposiciones del Tribunal Andino de Justicia, que establece que resultados y mejoras sorprendentes constituyen indicios de no obviedad, y que el Manual Andino de Patentes indica que las propiedades ventajosas constituyen indicios aceptables de nivel inventivo, además de una estructura inesperada, las formas cristalinas reclamadas en el Capitulo Reivindicatorio Modificado gozan de nivel inventivo frente a los antecedentes citados por el Examinador.

Se le recalca nuevamente al solicitante que el resultado obtenido en la presente solicitud (formas cristalinas I y II de la sal del ácido málico del compuesto N-[2 (dietilamino)etil]-5-[(5-fluoro-2oxo-3H-indol-3-ilideno) metil]-2,4-dimetil-1H-pirrol-3-

carboxamida con propiedades beneficiosas incluidas la estabilidad térmica, menor higroscopicidad y una alta pureza) no es sorprendente, ni inesperado para la persona medianamente versada en la materia, ya que si bien es cierto que el documento D1 no insinúa ni sugiere la presencia de otras formas cristalinas de la I de la sal del ácido málico del compuesto N-[2 (dietilamino)etil]-5-[(5-fluoro-2oxo-3H-indol-3-ilideno) metil]-2,4-dimetil-1H-pirrol-3-carboxamida es obvio para un experto medio con conocimientos en el arte (evidenciados en el libro de texto citado D3, Farmacia de Remington), que una vez se ha determinado que un compuesto presenta polimorfismo se procede a preparar las diferentes formas cristalinas por métodos convencionales , caracterizarlas por técnicas conocidas (patrón de difracción de rayos X, calorimetría etc..) y por ultimo determinar las características de cada forma cristalina (como por ejemplo la estabilidad térmica, la higroscopicidad, pureza entre otras), lo cual debe quedar claro no se considera un resultado patentable porque es el normal resultado obtenido de pruebas habituales por ensayo y error. Además, no hay un efecto diferente o mejorado de la sal del ácido málico del compuesto N-[2 (dietilamino)etil]-5-[(5-fluoro-2oxo-3H-indol-3-ilideno) metil]-2,4-dimetil-1H-pirrol-3-carboxamida puesto que tanto la forma conocida de este compuesto en D1, como los polimorfos de la actual solicitud presentan la misma actividad terapéutica (inhibidores de la enzimas PK) donde lo único que cambia son sus propiedades fisicoquímicas como ocurre con todos los polimorfos y es ampliamente divulgado en D3, debido a que las formas cristalinas se conforman de manera diferente entre ellas, por lo que este cambio en los parámetros fisicoquímicos de una forma cristalina a otra o con respecto a su forma amorfa era de esperarse para la persona normalmente versada en la materia;

10. *Por último, me permito reiterar que la presente invención ha sido concedida en numerosas jurisdicciones, como se demuestra en el listado adjunto.*

    Si bien la SIC repetidamente ha argumentado que las concesiones de patentes en otros países no influencian la decisión de patentamiento de solicitudes nacionales en Colombia, en cuanto a nivel inventivo se refiere, llamo la atención del Examinador sobre el hecho que la evaluación del requisito de nivel inventivo es equivalente entre muchas de las legislaciones que han concedido esta patente y la legislación colombiana, por lo que resulta a todas luces inconsecuente e incoherente que hasta el momento en esas jurisdicciones se acepte el carácter inventivo de la invención reivindicada, pero en la colombiana, aunque conceptualmente el significado sea el mismo, se niegue, por lo de más, basándose en premisas subjetivas, tal como lo demostré anteriormente[...].

    De nuevo, aunque las concesiones de patentes en oficinas de otros países no son vinculantes en el sentido que obliguen a la SIC a tomar una decisión en el mismo sentido, es completamente cierto que los criterios de patentabilidad en los que deben fundamentarse el análisis de solicitudes de patente, en particular en cuanto al nivel inventivo se refiere, son análogos globalmente, y concesiones en otras jurisdicciones sugieren el cumplimiento de dichos requisitos, ya que claramente, los criterios de evaluación y estudio aplicados para determinar la novedad y nivel inventivo resultan equivalentes a los que la Superintendencia debe emplear, establecidos de manera semejante por las legislaciones de patentes a lo largo del mundo entero.

    Así las cosas, solicito respetuosamente a ese despacho pronunciarse claramente sobre los hechos y elementos concluyentes que conducen a la SIC a evaluar el nivel inventivo de una manera diferente a la realizada por ejemplo por las oficinas de incluidas en el listado adjunto. No se entiende como pueden todas estas oficinas de manera independiente llegar a la misma conclusión, y la SIC a una exactamente contraria, aunque la legislación que define el requisito de nivel inventivo sea equivalente entre todas estas jurisdicciones.

El solicitante tiene razón en que a nivel mundial los requisitos de novedad y nivel inventivo que debe ostentar una invención son esencialmente equivalentes. Sin embargo se recalca nuevamente que la determinación de patentabilidad, depende de otros factores como por ejemplo los documentos o anterioridades utilizados por cada oficina durante el examen y los conocimientos comunes de la persona

to effective delivery via the intravenous route [71]. By contrast, immune responses could also enhance the efficacy of oncolytic viral therapy, as suggested by a significant number of studies investigating viruses that express immunostimulatory proteins [4,72]. Cytotoxic T-cell responses that are directed against the tumor have been identified as a potentially important therapeutic factor [72]. Therefore, several methods that eliminate undesired immune effects while preserving beneficial properties have been proposed. The production of neutralizing antibodies could be transiently ablated by administration of anti-CD20 antibodies (rituximab) against B-lymphocytes before oncolytic virotherapy. Alternatively, the exchange of blood plasma (plasma pheresis) will enable the elimination of antibodies that are directed against viruses from the bloodstream [68]. To prevent inactivation of administered viruses by the complement, the complement could be transiently neutralized by administration of cobra venom factor or cyclophosphamide (CPA) [73]. In addition, the use of virus mutants that incorporate complement resistance factors into the outer membrane, which therefore conveys resistance to the complement, has been suggested [68]. A major factor that could potentially lead to the rapid clearance of viruses from the bloodstream could be uptake into Kupffer cells, which are extremely active phagocytic cells that line the walls of the sinusoids of the liver [68]. However, preclinical experiments with several vectors have shown that systemic metastases can be targeted following intravenous administration, despite a level of clearance by the liver. Finally, sufficient expression of viral receptors on malignant cells is required for therapeutic efficacy [68] – a factor that has been identified as a potential limitation for oncolytic adenoviruses. Expression of the key receptor for adenoviral uptake (CAR) appears to be downregulated during tumor progression, presumably by activation of oncogenic signaling pathways that render highly malignant cells less susceptible to therapy [74]. However, strategies have been suggested to overcome these prospective obstacles, such as the pharmacological inhibition of the Ras-signaling pathway [e.g. by using inhibitors of the mitogen-activated protein kinase kinase (MEK)], which increases the expression of CAR on the cell surface and consequently restores the permissiveness of these cells to viral uptake [75]. In addition, retargeted viruses are being explored that comprise new receptor-binding motifs within their coat that enable CAR-independent infection [43].

Conclusion and future perspectives

Replication-competent oncolytic viruses, either naturally occurring or genetically engineered, represent a promising new class of agents that are in development for the treatment of human cancer. Potential hurdles have been identified, and solutions to these problems are currently being explored in preclinical studies and clinical trials. To date, clinical experience indicates that these agents are safe. To increase potency, two key strategies are being pursued. Combination of oncolytic virotherapy with traditional chemotherapy and radiotherapy significantly enhances the efficacy of virotherapy, frequently on a synergistic basis [76]. In parallel, additional antitumor mechanisms are applied to oncolytic viruses to arm them with therapeutic transgenes (e.g. prodrug-converting enzymes and anti-angiogenic or immunomodulatory proteins) that induce bystander effects that are capable of eliminating tumor cells that are not directly killed through viral oncolysis [4].

Randomized Phase III clinical trials are urgently needed to assess the clinical efficacy of these promising biological agents. The availability of systemic therapy in conjunction with oncolytic viruses will enhance the potential of oncolytic viruses to become a viable new therapeutic approach for the treatment of cancer.

Acknowledgements

We gratefully acknowledge Carola Ries for her careful review of this manuscript and for crucial discussions. We apologize to those authors whose work is not cited because of space constraints.

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No puede olvidarse que la búsqueda de polimorfos no es predecible, por lo que siempre los científicos investigadores deben explotar intensamente su conocimiento y creatividad con el fin de desarrollar nuevas formas cristalinas. [...]Ni siquiera un conocimiento profundo de la teoría de cristales o una amplia experiencia específica en el área, permitirían a una persona medianamente versada en la materia anticipar inequívocamente los anteriores hechos, basándose en la divulgación general del compuesto de D1 y la teoría de cristales sólidos, pues solamente con la invención de la presente solicitud se logró la obtención de las formas cristalinas reivindicadas, sin seguir ningún tipo de indicación o enseñanza del arte previo.

Evidencia de lo anterior se puede encontrar en el artículo "An experiment in crystal structure prediction by popular vote" de Graeme et al, adjunto al presente memorial como "Anexo 1 ", en donde se evidencia la no predictibilidad de una forma cristalina de un compuesto dado. En este estudio, se evalúa la habilidad de diferentes experimentadores cristalógrafos expertos para predecir estructuras cristalinas correctas a partir de una lista de estructuras cristalinas diferentes para un compuesto dado. La metodología del estudio involucra dos compuestos específicos (5- fluoro-2-oxindol y 3-quinoclidinol racémico) con estructuras rígidas, que no se encontraban publicadas en la base de datos de Cambridge (Cambridge Structural Database -CSD-), para las cuales se generaron diferentes estructuras cristalinas por métodos computacionales, en las que se buscaba el menor empaquetamiento energético posible, de las cuales se escogieron 5 estructuras para ser incluidas en el test.[...] Los resultados demostraron que la estructura de la forma cristalina real del compuesto 1 (5- fluoro-2-oxindol) fue la menos popular entre dichos expertos por un margen significativo (página 1 989, párrafo de conclusiones, línea 7-9). Para la segunda molécula, que resultaba ser más difícil de analizar (sólo se recibieron 38 análisis, mientras que para la molécula 1, se recibieron 50), ya que las diferentes estructuras cristalinas se podían distinguir menos, no se pudo establecer una marcada diferencia entre la popularidad de las diferentes estructuras cristalinas, sin embargo, la estructura cristalina obtenida experimentalmente fue de nuevo la menos popular.

De lo anterior se puede concluir que el estudio de las diferentes formas cristalinas de una molécula específica no es tarea fácil, requiere experiencia y experticia, así como no es posible predecir a partir de técnicas rutinarias (como lo señala el Examinador) y tampoco mediante técnicas computacionales a disposición de los expertos.

Este Despacho no deja de reconocer que el polimorfismo es un problema en la industria farmacéutica. También es cierto que solamente algunos polimorfos presentan propiedades deseables. Sin embargo en este caso particular las formas cristalinas reivindicadas son el resultado de un trabajo rutinario o habitual, en el cual el cometido inicial del investigador (en este caso preformulador, persona versada en la materia) es determinar si el compuesto de la solicitud (sal del ácido málico del compuesto -[2 (dietilamino)etil]-5-[(5-fluoro-2oxo-3H-indol-3-ilideno) metil]-2,4-dimetil-1H-pirrol-3-carboxamida puede existir en diferentes formas cristalinas y para esto ya se tienen procedimientos establecidos (Ver folio 254, página 2227). Una vez que el preformulador determina que la molécula presenta formas cristalinas diferentes (polimorfismo) existen procedimientos con los cuales el preformulador puede prepararlas en cantidades más grandes, en donde lo que se hace es establecer las condiciones en que se pueden producir cada una de ellas (Ver folio 255, página 2229). Si bien es cierto que el establecimiento de dichas condiciones requiere una rigurosa atención de los detalles referentes a las condiciones de preparación , lo que asegura la reproducibilidad del método para la preparación de cierta forma específica, la determinación y control de factores como el solvente utilizado y su pureza, el grado de agitación de la solución, la temperatura, la saturación la solución, la tasa de enfriamiento necesaria, los métodos de aislamiento entre otras resultan ser destrezas básicas para cualquier persona con formación en química [(1)]

Luego de obtener las diferentes formas cristalinas el preformulador procede a identificarlas o caracterizarlas mediante técnicas que son del conocimiento común,

[(1)] **Helman José. Farmacotecnia teórica y práctica. Tomo IV. Editorial Continental, Tercera impresión, México, Noviembre de 1982. Capítulo 30 Cristalización pág 1103 a 1143.**

tales como patrón de difracción de rayos X, IR, Ramán *y ^{13}C RMN*, y luego se diseña una serie de experimentos para determinar si sus propiedades difieren o no lo suficiente para alterar su comportamiento farmacéutico o biológico (estabilidad, punto de fusión, las gamas de estabilidad térmica, solubilidades, higroscopicidad entre otras) .(Ver folio 254 páginas 2227 a 2228)

Este trabajo es importante para establecer qué tipo de polimorfos hay que escoger para el desarrollo apropiado de las formulaciones, con el fin de evitar por ejemplo la falta de uniformidad, mal aspecto, mala biodisponibilidad u otro tipo de transformaciones durante la molienda u otros procedimientos de preparación que alteran las características físicas y biológicas, respuesta farmacológica inadecuada o mala estabilidad química entre otros, que son los problemas comunes y rutinarios que se presentan en la industria farmacéutica (Ver folio 256, página 2231).

Además, si bien es cierto que no existen reglas que permitan predecir que tipo de polimorfos o cuántos producirá una sustancia, la persona del oficio medianamente versada en la materia con base en sus conocimientos puede variar condiciones de cristalización como presión, temperatura, solventes etc... de manera aleatoria lo que permite obtener diferentes formas cristalinas de las cuales selecciona la(s) forma(s) cristalina(s) deseada(s) de la sal del ácido málico del compuesto -[2 (dietilamino)etil]-5-[(5-fluoro-2oxo-3H-indol-3-ilideno) metil]-2,4-dimetil-1H-pirrol-3-carboxamida por pruebas de ensayo -error (en este caso la que sea menos higroscópica , más estable y más pura) sin que esto represente una actividad inventiva.(Ver folio 255, página 2229 , folio 253, página 2226 y folio 254, página 2227)

Por lo tanto es válido aseverar, que la presente solicitud carece de nivel inventivo ya que se refiere a la obtención de un polimorfo mediante un procedimiento básico del conocimiento común (cristalización- recristalización) y su caracterización, que cualquier persona versada en la materia (farmacéutico formulador) debe realizar como una TAREA OBLIGATORIA dentro de las etapas de preformulación a pesar de que esta labor sea ardua y extensa; particularmente porque es necesaria en el campo farmacéutico, donde la seguridad y eficacia de los fármacos puede depender directamente del reconocimiento las diferentes formas cristalinas que se puedan encontrar .

6. *Dado el esfuerzo involucrado en el desarrollo de estas mejoras, y dado que éstas proporcionan ventajas comerciales competitivas, y se derivan directamente de la investigación y creatividad humanas, el sistema de patentes debe garantizar su protección. El Examinador no puede basarse en apreciaciones subjetivas para privar al inventor de la protección merecida para su desarrollo novedoso y no sugerido por el estado de la técnica. En el mundo entero las formas cristalinas específicas son consideradas no-obvias y por ende, materia patentable. [...]El Examinador debe tener en cuenta que el análisis de nivel inventivo no puede estar basado en si es o no posible obtener la invención reclamada, pues claramente era posible, toda vez que las formas polimórficas reivindicadas existen. Esta aproximación conllevaría a que ninguna invención sería patentable, volviendo así inoperante el sistema de patentes. En este caso, el hecho que pudiera existir eventualmente una mínima motivación general para llevar a cabo trabajo experimental para determinar si hay otras formas físicas (formas cristalinas), no hace el resultado positivo obtenido (es decir la invención) obvio o evidente; si así fuera, el trabajo experimental sería innecesario. El que sea obvio intentar, no necesariamente hace obvia la invención.*

   Recordemos también que el hecho de utilizar herramientas conocidas en el estado de la técnica no desvirtúa automáticamente el nivel inventivo de una invención, dado que es necesario valerse de las herramientas existentes en la técnica para así poder seguir avanzando en los desarrollos tecnológicos.[...] Así las cosas, durante el análisis de una solicitud de patente, el Despacho debe poder citar anterioridades que específicamente motiven a una persona versada en el arte a combinar los elementos del estado de la

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técnica y a intentar las opciones específicas para llegar a la solución inventiva reclamada. No es suficiente alegar que la persona versada podría llegar a la invención, puesto que, como se ha dicho, este criterio desdibujaría por completo el sistema de patentes, elevando el requisito de nivel inventivo a tal punto que sería patentable e indicaría que en todos los casos no hubo creatividad inventiva en el desarrollo de nuevas invenciones, porque éstas siempre serían vistas cómo logros apuntados por el estado de la técnica.

Al respecto resulta preciso aclarar que aunque los polimorfos reivindicados (Formas cristalinas I y II) no fuesen posibles de predecir este solo hecho no las hace patentables, porque si bien no se conocían puesto que las anterioridades no mencionan expresamente estas formas cristalinas, su obtención no es fruto de una actividad creativa del hombre que implique un salto cualitativo en relación con la técnica existente, ya que el polimorfismo es una característica inherente a los compuestos es su capacidad natural para ordenar sus moléculas de un modo muy regular y simétrico dando lugar a diferentes formas cristalinas, por pruebas de ensayo –error (variando las condiciones de su entorno (como presión, temperatura, secado, solvente) [1], con propiedades fisicoquímicas diferentes entre ellas tales como velocidad de disolución, estabilidad térmica, higroscopicidad pureza entre otras. (Ver D3, folio 253)

Además, diversas autoridades en cristalografía y publicaciones relacionadas sobre el tema cuando al abordar cuestiones como la posibilidad de predecir a partir de un diseño creativo el producto que se hará de obtener con el manejo de variables termodinámicas y cinéticas, han señalado que estamos lejos de esta clase de logros [2] . Si bien los parámetros pueden ser controlables y modificables, el investigador no tiene control sobre el producto (en este caso las formas cristalinas I y II de la actual solicitud) que se habrá de obtener y tampoco lo puede determinar como resultado de su actividad inventiva, concluyendo que no se puede afirmar que el investigador puede realizar un diseño cristalográfico por su actividad creativa, toda vez que el resultado de este control de variables dependerá única y exclusivamente de la naturaleza de la molécula.

De otra parte, tampoco se puede afirmar que la actividad creadora reside en el hecho de encontrar formas cristalinas (como en el caso de las formas cristalinas I y II de la sal del ácido málico del compuesto 2 (dietilamino)etil]-5-[(5-fluoro-2oxo-3H-indol-3-ilideno) metil]-2,4-dimetil-1H-pirrol-3-carboxamida) , que no se habían reportado con anterioridad, porque el hecho de haberlas hallado no implica que hubiera sido el resultado de su incidencia directa al establecer un diseño de tipo matemático y físico creado y premeditado con el único fin de reducir la entropía del estado sólido de la mencionada sal y conseguir su cristalización en un ordenamiento específico, ya que como lo afirma Mc Crone (1965) [3] *"en general, el número de formas conocidas para un compuesto dado, es proporcional al tiempo y dinero gastado en la investigación sobre aquel compuesto" "Todos los compuestos comunes (y elementos) muestran polimorfismo"*. Y en palabras de Buerger y Bloom (1937) [4] *"el polimorfismo es una propiedad inherente del estado sólido y sólo se presenta sólo bajo condiciones especiales"*. En 1951 Findlay señaló en su libro que *"el polimorfismo: "... es ahora reconocido como un hecho muy frecuente en verdad"* [5]. De tal manera, este Despacho no comparte la opinión del señor solicitante en lo referente a la existencia de una altura inventiva en torno a las formas polimórficas I y II aquí reclamadas.

[1] Helman José, Farmacotecnia teórica y práctica. Tomo IV. Editorial Continental, Tercera impresión, México, Noviembre de 1982. Capítulo 30 Cristalización pág 1103 a 1143.

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7. *De hecho, quiero anotar que el desconocimiento de las estructuras cristalinas polimórficas específicamente reivindicadas en la presente solicitud, y la especificidad de los métodos reclamados y sus condiciones, constituye innegablemente la evidencia de que se está frente a una "estructura cristalina inesperada", con lo cual cumpliría el requisito de patentabilidad de nivel inventivo, como bien lo señala el Examinador al establecer que dichas formas son novedosas. El Examinador parece rechazar el principio establecido por el Manual Andino de Patentes que establece que un compuesto tiene nivel inventivo cuando (i) el compuesto tiene estructura inesperada, o ii) cuando el compuesto exhibe un uso o un efecto sorprendente.*

Para responder a estas manifestaciones del solicitante resulta pertinente precisar, en primer término, que el mencionado Manual Andino de Patentes es sólo una guía para los examinadores de patentes y no constituye en modo alguno legislación aplicable propiamente dicha. Pero aún así, no resulta aplicable lo citado del dicho Manual, comoquiera que la situación que plantea esta solicitud es diferente. En efecto, como bien lo cita el solicitante la existencia de un efecto o uso sorprendente es la manera más común de establecer altura inventiva para los compuestos nuevos.

De acuerdo con lo anterior, debe partirse de la base que se trata de una estructura química nueva o dicho en otras palabras de una "nueva entidad química". Parece que el error se deriva del hecho de que el solicitante olvidó o no contextualizó adecuadamente la referencia citada. Volviendo a esta cabe reformularse la pregunta a indagar **b) Que es necesario considerar para que un compuesto nuevo tenga nivel inventivo?.** A lo cual debe responderse que en este caso el compuesto no es nuevo puesto que la sal del málica del compuesto N-[2 (dietilamino)etil]-5-[(5-fluoro-2oxo-3H-indol-3-ilideno) metil]-2,4-dimetil-1H-pirrol-3-carboxamida que se obtiene por la reacción de ácido málico con la N-[2 (dietilamino)etil]-5-[(5-fluoro-2oxo-3H-indol-3-ilideno) metil]-2,4-dimetil-1H-pirrol-3-carboxamida era conocido en D1 (Ver folio página 39, compuesto 80 , folio 173, página 87, líneas 9 y 11).

Por último para este Despacho resulta importante aclarar, que no debe confundirse el término "forma cristalina o cristal" con los términos: sustancia, entidad química o compuesto químico, dado que el cristal es tan sólo la forma externa que adopta el compuesto químico, el cual en este caso es conocido en el estado de la técnica.

Por lo tanto, los argumentos expresados por el solicitante basados en el Manual Andino no encuentran fundamento de acuerdo a lo reclamado en la actual solicitud.

8. *Como lo mencioné en mi respuesta al primer examen de fondo, el carácter novedoso e inventivo de un polimorfo en particular y de los polimorfos de la presente invención yace en el hecho de que, (i) es imposible para una persona medianamente versada en el arte, determinar, a priori, la existencia de polimorfos específicos para un compuesto dado; (u) es imposible saber, a priori, si una forma cristalina específica existe hasta que no se haga el trabajo experimental que conduzca a la obtención de dicho cristal; (iii) es imposible predecir el acercamiento experimental para obtener un cristal de manera eficiente ya que su producción depende de múltiples factores como son la temperatura, la agitación, la velocidad de enfriamiento, la naturaleza del disolvente(s), la cantidad de disolvente(s), la pureza del disolvente, etc.; (iv) si el cristal llega a producirse eficientemente, no es posible predecir sus parámetros fisicoquímicos, como tampoco es posible predecir cual será por ejemplo sus propiedades específicas.*

   Adicionalmente, me permito señalar que el Examinador no da respuesta a la solicitud realizada en mi respuesta al primer examen de fondo, en donde respetuosamente insto al Examinador a indicar por qué si la SIC ha determinado que un experto en la materia no puede (sin necesidad de experimentación excesiva) predecir la estructura de formas

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Journal of Virology

A Recombinant Newcastle Disease Virus (NDV) Expressing VP2 Protein of Infectious Bursal Disease Virus (IBDV) Protects against NDV and IBDV

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J. Virol. 2004, 78(18):10054. DOI: 10.1128/JVI.78.18.10054-10063.2004.

JOURNAL OF VIROLOGY, Sept. 2004, p. 10054–10063
0022-538X/04/$08.00+0 DOI: 10.1128/JVI.78.18.10054–10063.2004

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A Recombinant Newcastle Disease Virus (NDV) Expressing VP2 Protein of Infectious Bursal Disease Virus (IBDV) Protects against NDV and IBDV

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Received 24 February 2004/Accepted 7 May 2004

Infectious bursal disease virus (IBDV) causes a highly immunosuppressive disease in chickens. Currently available, live IBDV vaccines can lead to generation of variant viruses. We have developed an alternative vaccine that will not create variant IBDV. By using the reverse genetics approach, we devised a recombinant Newcastle disease virus (NDV) vector from a commonly used vaccine strain LaSota to express the host-protective immunogen VP2 of a variant IBDV strain GLS-5. The gene encoding the VP2 protein of the IBDV was inserted into the most 3′-proximal locus of a full-length NDV cDNA for high-level expression. We successfully recovered the recombinant virus, rLaSota/VP2. The rLaSota/VP2 was genetically stable, at least up to 12 serial passages in chicken embryos, and was shown to express the VP2 protein. The VP2 protein was not incorporated into the virions of recombinant virus. Recombinant rLaSota/VP2 replicated to a titer similar to that of parental NDV strain LaSota in chicken embryos and cell cultures. To assess protective efficacy of the rLaSota/VP2, 2-day-old specific-pathogen-free chickens were vaccinated with the recombinant virus and challenged with a highly virulent NDV strain Texas GB or IBDV variant strain GLS-5 at 3 weeks postvaccination. Vaccination with rLaSota/VP2 generated antibody responses against both NDV and IBDV and provided 90% protection against NDV and IBDV. Booster immunization induced higher levels of antibody responses against both NDV and IBDV and conferred complete protection against both viruses. These results indicate that the recombinant NDV can be used as a vaccine vector for other avian pathogens.

Infectious bursal disease virus (IBDV) is a pathogen of major economic importance in the poultry industry worldwide. IBDV replicates specifically in developing B-lymphoid cells, resulting in the destruction of the precursors of antibody-producing B cells in the bursa of Fabricius, and consequently, the immunosuppression, which leads to vaccination failures and susceptibility to other infections and diseases (25). Vaccination is the principal method used for the control of infectious bursal disease in chickens. Although IBDV strains of different antigenic types have been incorporated into vaccines, IBDV remains a major problem for the poultry industry. The efficacy of current live IBDV vaccines decreases in the presence of maternal antibodies, which are essential for the protection of young chickens for the critical first few weeks of life. Live IBDV vaccines also cause various degrees of bursal atrophy and may contribute to the emergence of antigenic variant viruses (25). The first antigenic variant strain of IBDV was isolated from vaccinated flocks on the Delmarva Peninsula in 1985 (40). Other variant strains were subsequently isolated in the United States and other countries (17, 35, 41, 42, 46). The antigenic variant strains were serologically different from the so-called classic isolates most typically isolated before 1985 (25, 42, 46). These variant strains lack the epitope(s) defined by neutralizing monoclonal antibodies (MAbs) B69 and R63, which were prepared with classical isolates (40, 41). The variant viruses infect chickens, even those with relatively high levels of maternal antibodies, and cause great economic losses (17, 35, 42, 46). The apparent inability to control IBDV infection through current vaccination warrants a necessity to develop alternate IBDV vaccine strategies that would not result in variant viruses.

IBDV is a member of the genus *Avibirnavirus* in the family *Birnaviridae*, and its genome is composed of two segments of double-stranded RNA (25). The smaller segment B encodes VP1, a 90,000-molecular-weight RNA-dependent RNA polymerase. The larger segment A contains two partially overlapping open reading frames (ORFs). The first, smaller ORF encodes a nonstructural protein VP5; whereas the second ORF encodes a precursor polyprotein, which is subsequently cleaved into VP2, VP4, and VP3. VP2 and VP3 are the major capsid proteins of IBDV, constituting 51 and 40% of the viral proteins, respectively (25). The VP2 protein has been identified as the major host-protective immunogen of IBDV and contains major epitopes responsible for eliciting neutralizing antibodies (3, 4, 16). Passive antibodies to VP2 were found to protect chickens (12, 25, 44). Many attempts have been made to express the structural proteins of IBDV as subunit vaccines for the control of this disease. It has been demonstrated that the recombinant VP2 protein expressed in different expression systems provided significant protection against the disease (5, 37, 39, 43, 45). However, those efforts have not been translated for practical use, due to limitations of the delivery systems (11, 21, 34, 48).

The recently developed reverse genetics system to engineer negative-strand RNA viruses (NSV) has provided a new method

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to express foreign genes (9, 30). Owing to the modular nature of their genomes, it is easy to engineer additional genes into the genomes of NSV. A number of recombinant NSV containing additional foreign genes have been engineered (6, 14, 15, 27, 31, 38, 47). It has also been shown that NSV can serve as highly effective vaccine vectors for protection against other pathogens (7, 13, 29, 36). Earlier studies have shown that recombinant Newcastle disease virus (NDV), an NSV, can be used to express heterologous genes (19, 22, 29).

NDV is a member of the genus *Avulavirus* in the family *Paramyxoviridae* (24, 26). The genome of NDV is a nonsegmented, negative-stranded RNA of 15,186 nucleotides (nt) containing six genes in the order of 3′-NP-P-M-F-HN-L-5′ (10, 23). Expression levels of the proteins are attenuated in a sequential manner from the 3′ end to the 5′ end of the viral genome (24, 32). NDV causes an economically important disease in all species of birds worldwide (2). Newcastle disease can vary from clinically inapparent to highly virulent forms (depending on the virus strains and host species (2). Currently, naturally occurring avirulent NDV strains are routinely used as live vaccines throughout the world.

Several characteristics of NDV suggest that recombinant NDVs expressing a protective antigen of another avian pathogen would be a very good multivalent vaccine for poultry. Live NDV vaccines are widely used in commercial chickens with proven track records of efficacy and safety. NDV grows to very high titers in many cell lines and embryonated eggs and elicits strong humoral and cellular immune responses in vivo. NDV naturally infects the chicken via the upper respiratory tract and induces strong secretory immunoglobulin A (IgA), which is a critical component of the immunological repertoire to prevent mucosal infections (2, 18). Therefore, we explored the potential of recombinant NDV as a vaccine vector for poultry.

In this paper, we describe a recombinant NDV strain LaSota expressing the host-protective immunogen VP2 of a variant IBDV strain GLS-5. The recombinant rLaSota/VP2 grew to a level comparable to the parental virus. The VP2 gene was stably maintained and expressed even after serial passages in embryonated eggs. Vaccination with the recombinant rLaSota/VP2 induced strong humoral immune responses against both NDV and IBDV and conferred complete protection against both viruses after secondary immunization. These results clearly suggest that it is possible to develop a commercial bivalent recombinant NDV/IBDV vaccine that would provide protection against both of these economically important diseases.

MATERIALS AND METHODS

Cells and viruses. DF1 (a chicken embryo fibroblast cell line) cells were grown in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum. Vero and HEp-2 cells were grown in Eagle's minimum essential medium containing 10% fetal bovine serum. Recombinant and wild-type NDV strains were grown in 9-day-old specific-pathogen-free (SPF) embryonated chicken eggs. The highly virulent NDV strain Texas GB used for challenge studies was originally received from National Veterinary Services Laboratory, Ames, Iowa. The wild-type and cell culture-adapted IBDV variant GLS-5 strains were kindly provided by Vikram Vakharia, University of Maryland Biotechnology Institute. The modified vaccinia strain Ankara expressing the T7 RNA polymerase (a generous gift of Bernard Moss, National Institutes of Health) was grown in primary chicken embryo fibroblast cells.

Plasmid construction and recovery of recombinant virus. A cDNA fragment containing the VP2 gene (size of VP2 ORF, 1,356 nt; GenBank access number, M97346) of the cell culture-adapted GLS-5 strain was obtained by reverse transcription (RT)-PCR. Briefly, the IBDV strain GLS-5 was grown in Vero cells. Virus was purified from clarified supernatant by centrifugation at 70,000 × *g* for 2 h on a 26% sucrose cushion. The partially purified virus pellet was resuspended in phosphate-buffered saline (PBS) and treated with sodium dodecyl sulfate (SDS) (0.1%) and proteinase K (100 μg/ml) for 2 h. IBDV genomic RNAs were extracted with TRIzol reagent (Invitrogen, Carlsbad, Calif.) according to the manufacturer's instruction. The VP2 gene was amplified by RT-PCR using IBDV specific primers. The amplified product was sequenced to confirm the identity of the VP2 gene.

Construction of plasmid pLaSota carrying the full-length cDNA of the NDV vaccine strain LaSota has been described previously (19). To facilitate insertion of the VP2 gene of IBDV into the most 3′-proximal locus of NDV antigenome, the AscI and SacII fragment from pLaSota was subcloned into pGEM-7Z (+) (Promega, Madison, Wis.) using XbaI and HindIII overhang primers. An 18-nt insert (5′-GGCCGGCCTCTGCCAACT-3′) with an FseI site was introduced just before the NP gene ORF as described elsewhere (19). The VP2 gene of IBDV was amplified by PCR with FseI-tagged forward primer 5′-act**ggccggcc**AATGACAAACCTGCAAGATCAAACCCAACAG-3′ and reverse primer 5′-act**ggccggcc**ttctaccccgtcttttttctaatgcctcaCCTTATGGCCCGGATTATGTCTTTG-3′ (the FseI site is in bold; the NDV gene start and gene end sequences are underlined; sequence specific to the IBDV VP2 gene is in uppercase), digested with FseI, and cloned into the pGEM-7Z (+) subclone. A termination codon (TGA) was placed immediately after the VP2 gene. Four additional nucleotides (GGCA, antigenome sense) were added after the termination codon to maintain the "rule of six" (8, 32, 33). The resulting AscI and SacII fragment in the subclone was excised and used to replace the corresponding part in pLaSota. The AscI and SacII region was sequenced to confirm the presence of the VP2 gene.

The recovery of infectious LaSota virus entirely from cloned cDNA using a mixture of three expression plasmids encoding NP, P, and L proteins of NDV strain LaSota and a fourth plasmid encoding the NDV plus IBDV VP2 gene was carried out following the procedures described previously (19). Virus recovered in the supernatant was plaque purified prior to amplification and characterization. The recovered recombinant virus was designated rLaSota/VP2.

Identification of recombinant virus by RT-PCR. Recombinant virus was grown in 10-day-old embryonated SPF chicken eggs. The virus was purified as described previously (20, 22). Genomic RNA was extracted from partially purified virus using TRIzol reagent (Invitrogen). The genomic RNA was reverse transcribed using ThermoScript reverse transcriptase (Invitrogen) and an antigenomic-sense primer 5′-^{35}CGAAGGAGCAATTGAAGTCGCACG^{32}-3′. Two sets of PCR were performed using the above reverse-transcribed cDNA as templates. The first set of PCR was performed with the same antigenomic-sense primer used for RT and a genomic-sense oligonucleotide, 5′-TCTGCTGGTTTACCCTGGGCTGT-3′, which is at position 1959 to 1981 in the NDV genome. The second PCR was performed using the same antigenomic-sense primer and a negative-sense internal IBDV VP2 gene-specific primer, 5′-TAGCCAAACCTGCGTCTGCTAGA-3′, at position 1371 to 1393 in the IBDV genome. Aliquots of the PCR products were analyzed on a 1% agarose gel. The PCR products were purified using the PCR purification kit (QIAGEN, Hilden, Germany) and sequenced to entirety to confirm the presence of the VP2 gene of IBDV in the recovered virus.

Expression of IBDV VP2 protein by recombinant virus. The expression of VP2 protein was demonstrated in DF1 cells infected with virus stock by immunofluorescence assay. Briefly, confluent DF1 cells on six-well plates were infected with virus at a multiplicity of infection (MOI) of 0.1. After 24 or 48 h, the cells were washed with PBS and fixed with 3% paraformaldehyde in PBS for 20 min at room temperature. The cells were washed three times and permeabilized with PBS containing 0.05% Tween 20 (PBS-T) for 30 min. After further washing with PBS, the cells were incubated with 1:700 dilution of a rabbit anti-IBDV antiserum (a gift from Vikram Vakharia) for 1 h. The cells were rinsed with PBS and incubated with fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit immunoglobulin G antibody (Kirkegaard & Perry Laboratories [KPL], Gaithersburg, Md.) for 45 min. The cells were washed again with PBS and visualized using a Nikon Eclipse TE (Nikon, Tokyo, Japan) fluorescent microscope.

To further confirm the expression of the VP2 protein by the recombinant virus, Western blot analysis was performed using infected cell lysate and purified recombinant virus. Cell lysate was prepared from DF1 cells infected with the recombinant virus (MOI, 3) at 20 h postinfection. Cells were washed with PBS, scraped, collected by low-speed centrifugation, and lysed with lysis buffer (0.25 mM Tris [pH 6.8], 1% SDS, 10% glycerol, 6 M urea, 0.01% bromophenol blue, 0.01% phenol red) for 30 min on ice. The lysates were clarified by centrifugation at 7,000 × *g* for 10 min and used for Western blot analysis. The recombinant virus was purified from infective allantoic fluid and subjected to Western blot analysis. To examine whether the VP2 protein is incorporated into the virion,

TABLE 1. Experimental design

Expt group	No. of chickens	Age (days) at vaccination		Vaccine[a]	Age at challenge (days)	Challenge virus[b]
		Primary	Secondary			
1	10	2	—	Commercial NDV vaccine	23	Texas GB
2	10	2	—	Commercial IBDV vaccine	23	GLS-5
3	10	2	—	rLaSota/VP2	23	Texas GB
4	10	2	—	rLaSota/VP2	23	GLS-5
5	10	—[c]	—	None	23	Texas GB
6	10	—	—	None	23	GLS-5
7	5	—	—	None	—	None
8	10	2	23	Commercial NDV vaccine	47	Texas GB
9	10	2	23	Commercial IBDV vaccine	47	GLS-5
10	10	2	23	rLaSota/VP2	47	Texas GB
11	10	2	23	rLaSota/VP2	47	GLS-5
12	10	—	23	None	47	Texas GB
13	10	—	23	None	47	GLS-5
14	5	—	—	None	—	None

[a] Vaccination was given by eyedrop instillation with commercial vaccines (manufacturer's recommended dose/chicken) or rLaSota/VP2 (1×10^6 EID_{50}/chicken).
[b] Chickens were challenged with virulent NDV strain Texas GB (1×10^6 EID_{50}/chicken) intramuscularly or virulent IBDV strain GLS-5 (1×10^5 EID_{50}/chicken) ocularly.
[c] —, no vaccination or no challenge.

...ungates of the cell lysate and the purified virions were mixed with Laemmli sample buffer (Bio-Rad Hercules, Calif.) and boiled for 3 min before electrophoresis. The boiled samples were separated by SDS-polyacrylamide gel electrophoresis (PAGE) on a 12% gel, and the resolved proteins were transferred to polyvinylidene difluoride membranes (Millipore, Bedford, Mass.). The membranes were probed with the rabbit anti-IBDV antiserum, washed with PBS-T, and subsequently incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG antibody (KPL). Proteins were visualized after incubation with 3,3',5,5'-tetramethyl benzidine (TMB) peroxidase substrate (KPL).

To examine whether the VP2 protein expressed by the recombinant virus retained the conformational antigenic epitopes, antigen capture enzyme-linked immunosorbent assay (AC-ELISA) was performed using a commercial kit (Synbiotics, San Diego, Calif.). The assay utilized a panel of MAbs raised against neutralizing epitopes of IBDV to examine the reactivity profile of the expressed VP2 from both cell lysates and supernatant of virus-infected cells. Briefly, cell lysate or supernatant from virus-infected DF1 cells was diluted (1:50) with antigen dilution buffer (Synbiotics) and added to the wells of MAb-coated plates. The plates were incubated at 4 to 8°C overnight and then washed three times with PBS-T. A positive chicken antiserum against IBDV was added to the plates. After 30 min at room temperature, the plates were washed three times and reacted with peroxidase-labeled goat anti-chicken IgG for 30 min. Following another three washings with PBS-T, the substrate 2,2'-azinobis(3-ethylbenzthiazoline-sulfonic acid) (ABTS) was added. Color development was stopped after 15 min with stop solution. Optical density (OD) values were read on an ELISA reader at 405 nm, and the reactivities of each monoclonal were inferred from the OD readings compared to the controls, according to the manufacturer's instructions (Synbiotics).

Virus growth in cell cultures and embryonated SPF chicken eggs. The growth of the recombinant virus was assessed by a multistep growth assay in DF1 cells. DF1 cells were infected at an MOI of 0.01 and incubated at 37°C in DMEM containing 5% fetal bovine serum and 1 μg of acetyl-trypsin/ml. Supernatants were harvested at 8-h intervals and titrated by plaque assay in DF1 cells. To compare the growth of the recombinant viruses in SPF chicken embryonated eggs, 2×10^3 PFU of each virus in a volume of 100 μl were inoculated into the allantoic cavity of 10-day-old embryonated chicken eggs. Infective allantoic fluids were harvested 60 h postinoculation for virus titration by plaque assay.

Characterization of recombinant virus in vivo. The mean death time (MDT) was determined to assess the pathogenicity of the recombinant viruses in embryonated SPF chicken eggs as described previously (1). Briefly, fresh infective allantoic fluid was diluted in PBS to give a 10-fold dilution series. For each dilution, 100 μl was inoculated into the allantoic cavity of each of five 9-day-old embryonated SPF chicken eggs. The eggs were incubated at 37°C and examined three times daily for 7 days. The time that each embryo was first observed dead was recorded. The highest dilution that killed all embryos was considered the minimum lethal dose. The MDT was calculated as the mean time in hours for the minimum lethal dose to kill the embryos.

Evaluation of the immunogenicity and protective efficacy of the recombinant virus in chickens. We evaluated the immunogenicity and protective efficacy of rLaSota/VP2 in chickens at our U.S. Department of Agriculture-approved biosafety level 3 animal facility. One-day-old SPF white leghorn chickens (SPAFAS, Norwich, Conn.) were randomly assigned to 12 treatment groups of 10 birds each and 2 groups of 5 birds each, as shown in Table 1. Chickens in groups 1 to 7 were used for primary vaccination and challenge, whereas groups 8 to 14 were kept for secondary immunization and challenge later. All chickens were housed in separate poultry isolation chambers with ad libitum access to feed and water. At 2 days of age, chickens were vaccinated via the ocular route with either rLaSota/VP2 (1×10^6 50% embryo lethal doses [ELD_{50}] per bird) or commercial NDV LaSota vaccine and commercial live IBDV vaccine at the manufacturer's recommended dose. Chickens from each group were bled at 3 weeks postimmunization for assessing NDV or IBDV antibodies using commercially available ELISA kits (Synbiotics). Virus-neutralizing antibody titers in blood samples were also assessed by microtiter virus neutralization (VN) test with the constant-virus diluting-serum technique using homologous viruses. The VN titers were expressed as the reciprocal of the last serum dilution that was capable of neutralizing 100 mean 50% tissue culture infectious doses ($TCID_{50}$). A geometric mean titer was calculated for each group. Birds in groups 1 to 7 were challenged with virulent NDV strain Texas GB at 1×10^6 ELD_{50} per bird intramuscularly or virulent IBDV GLS-5 strain 1×10^5 embryo infectious dose (EID_{50}) per bird ocularly, at 23 days of age, as indicated in Table 1. The birds in groups 8 to 14 were boosted with commercial NDV or IBDV vaccines or recombinant rLaSota/VP2 at 23 days of age. Two weeks after the secondary vaccination, all the chickens were bled for immunological assessment of NDV or IBDV antibodies by ELISA and then challenged as described above.

The birds challenged with IBDV were examined for clinical signs and mortality for 72 h postchallenge. Three days after challenge, the chickens were euthanized and weighed. Bursa samples from all chickens were weighed for comparison of bursa-to-body weight ratio (BBWR). The gross lesions of the bursa were examined. The obtained bursa was divided into two parts. One part was used for viral antigen detection by AC-ELISA, and the other part was fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hemosylin and eosin for histopathological examination. Indicators of protection from IBDV challenge were based on the following criteria: (i) lack of morbidity or mortality, (ii) lack of histological lesions in the bursa, (iii) undetectable IBDV antigen in the bursa, and (iv) the individual value of BBWR within 2 standard deviation units of the mean of the corresponding control group, which indicates bursal integrity. To evaluate whether the expression of VP2 protein by recombinant rLaSota/VP2 interferes with protective immunity against NDV, birds challenged with NDV strain Texas GB were observed daily for a period of 10 days for mortality and clinical signs of neurotropic Newcastle disease. Birds were considered protected if they showed no central nervous system signs and survived.

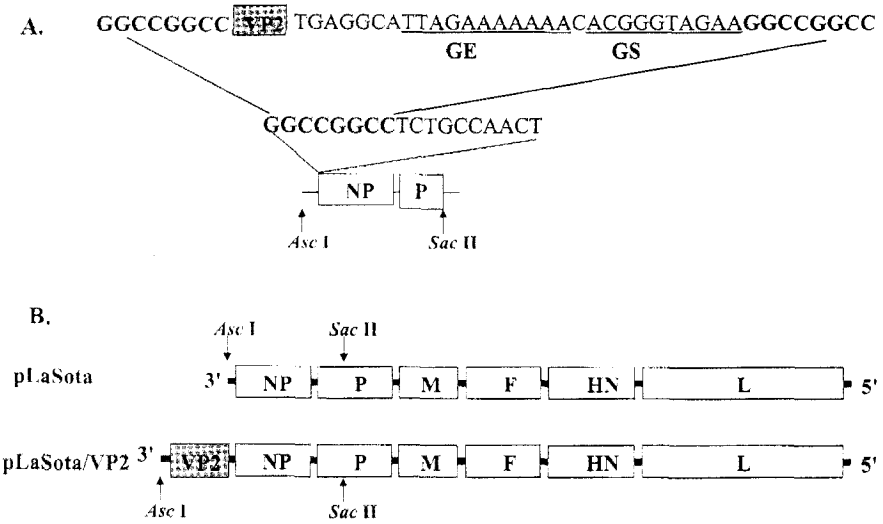

FIG. 1. Construction of the recombinant NDV expressing IBDV VP2 protein. (A) The VP2 gene of IBDV was amplified by RT-PCR with a pair of FseI-tagged primers, digested with FseI, and introduced into the FseI site in the noncoding region of the NP gene. A termination codon (TGA) was placed immediately after the VP2 gene, followed by four additional nucleotides (GGCA) to maintain the rule of six. The VP2 gene was flanked by NDV gene end (GE), intergenic sequence, and gene start (GS) signals. (B) The fragment containing the VP2 gene was excised with AscI and SacII and used to replace the corresponding fragment in pLaSota. The resulting pLaSota/VP2 encodes an antigenomic RNA of 16,596 nt, which is a multiple of six.

RESULTS

Recovery of recombinant NDV expressing IBDV VP2 protein. We have previously constructed pLaSota, which encodes a complete 15,816-nt antigenome of NDV strain LaSota, and pLaSota/CAT containing the chloramphenicol acetyltransferase gene as an additional transcriptional unit (19). Here, we used the same approach to construct a plasmid pLaSota/VP2, in which the IBDV VP2 gene was inserted into the NDV antigenome upstream of the NP gene ORF (Fig. 1). IBDV genomic RNA extracted from the purified virus was subjected to RT-PCR to amplify the VP2 gene fragment as described previously (19). A termination codon and four additional nucleotides were inserted after the VP2 gene ORF to maintain the rule of six (8, 32). The resulting plasmid, designated as pLaSota/VP2, contained an additional transcriptional unit and encoded an antigenome of 16,596 nt (Fig. 1). Recombinant rLaSota/VP2 virus was recovered entirely from this cDNA by using our established reverse genetics procedures (19).

RT-PCR analysis of recombinant virus. To confirm the presence of the IBDV VP2 gene in the genome of the recovered rLaSota/VP2 viral RNA was extracted from purified virus and analyzed by RT-PCR. RT was performed with an antigenomic-sense primer that annealed to the leader sequence at NDV genome positions 35 to 58. Two sets of PCR were performed with the same antigenomic-sense primer along with two other negative-sense primers, as detailed in Materials and Methods. As expected, rLaSota yielded a PCR product of 1.9 kb, which would represent the segment without the foreign gene. In contrast, rLaSota/VP2 produced a single, longer PCR product corresponding to the predicted 3.3-kb fragment containing the inserted VP2 transcriptional unit. No PCR product was present when PCR was performed on extracted viral RNA without RT (data not shown). When using the VP2 gene internal primer for PCR, only rLaSota/VP2 yielded a PCR product of the predicted size of 0.5 kb and no product was observed with rLaSota virus (data not shown). The RT-PCR products were sequenced to confirm the identity of the IBDV VP2 gene.

To determine the stability of the VP2 gene in recombinant rLaSota/VP2 virus, the recovered virus was passaged 12 times in 9-day-old embryonated SPF chicken eggs, and the presence of the VP2 gene in the virus from each passage was examined by RT-PCR. Our results showed that the sequence identity of the VP2 gene was preserved and stably maintained even after 12 passages in developing chicken embryos.

Expression of IBDV VP2 protein by recombinant virus. To examine the expression of VP2 protein by the rLaSota/VP2, DF1 cells were infected with rLaSota/VP2 of the 12th passage at an MOI of 0.1. At 24 or 48 h postinfection, the cells were fixed and incubated with a rabbit antiserum directed against IBDV, followed by immunostaining with FITC-conjugated goat anti-rabbit IgG. The results indicated that there was extensive expression of VP2 protein at 24 h postinfection (Fig. 2C), which further increased at 48 h postinfection (data not shown). Compared to rLaSota/VP2, cell culture-adapted IBDV strain GLS-5 displayed low levels of fluorescence at 24 h postinfection (Fig. 2), suggesting a slower replication cycle for IBDV. However, the fluorescence intensities and cellular distribution of VP2 antigen in rLaSota/VP2- and IBDV GLS-5-infected cells were similar at 48 h postinfection (data not shown). Western blot analysis of infected DF1 cell lysates further confirmed the expression of VP2 protein by the rLaSota/VP2 virus (data not shown). We were also able to demonstrate the VP2 protein from the supernatant of rLaSota/VP2-infected cells by ELISA. Western blotting of purified rLaSota/VP2 virus showed no detectable amount of the VP2 protein (data not shown). Taken together, these results suggested that the recombinant rLaSota/VP2 stably expressed the

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/303,034	12/01/2008	Jong Soo Woo	Q111239	2061

23373 7590 06/28/2011

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EXAMINER	
CHOI, FRANK I	

ART UNIT	PAPER NUMBER
1616	

NOTIFICATION DATE	DELIVERY MODE
06/28/2011	ELECTRONIC

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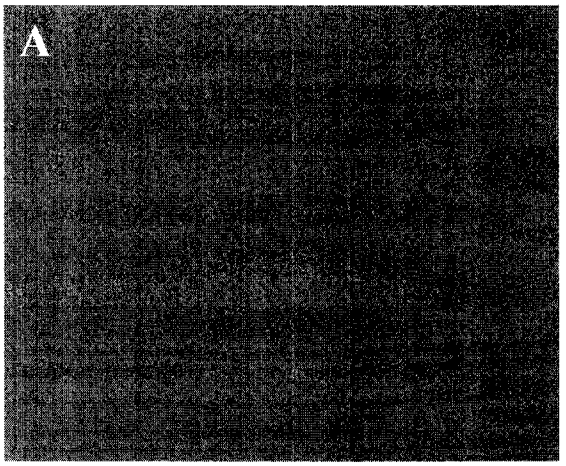

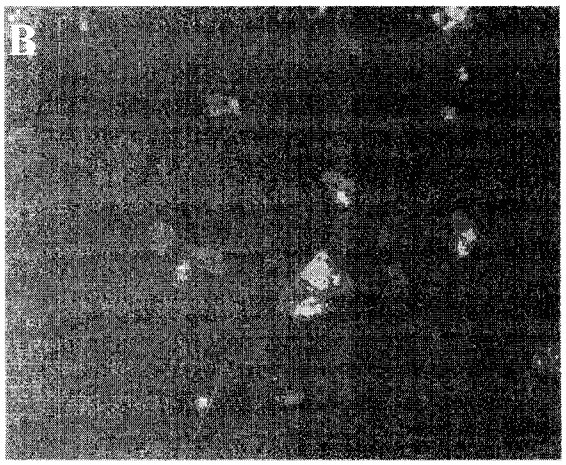

FIG. 2. Immunofluorescence analysis of IBDV VP2 protein expression. Confluent DF1 cells were infected with rLaSota (A), cell culture-adapted GLS-5 (B), or rLaSota/VP2 at 12th passage (C) at an MOI of 0.1. The infected cells were fixed, permeabilized, and probed with rabbit anti-IBDV antiserum, followed by incubation with FITC-conjugated goat anti-rabbit IgG antibody. The cells were visualized under an Eclipse TE (Nikon) fluorescent microscope. Magnification, ×400. Data are shown at 24 h postinfection.

VP2 protein and that the VP2 protein was probably not incorporated into the virions.

MAb reactivity profile of rLaSota/VP2. To assess whether the VP2 protein expressed by rLaSota/VP2 virus contains the neutralizing epitopes, both the cell lysate and the supernatant from infected cells were analyzed by AC-ELISA with a panel of well-characterized epitope-specific MAbs. Five virus-neutralizing MAbs (8, 10, 57, R63, and B69) corresponding to epitopes residing on the VP2 protein were used to analyze the antigenic properties of the expressed protein (40, 41). Samples were considered positive if their OD values were equal to or greater than 0.6. MAb 57 corresponds to a unique neutralizing epitope in the VP2 protein of variant GLS-5 strain; whereas MAbs 8 and 10 define neutralizing epitopes present both in the classic and variant GLS-5 strains of IBDV (40, 41, 42). The expressed VP2 protein reacted with both MAbs 57 and 10, as did the variant GLS-5 strain (Table 2). However, the cell lysate and supernatant from rLaSota/VP2-infected cells did not bind to MAb 8, indicating that the presence of this epitope may depend on the presence of other virus-associated proteins. Epitopes defined by MAbs R63 and B69 are present only in classical strains but absent in the GLS-5 strain (41, 42). The recombinant VP2 protein did not bind to R63 and B69 (Table 2). These results suggested that the VP2 protein expressed by rLaSota/VP2 was specific to the GLS-5 strain and retained the neutralizing epitopes. Since these neutralizing epitopes are

TABLE 2. Antigenic characterization of the VP2 protein expressed by rLaSota/VP2 with IBDV-neutralizing MAbs by AC-ELISA[a]

MAbs[b]	Antigenic reactivity of IBDV[c]			
	Classic[d]	GLS-5	rLaSota/VP2	rLaSota
8	+	+	−	−
10	+	+	+	−
57	−	+	+	−
R63	+	−	−	−
B69	+	−	−	−

[a] Cell lysates from DF1 cells infected with recombinant rLaSota/VP2, rLaSota and GLS-5, and homogenized bursa of IBDV classic strain D78, were used for AC-ELISA.

[b] Neutralizing MAbs with known reactivity to epitopes on VP2 protein of IBDV.

[c] Antigenic reactivity determined by AC-ELISA. The presence (+) and the absence (−) of the epitope in viruses are indicated.

[d] IBDV classic strain D78.

EXPEDIENTES CONTRALORIA - URGENTES

No	Expediente	Presentación	Trámite	Estado	Persona	Fecha	sector	Estado	fecha	observacion	Situación actual	Examinador	1	2	3	Fecha Venc	F. creac div
11	5-125373	2005-12-13 11:17:08	11	REASIGNACION	JOSE LUIS SALAZAR LOPEZ	2012-04-30 09:32:46	QF	45-REQUERIMIENTO 45	2011-11-03 14:08:29	oficio-17376-del-03/11/2011 vence-02/02/2012. Respuesta:NO Prorroga:24/01/2012 Vence Prorro: 06/03/2012 Rta Prorro:13/03/2012	3er Art 45, para correcciones resolución	ANDRES GIL HELFFHRITTZ	R				
12	5-125373A	2005-12-13 11:17:08	11	DEVOLUCION	JANNETH EMILSE MARTINEZ CASTRO	2012-04-27 10:09:51	QF	45-REQUERIMIENTO 45	2012-04-24 10:00:20	oficio-6696-del-24/04/2012 vence-26/07/2012. Respuesta:NO Prorroga:NO Rta Prorro:	1er Art 45, Vence 26 julio 2012	ANDRES GIL HELFFHRITTZ	T	DV	DV	24/04/2012	24/04/2012
13	6-2587	2006-01-13 14:10:10	11	REASIGNACION	ANA DELIA PINZON GARCIA	2012-05-02 09:48:56	BT	45-REQUERIMIENTO 45	2011-12-15 14:32:23	oficio-19537-del-15/12/2011 vence-12/03/2012. Respuesta:NO Prorroga:29/02/2012 Vence Prorro: 16/04/2012 Rta Prorro:20/04/2012	3er Art 45, En estudio 2 mayo 2012	OSCAR JAVIER MARTINEZ	E				
14	6-8335A	2006-01-30 17:23:03	11	REASIGNACION	JOSE LUIS SALAZAR LOPEZ	2012-03-28 14:00:00	QF	45-REQUERIMIENTO 45	2011-12-14 14:10:56	oficio-19430-del-14/12/2011 vence-09/03/2012. Respuesta:28/02/2012 Prorroga:NO Rta Prorro:NO	3er Art 45, Ya salio Decisión corregir sistema	LYNA MORDECAY	R	DV	DV		
15	6-9419	2006-02-01 16:27:48	11	REASIGNACION	ANA DELIA PINZON GARCIA	2012-02-17 16:46:29	BT	45-REQUERIMIENTO 45	2011-01-13 15:05:58	oficio-627-del-13/01/2011 vence-11/04/2011. Respuesta:NO Prorroga:08/04/2011 Vence Prorro: 24/05/2011 Rta Prorro:25/05/2011	2do Art 45, En estudio 17 febrero 2012	OSCAR JAVIER MARTINEZ / ANA DELIA PINZON GARCIA	E				
16	6-11050	2006-02-06 15:41:57	11	DEVOLUCION	JANNETH EMILSE MARTINEZ CASTRO	2012-01-23 14:24:54	QF	45-REQUERIMIENTO 45	2012-01-04 12:41:46	oficio-9-del-04/01/2012 vence-02/04/2012. Respuesta:NO Prorroga:30/03/2012 Vence Prorro: 16/05/2012 Rta Prorro:NO	4to Art 45, Vence prorroga 17 Mayo 2012	MARIA ALEJANDRA SANCHEZ	T			17/05/2012	
17	6-11103A	2006-02-06 16:52:43	11	REASIGNACION	YENNY MARCELA CAMPOS BORDA	2012-03-16 12:51:24	IQ	45-REQUERIMIENTO 45	2011-12-14 12:22:59	oficio-19410-del-14/12/2011 vence-09/03/2012. Respuesta:01/03/2012 Prorroga:NO Rta Prorro:NO	2do Art 45, En estudio 16 marzo 2012	CARLOS ANTONIO GOMEZ	E	DV	DV		08/04/2010
18	6-14624A	2006-02-14 17:41:40	11	DEVOLUCION	JANNETH EMILSE MARTINEZ CASTRO	2012-01-23 14:24:54	BT	45-REQUERIMIENTO 45	2012-01-04 12:49:30	oficio-14-del-04/01/2012 vence-02/04/2012. Respuesta:NO Prorroga:28/03/2012 Vence Prorro: 14/05/2012 Rta Prorro:NO	1er Art 45, Vence prorroga 17 mayo 2012	NATALIA LAMPREA BERMUDEZ	T	DV	DV	17/05/2012	04/01/2012
19	6-14919A	2006-02-15 13:52:42	11	PRESTAMO	JENY PAOLA VILLATE BECERRA	2012-04-24 16:58:29	QF	45-REQUERIMIENTO 45	2012-01-04 12:43:09	oficio-10-del-04/01/2012 vence-02/04/2012. Respuesta:29/03/2012 Prorroga:NO Rta Prorro:NO	1er Art 45, Vence 2 abril 2012	ANDRES GIL HELFFHRITTZ	E	DV	DV	02/04/2012	04/01/2012
20	6-22158	2006-03-06 16:48:34	11	PRESTAMO	ANA DELIA PINZON GARCIA	2012-04-25 16:52:53	BT	45-REQUERIMIENTO 45	2011-12-14 12:23:49	oficio-19411-del-14/12/2011 vence-09/03/2012. Respuesta:NO Prorroga:07/03/2012 Vence Prorro: 23/04/2012 Rta Prorro:19/04/2012	3er Art 45, Vence 25 abril 2012	OSCAR JAVIER MARTINEZ	E				

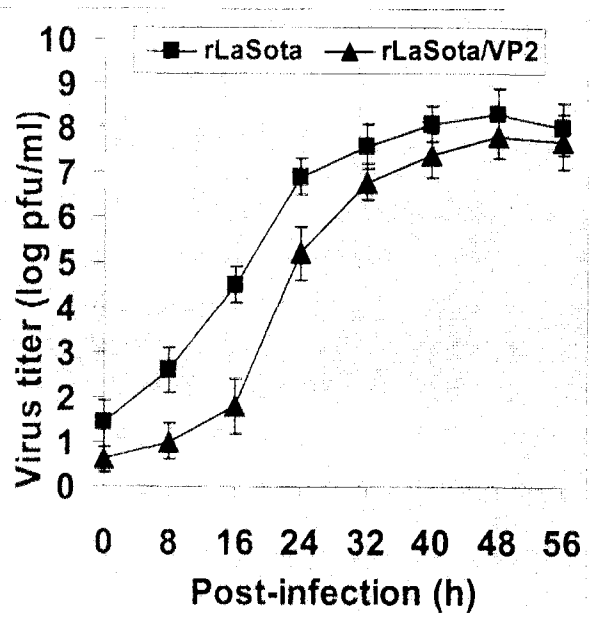

FIG. 3. Multistep growth curve of rLaSota and rLaSota/VP2 in cell culture. Monolayers of DF1 cells in 25-cm^2 flasks were infected with viruses at an MOI of 0.01 and incubated at 37°C in DMEM supplemented with 5% fetal calf serum and 1 μg of acetyl-trypsin/ml. Supernatants were harvested at 8-h intervals for virus titration. Values are from two independent experiments, each performed in triplicate. Bars show standard deviations.

conformation dependent, the expressed VP2, therefore, should be forming conformationally correct structures for immune recognition. Although direct measurement of the amount of recombinant proteins was not carried out, the results from immunostaining and AC-ELISA suggested that the expression of VP2 protein was at a higher level.

Growth characteristics of the recombinant virus. To determine the effect of VP2 protein on growth of recombinant rLaSota/VP2, we analyzed the kinetics and final virus yield under multistep growth conditions on DF1 cells. Triplicate monolayers of DF1 cells were infected with either rLaSota/VP2 or rLaSota virus at an MOI of 0.01, and samples were collected at 8-h intervals to assess the virus titers by plaque assay. The results showed that the kinetics and magnitude of replication for both rLaSota and rLaSota/VP2 were very similar, though the replication of rLaSota/VP2 was delayed slightly (Fig. 3). The final virus yields in DF1 cells and in embryonated SPF chicken eggs 64 h postinfection were comparable for both recombinant viruses. Moreover, there were no significant differences in the plaque size and morphology between the rLaSota/VP2 and the parental rLaSota (data not shown).

MDT in chicken embryos. The virulence of the parental virus rLaSota and that of the recombinant rLaSota/VP2 were determined by MDT in 10-day-old embryonated SPF chicken eggs. The MDT is one of the internationally accepted methods for assessing the pathogenicity of NDV strains. Strains of NDV are categorized into three groups on the basis of their MDTs: velogenic (less than 60 h), mesogenic (60 to 90 h), and lentogenic (greater than 90 h) (1, 2). The values of MDT for rLaSota and rLaSota/VP2 were 98 and 106 h, respectively. These results indicated that the rLaSota/VP2 virus took slightly longer to kill the embryos and that the pathogenicity of the recombinant virus was not increased after insertion of the IBDV VP2 gene.

Immunogenicity and protective efficacy against virulent IBDV challenge. To determine the immunogenicity and protective efficacy of recombinant rLaSota/VP2, groups of 2-day-old chickens were vaccinated with a commercial IBDV, or commercial NDV vaccine, or rLaSota/VP2 (Table 1). Three weeks after vaccination, blood samples (prechallenge) were analyzed by ELISA for the presence of antibodies to NDV or IBDV with a commercially available ELISA kit (Synbiotics) and by VN test for neutralizing antibodies. Our results showed that antibody titers from the ELISA and VN test were highly correlated (Table 3). The ELISA titers and VN titers against IBDV were at high levels in chickens vaccinated with rLaSota/VP2 and commercial IBDV vaccine (Table 3). Both the ELISA titers and VN titers against NDV were comparable in birds immunized with rLaSota/VP2 and commercial NDV vaccine. No detectable amounts of NDV or IBDV antibodies were present in the blood samples from the unvaccinated control group (Table 3). The data suggested that the VP2 protein expressed by rLaSota/VP2 induced a very good antibody response in chickens and that the expression of the VP2 protein did not interfere with the induction of NDV antibodies.

Immunized chickens, along with unvaccinated control birds,

TABLE 3. Immune responses to NDV or IBDV induced by vaccination

Group	Vaccine[a]	Antibody titers[b]							
		Against NDV				Against IBDV			
		Primary vaccination		Secondary vaccination		Primary vaccination		Secondary vaccination	
		ELISA	VN[c]	ELISA	VN	ELISA	VN	ELISA	VN
1	Commercial NDV vaccine	4,720	336	6,960	683	<10	≤2	<10	≤2
2	Commercial IBDV vaccine	<10	≤2	<10	≤2	7,870	687	8,610	1,056
3	rLaSota/VP2	5,105	294	7,030	629	6,580	576	7,120	982
4	None	<10	≤2	<10	≤2	<10	≤2	<10	≤2

[a] Vaccination was given by eyedrop instillation with commercial vaccines (manufacturer's recommended dose/chicken) or rLaSota/VP2 (1×10^4 EID_{50}/chicken).
[b] Immune responses were performed 3 weeks after primary vaccination or 2 weeks after secondary vaccination.
[c] Value was the reciprocal geometric mean titer from ELISA.
[d] VN titer was expressed as geometric mean titer of serum that neutralized 100 50% tissue culture infective doses of the homologous virus.

Office Action Summary	Application No.	Applicant(s)
	12/303,034	WOO ET AL.
	Examiner	Art Unit
	FRANK CHOI	1616

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) 1-13 is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) 1-13 is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on 01 December 2008 is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date 20101027,20100823,20100106,20081201.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

TABLE 4. Protection efficacy for primary and secondary vaccination against IBDV challenge[a]

Vaccination	Primary vaccination				Secondary vaccination			
	BBWR[b]		AC-ELISA[c] (no. protected/total)	Overall protection (%)	BBWR		AC-ELISA (no. protected/total)	Overall protection (%)
	Mean ± SD	No. protected/total			Mean ± SD	No. protected/total		
Commercial IBDV vaccine	5.04 ± 0.77 (A)	9/10	9/10	90	4.71 ± 0.44 (A)	10/10	10/10	100
rLaSota/VP2	5.12 ± 0.78 (A)	8/10	8/10	80	4.91 ± 0.51 (A)	10/10	10/10	100
Unvaccinated, challenged	2.99 ± 0.57 (B)	0/10	0/10	0	2.89 ± 0.58 (B)	0/10	0/10	0/10
Unvaccinated, unchallenged	5.31 ± 0.79 (A)	5/5	5/5	100	5.26 ± 0.62 (A)	5/5	5/5	100

[a] Chickens were vaccinated by eyedrop instillation and challenged ocularly with virulent IBDV strain GLS-5 3 weeks after primary or 2 weeks after secondary vaccination.

[b] BBWR value represents BBWR × 1,000. Data presented in the Mean ± SD column are the group means ± standard deviations followed by a letter (A or B) indicating statistical significance. Group means were assigned to similar groups (A and B) by the Kruskal-Wallis test. Within each column, group means with different letters are statistically different ($P < 0.05$). Protection was defined as individual value of BBWR within 2 standard deviation units of the mean of the unvaccinated unchallenged control group, with data showing the numbers protected and the total in each group.

[c] Protection was determined by the absence of viral antigen in bursa 3 days postchallenge by AC-ELISA.

were challenged with virulent IBDV strain GLS-5 at 3 weeks postvaccination. Unvaccinated chickens were fully susceptible to the challenge, showing either mortality (10%) or clinical signs (100%). In contrast, no clinical signs or mortality were observed in chickens vaccinated with either rLaSota/VP2 or commercial live IBDV vaccine. Of chickens vaccinated with rLaSota/VP2 or commercial live IBDV vaccine, 20 and 10% had bursal atrophy after challenge, respectively, as indicated by BBWR. The group mean of BBWR was assigned to similar groups by the Kruskal-Wallis test, showing that both commercial IBDV vaccine and rLaSota/VP2 offered significant protection against challenge (Table 4, primary vaccination). Analysis by AC-ELISA showed a protective pattern similar to that of BBWR, with 20 and 10% bursal samples displaying the presence of IBDV antigens (Table 4, primary vaccination). Histopathological examination of representative samples revealed that chickens vaccinated with commercial live IBDV vaccine or rLaSota/VP2 and challenged with GLS-5 virus showed no to only mild bursal lesions, whereas unvaccinated challenged chickens showed severe bursal lesions (Fig. 4). Overall, based on these criteria, primary vaccination with rLaSota/VP2 and commercial IBDV live vaccine conferred 80 and 90% protection, respectively, against challenge with virulent IBDV strain GLS-5 after primary vaccination. Of chickens vaccinated with either commercial live NDV vaccine or rLaSota/VP2, 1 out of 10 in each group died after lethal challenge with virulent NDV strain Texas GB. All other birds were normal and healthy, suggesting 90% protection level for both live NDV vaccine and rLaSota/VP2 after primary vaccination.

Secondary immunization at 23 days of age with commercial live NDV or IBDV vaccines induced higher levels of antibodies to NDV and IBDV, respectively, than did the primary vaccination (Table 3). Revaccination with rLaSota/VP2 enhanced antibody responses to both NDV and IBDV, as reflected by the higher antibody titers (Table 3). Two weeks after secondary immunization, chickens were challenged with either virulent IBDV strain GLS-5 or NDV strain Texas GB as described above. As indicated by antigen detection, BBWR, and histopathology, both the commercial live IBDV vaccine and recombinant rLaSota/VP2 conferred complete protection against virulent IBDV strain GLS-5 challenge; whereas the unvaccinated control chickens were fully susceptible (Table 4). Chickens vaccinated with rLaSota/VP2 and commercial NDV vaccines were also protected against challenge with virulent strain NDV Texas GB after secondary immunization (Table 5). Overall, the results showed that the levels of protection against both IBDV and NDV challenge were very well correlated with the antibody titers induced by the vaccination and that expression of the IBDV VP2 protein by rLaSota/VP2 would not interfere with immunity against NDV.

DISCUSSION

The use of biotechnology to create recombinant viral-vectored vaccines holds many promises for the future. In the poultry industry, so far, fowl poxvirus, herpesvirus of turkeys, Marek's disease virus, adenovirus, and members of the retrovirus family have been used most extensively as expression vectors (11, 21, 48). These recombinant viruses have demonstrated protective efficacy against a variety of avian diseases. However, their practical use still needs to be evaluated, particularly with regard to factors such as safety, route of delivery, efficacy, and production cost (11, 21, 48).

NDV provides an efficient vector system for the delivery of protective antigens of other avian pathogens, such as those of the IBDV or infectious bronchitis virus. Live NDV vaccines, such as the LaSota strain, are widely used in poultry industries around the world with proven track records in safety and efficacy. Immunization with LaSota induces not only long-lasting humoral and cellular immunity but also a strong mucosal immunity. NDV grows to very high titers in many cell lines and embryonated eggs, which allows cost-effective and easy manufacture of the vaccine. Unlike other viral vectors, NDV has a simple genome encoding only a few proteins. For generation of specific immune responses, it would be advantageous to have only a limited number of proteins expressed.

IBDV is a pathogen of major economic importance to the poultry industry worldwide. Currently, chickens are routinely vaccinated against IBDV (25). However, infectious bursal disease still has considerable socioeconomic importance at the international level, as the disease is present in almost every country because of the emergence of new antigenic variants or strains of increased virulence (25). Variant and hypervirulent strains probably arise through the accumulation of point mu-

EXPEDIENTES CONTRALORIA - URGENTES

No	Expediente	Presentación	Trámite	Estado	Persona	Fecha	sector	Estado	fecha	observacion	Situación actual	Examinador	1	2	3	Fecha Venc	F. creac div
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2	**5-7266B**	2005-01-28 15:39:37	11	ENVIO PRESTAMO	JOSE LUIS SALAZAR LOPEZ	2012-02-23 11:00:52	BT	45-REQUERIMIENTO 45	2011-11-24 15:03:28	oficio-18381-del-24/11/2011 vence-21/02/2012. Respuesta:16/02/2012 Prorroga:NO Rta Prorro:NO	2do Art 45, Vence 21/02/2012 pend reasignar	NATALIA LAMPREA BERMUDEZ	B	DV	DV		20/05/2011
3	5-22450A	2005-03-10 17:27:31	11	REASIGNACION	LYNA DEL CARMEN MORDECAY DUNOYER	2012-03-13 17:02:10	QF	45-REQUERIMIENTO 45	2011-11-30 15:27:08	oficio-18546-del-30/11/2011 vence-27/02/2012. Respuesta:23/02/2012 Prorroga:NO Rta Prorro:NO	3er Art 45, En estudio 13 marzo 2012, para revisión de la resolución	LYNA DEL CARMEN MORDECAY	E	DV	DV		19/05/2011
4	5-26162	2005-03-22 17:05:39	2	REASIGNACIÓN	ZULEMA ROSADO BAYONA	2012-03-23 17:24:12	IQ	45-REQUERIMIENTO 45	2011-11-09 11:38:22	oficio-17445-del-09/11/2011 vence-07/02/2012. Respuesta:NO Prorroga:06/02/2012 Vence Prorro: 20/03/2012 Rta Prorro:13/03/2012	3er Art 45,Nvo examinador En estudio 23 marzo 2012	ANDRES EDUARDO MÉNDEZ JAIMES/ZULEMA ROSADO BAYONA	E				
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6	5-35881	2005-04-15 17:50:26	11	ENVIO PRESTAMO	JUANITA CATALINA RICO VALBUENA	2012-04-26 14:46:12	QF	45-REQUERIMIENTO 45	2011-12-14 11:53:30	oficio-19404-del-14/12/2011 vence-09/03/2012. Respuesta:NO Prorroga:21/02/2012 Vence Prorro: 04/04/2012 Rta Prorro:17/04/2012	3er Art 45, Vence 25/04/2012	GLORIA JACQUELINE ALFONSO ROZO	E				
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8	5-102278B	2005-10-07 14:08:46	11	REASIGNACION	JOSE LUIS SALAZAR LOPEZ	2012-04-30 09:33:23	QF	45-REQUERIMIENTO 45	2011-12-15 14:30:59	oficio-19535-del-15/12/2011 vence-12/03/2012. Respuesta:NO Prorroga:27/02/2012 Vence Prorro: 12/04/2012 Rta Prorro:17/04/2012	1er Art 45, Para revisión de la resolución	LYNA DEL CARMEN MORDECAY	R	DV	DV	26/04/2012	15/12/2011
9	5-106147A	2005-10-19 14:32:07	11	DEVOLUCION	JANNETH EMILSE MARTINEZ CASTRO	2012-02-29 12:12:49	QP	45-REQUERIMIENTO 45	2012-02-23 14:26:28	oficio-2756-del-23/02/2012 vence-25/05/2012. Respuesta:NO Prorroga:NO Rta Prorro:	1er Art 45, Vence 25/05/2012	MARIA GRACIELA OVALLE GAITAN	T	DV	DV	25/05/2012	23/02/2012
10	5-122635A	2005-12-02 16:06:08	11	REASIGNACION	ANA DELIA PINZON GARCIA	2012-05-02 09:49:52	BT	45-REQUERIMIENTO 45	2011-12-14 12:00:39	oficio-19406-del-14/12/2011 vence-09/03/2012. Respuesta:NO Prorroga:06/03/2012 Vence Prorro: 20/04/2012 Rta Prorro:24/04/2012	1er Art 45, Vence prorroga 25/04/2012	ANA DELIA PINZON GARCIA	E	DV	DV	25/04/2012	14/12/2011

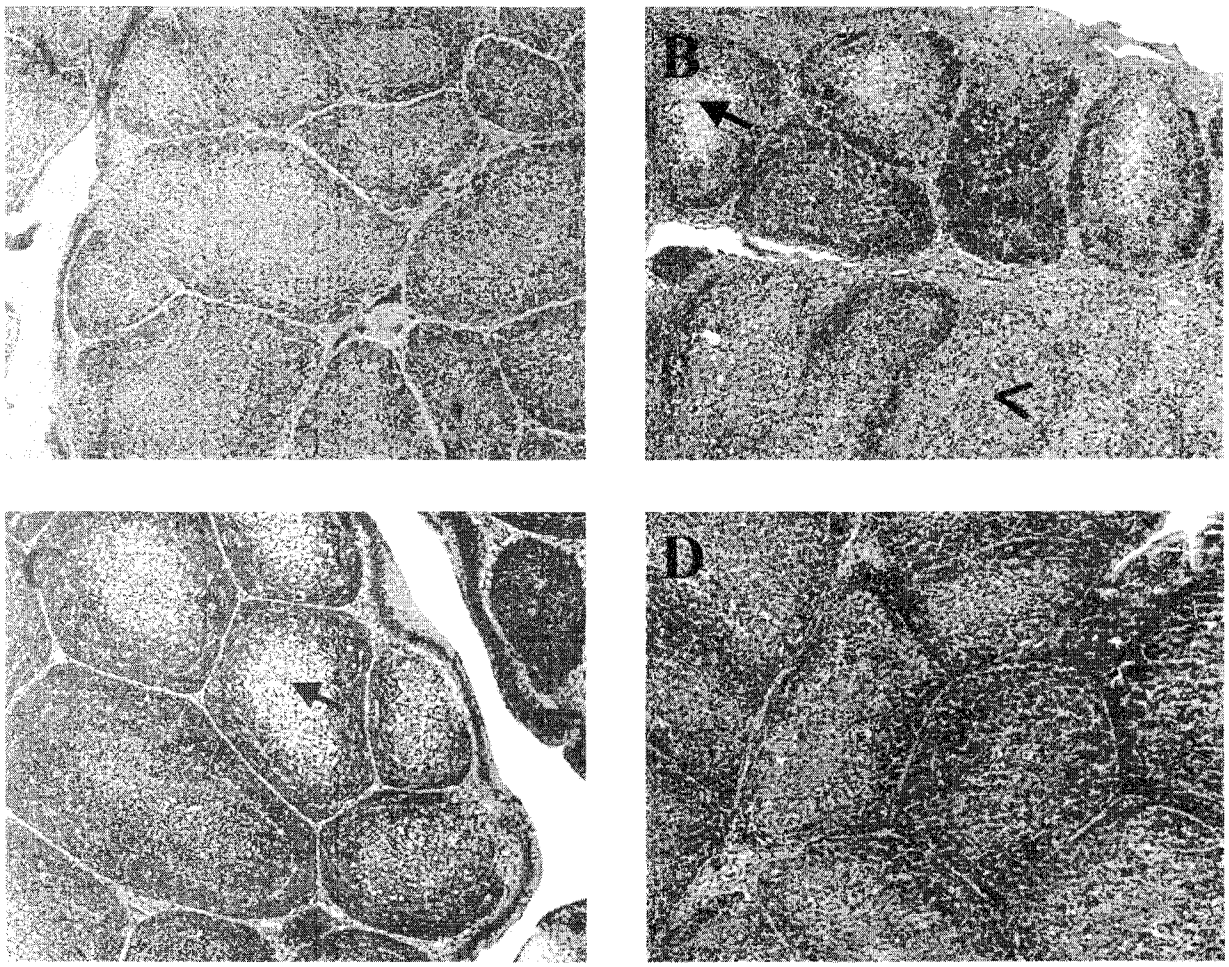

FIG. 4. Histopathology of bursa of Fabricius 3 days after challenge. Chickens were vaccinated and boosted with PBS (A and B), commercial IBDV vaccine (C), or rLaSota/VP2 (D) and challenged with virulent IBDV strain GLS-5 (B, C, D). Panel A shows an unchallenged control. Seventy-two hours after challenge, the bursa was removed and fixed in neutral buffered formalin. Tissues were embedded in paraffin, sectioned, and stained with hemoxylin and eosin for microscopic examination. Magnification, ×40. (A) Bursa from normal chicken: large active follicles with little, interfollicular tissue. (B) Bursa from unvaccinated challenged chicken: severe multifocal medullary vacuolation and lymphocytic depletion (arrow), and severe follicular degeneration (arrowhead). (C) Bursa from chicken vaccinated with commercial IBDV vaccine and challenged: some mild multifocal medullary vacuolation and lymphocytic depletion (arrow). (D) Bursa from chicken vaccinated with rLaSota/VP2 and challenged: no histologic lesions observed.

tations due to the error-prone nature of viral RNA polymerase and through reassortment between circulating strains or between vaccines and circulating field strains (25, 42). Another factor that may be contributing to creation of the antigenic variant viruses is the wide use of live attenuated IBDV vaccines (25). It is possible that, over a period of time, these live attenuated IBDV vaccines may revert back to virulence and may change antigenicity (17, 41, 42). Further, live IBDV vaccines cannot break through the high levels of maternal antibodies in young chickens (25). Therefore, there is a need to develop alternative IBDV immunizing strategies, which will deter the development of variant and/or hypervirulent strains but still have the capability to induce local and systemic immunity critical to controlling the disease, in the presence of maternal antibodies.

TABLE 5. Protective efficacy of primary and secondary vaccination against NDV challenge[a]

Vaccine	Primary vaccination		Secondary vaccination	
	No. dead/total	Protection (%)	No. dead/total	Protection (%)
Commercial NDV vaccine	1/10	90	0/10	100
rLaSota/VP2	1/10	90	0/10	100
None	10/10	0	10/10	0

[a] Chickens were vaccinated by eyedrop instillation with commercial NDV vaccine or rLaSota/VP2 and challenged intramuscularly with virulent NDV strain Texas GB 2 weeks after primary or 2 weeks after secondary vaccination.

The VP2 protein is the major host-protective immunogen of IBDV, and most of the variations among isolates appear in this part of the viral genome (4, 12, 16, 40). Since a major neutralizing epitope of the virus is a conformational epitope, delivery of VP2 protein in native conformation is critical for correct antigen processing and presentation (3, 25). Furthermore, in

DETAILED ACTION

Information Disclosure Statement

The listing of references in the Search Report is not considered to be an information disclosure statement (IDS) complying with 37 CFR 1.98. 37 CFR 1.98(a)(2) requires a legible copy of: (1) each foreign patent; (2) each publication or that portion which caused it to be listed; (3) for each cited pending U.S. application, the application specification including claims, and any drawing of the application, or that portion of the application which caused it to be listed including any claims directed to that portion, unless the cited pending U.S. application is stored in the Image File Wrapper (IFW) system; and (4) all other information, or that portion which caused it to be listed. In addition, each IDS must include a list of all patents, publications, applications, or other information submitted for consideration by the Office (see 37 CFR 1.98(a)(1) and (b)), and MPEP § 609.04(a), subsection I. states, "the list ... must be submitted on a separate paper." Therefore, the references, WO 2004-075892, WO 2000-027397 and WO 1997-049392 cited in the Search Report and listed in the IDS (12/1/2008) have not been considered. Applicant is advised that the date of submission of any item of information or any missing element(s) will be the date of submission for purposes of determining compliance with the requirements based on the time of filing the IDS, including all "statement" requirements of 37 CFR 1.97(e). See MPEP § 609.05(a).

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

developing a poultry vaccine, a number of factors must be taken into account. Since the price of a single bird is relatively low, one of the most important factors is the cost of the vaccine. The research conducted here leads to the development of a bivalent vaccine candidate for NDV and IBDV and provides an inexpensive approach. This novel vaccine candidate has the following features. The IBDV VP2 protein was stably and correctly expressed and retained the host-protective conformational epitopes. The expressed VP2 protein was not incorporated into the recombinant virus, and the recombinant virus had the similar replicative kinetics as the parental rLaSota virus.

Overall, the recombinant rLaSota/VP2 has several advantages over the existing IBDV vaccines. First, the recombinant NDV/VP2 will be highly economical for the poultry industry, since the cost of current IBDV vaccination will be eliminated for the poultry producers. Second, the recombinant virus can break through and will not be neutralized by maternal immunity against IBDV; therefore, it can be used for vaccination even in the presence of maternal antibody to enhance protection in the first few critical weeks of chicken life. Third, the recombinant rLaSota/VP2 vaccine will not result in new IBDV variants, since no live IBDV will be used for vaccination. Finally, it was reported that the V protein of NDV is associated with viral pathogenesis and functions as an interferon antagonist (20, 28). It was also demonstrated that elimination of the V protein expression in NDV rendered the virus attenuated but still highly immunogenic and that the attenuated NDV vaccine strain would be administered in ovo in uniform dosage by automation to protect chickens with or without maternal antibodies (28). Therefore, the recombinant virus can be tailored with ease for in ovo use. Thus, the recombinant virus described here for the protection of both NDV and IBDV will be highly beneficial to the poultry industry.

Our results demonstrated, for a variant infectious bursal disease, the potential of using NDV recombinant vaccine to protect SPF chickens against a massive challenge dose of homologous virus strains. Successful immunization of commercial chickens against many different field strains is likely to be more complicated. However, we can devise strategies to improve the recombinant vaccines. Since additional, albeit minor, neutralizing epitopes are also present on VP3 subunit (25), it is reasonable to assume that the immunogenicity of the recombinant virus can be enhanced by inserting the gene encoding IBDV polyprotein into the NDV genome. Moreover, correct expression of the polyprotein may result in formation of virus-like particles, which would be more immunogenic and offer better protection (45). Currently, in the poultry industry, classic and variant strains of live IBDV vaccines are simultaneously used to obtain maximal cross-protection. We could make two recombinant viruses, each expressing the VP2 proteins of the classic or variant strains, respectively, and use them as combined products for vaccination. Alternatively, we could express both the VP2 proteins simultaneously from the same NDV genome as two separate transcriptional units. In the case of emergence of a new variant serotype for which current vaccines do not provide enough protection, we could use this system conveniently to update the antigenic specificity of vaccines to best control current circulating field strains. The fact that NDV grows in cell cytoplasm without a DNA stage and has extremely low frequency of recombination (24) would make the use of recombinant NDV expressing the VP2 protein of IBDV more appealing.

The present study demonstrated that recombinant NDV is an excellent vaccine vector for IBDV. The use of an NDV-based vaccine that would reduce the number of circulating IBDV strains may be highly beneficial to the poultry industry. Furthermore, our results suggested that NDV could be used as a vaccine vector for other avian pathogens. Moreover, NDV replicates preferentially in avian cells but is replication deficient in most mammalian cells; therefore, NDV could be exploited as a promising vaccine delivery vector for human pathogens. Armed with basic knowledge, the recombinant NDV could also be used in cancer treatment and gene therapy.

ACKNOWLEDGMENTS

We greatly acknowledge Chinta Lamichhane at Synbiotics Corporation for his advice and reagents in immunological assessment. We thank Daniel Rockemann and Peter Savage for their excellent technical assistance.

This work was partially supported by U.S. Department of Agriculture grant 2002-35204-1601.

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EXPEDIENTES CONTRALORIA - URGENTES

No	Expediente	Presentación	Trámite	Estado	Persona	Fecha	sector	Estado	fecha	observacion	Situación actual	Examinador	1	2	3	Fecha Venc	F. creac div
21	6-22161	2006-03-06 16:50:41	11	SOLICITUD PRESTAMO	JUANITA CATALINA RICO VALBUENA	03/05/2012	QP	45-REQUERIMIENTO 45	2012-01-25 14:08:58	oficio-1045-del-25/01/2012 vence-24/04/2012. Respuesta:20/04/2012 Prorroga:NO Rta Prorro:NO	3er Art 45, Vence 24 abril 2012	ANDREA DEL PILAR MOJICA CORTES	E			24/04/2012	
22	6-26817	2006-03-16 16:48:47	11	REASIGNACION	SANDRA ISABEL CALDERON RODRIGUEZ	2012-05-02 14:57:16	QP	45-REQUERIMIENTO 45	2011-12-14 12:24:34	oficio-19412-del-14/12/2011 vence-09/03/2012. Respuesta:NO Prorroga:07/03/2012 Vence Prorro: 23/04/2012 Rta Prorro:19/04/2012	3er Art 45, En estudio 2 mayo 2012	RAFAEL BARACALDO	E				
23	6-27209	2006-03-17 14:49:37	11	REASIGNACION	ANDREA DEL PILAR MOJICA CORTES	2012-03-16 11:02:29	QP	46-REQUERIMIENTO 46	2011-12-14 13:45:11	oficio-19422-del-14/12/2011 vence-09/03/2012. Respuesta:28/02/2012 Prorroga:NO Rta Prorro:NO	3er Art 45 y 46, En estudio 16 marzo 2012	ANDREA DEL PILAR MOJICA CORTES	E				
24	6-27690	2006-03-21 14:17:49	11	ENVIO PRESTAMO	CLARA INES BARRERA GARZON	2012-04-30 14:30:54	QF	45-REQUERIMIENTO 45	2011-12-14 12:26:48	oficio-19415-del-14/12/2011 vence-09/03/2012. Respuesta:NO Prorroga:01/03/2012 Vence Prorro: 17/04/2012 Rta Prorro:16/04/2012	Rec Rep Rech, 2do Art 45, Vence 25 abril 2012	GLORIA JACQUELINE ALFONSO ROZO	E			25/04/2012	
25	6-30672	2006-03-28 17:18:12	11	REASIGNACION	ANDREA DEL PILAR MOJICA CORTES	2011-12-28 14:42:24	QP	45-REQUERIMIENTO 45	2011-01-13 15:46:58	oficio-642-del-13/01/2011 vence-11/04/2011. Respuesta:NO Prorroga:06/04/2011 Vence Prorro: 20/05/2011 Rta Prorro:19/05/2011	1er Art 45, En estudio 28 diciembre 2011	MARIA GRACIELA OVALLE GAITAN/ ANDREA DEL PILAR MOJICA CORTES	E				
26	6-37892A	2006-04-21 14:29:17	11	REASIGNACION	JUAN CARLOS CHAPARRO VELEZ	2012-05-02 10:23:53	QF	45-REQUERIMIENTO 45	2011-12-14 13:53:56	oficio-19424-del-14/12/2011 vence-09/03/2012. Respuesta:NO Prorroga:02/03/2012 Vence Prorro: 18/04/2012 Rta Prorro:NO	1er Art 45, Negación X No Rta	ANDRES GIL HELFFHRITTZ	R	DV	DV	25/04/2012	14/12/2011
27	6-37892	2006-04-21 14:29:17	11	REASIGNACION	JUAN CARLOS CHAPARRO VELEZ	2012-05-02 10:23:30	QF	45-REQUERIMIENTO 45	2011-12-14 12:29:14	oficio-19416-del-14/12/2011 vence-09/03/2012. Respuesta:NO Prorroga:02/03/2012 Vence Prorro: 18/04/2012 Rta Prorro:NO	2do Art 45, Negación X No Rta	ANDRES GIL HELFFHRITTZ	R			25/04/2012	
28	6-42082	2006-05-04 17:37:28	11	REASIGNACION	CARLOS DANIEL LEMUS GIRALDO	2012-04-16 14:48:15	QF	45-REQUERIMIENTO 45	2011-12-22 15:27:05	oficio-20173-del-22/12/2011 vence-16/03/2012. Respuesta:15/03/2012 Prorroga:NO Rta Prorro:NO	1er Art 45, Para revisión de la resolución	GLORIA JACQUELINE ALFONSO ROZO	R				
29	6-49473	2006-05-23 17:39:04	11	REASIGNACION	EMILSE CANO URREGO	2012-02-29 17:16:03	QF	45-REQUERIMIENTO 45	2011-11-24 15:05:25	oficio-18383-del-24/11/2011 vence-21/02/2012. Respuesta:17/02/2012 Prorroga:NO Rta Prorro:NO	4to Art 45 y Art 46, En estudio 29 febrero 2012	MARIA ALEJANDRA SANCHEZ	E				
30	6-50507	2006-05-25 16:47:46	11	REASIGNACION	NATALIA LAMPREA BERMUDEZ	2012-05-02 09:54:54	BT	45-REQUERIMIENTO 45	2011-12-14 12:34:11	oficio-19418-del-14/12/2011 vence-09/03/2012. Respuesta:NO Prorroga:07/03/2012 Vence Prorro: 23/04/2012 Rta Prorro:19/04/2012	2do Art 45, En estudio 2 mayo 2012	NATALIA LAMPREA BERMUDEZ	E			25/04/2012	

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copending Application No. 12/072200 in view of Finnie (US 6391358). Although the conflicting claims are not identical, they are not patentably distinct from each other because the subject matter of Claims 1-22 of the conflicting copending Application differs from the current application in the type of gelling agent in the concentrate for preparing a bouillon, broth, soup etc. The gelling agent in the conflicting copending Application is konjac with optionally another gelling agent, which can be starch as per dependent claim 8 of conflicting copending application, and current application claims of starch. Further gelling agents, such as konjac and starch are well known in the art for producing gelled products and thus, are obvious variants of each other, as evidenced by **Finnie (US 6391358)**. Finnie teaches of gelled compositions comprising konjac, pectin, gelatin (see examples 1 and 2, columns 9-10). Further in Column 11, lines 60-65, Finnie clearly states that "suitable thickening and gel forming agents that may find advantageous use with the present invention include at least: gelatin, xanthan, curdlan, agar, alginates, carrageenans, guar, locust bean gum, tara, methocel, fenugreek, pectin, gellan, other derivatives of cellulose, and starch." which makes the gelling agents recited in the two applications obvious variants of each other. Thus, claims 1-3 and 8-20 of current application and claims 1-22 of conflicting copending application overlap in scope.
This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Claims 1-20 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over Claims 1-15 of copending Application No. 11/990967 in view of Finnie (US 6391358). Although the conflicting claims are not identical, they are not patentably distinct from each other because the subject matter of Claims 1-14 of the conflicting copending Application differs from the current application in the type of gelling agent in the concentrate for preparing a bouillon, broth, soup etc. The gelling agent in the conflicting copending Application is starch(0.1-10%)and gelatin (1.5-30%), and current application claims of

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starch (10-30%). Further gelling agents, such as gelatin and starch are well known in the art for producing gelled products either together or separately and thus, are obvious variants of each other, as evidenced by **Finnie (US 6391358)**. Finnie teaches of gelled compositions comprising gelatin (see examples 1-3, columns 9-11). Further in Column 11, lines 60-65, Finnie clearly states that "suitable thickening and gel forming agents that may find advantageous use with the present invention include at least: *gelatin*, xanthan, curdlan, agar, alginates, carrageenans, guar, locust bean gum, tara, methocel, fenugreek, pectin, gellan, other derivatives of cellulose, and *starch*." which makes the gelling agents recited in the two applications obvious variants of each other. Thus, claims 1-20 of current application and claims 1-14 of conflicting copending Application overlap in scope.
This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Claims 1-20 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over Claims 1-16 of copending Application No. 11/990969 in view of Finnie (US 6391358) for the same reasons as provided in double patenting rejection over application 11/990967 as claims of both applications recite same proportion of starch and gelatin combination as gelling agent. Thus, claims 1-20 of current application and claims 1-16 of conflicting copending Application overlap in scope.
This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Claim Objections

Claims 1-20 are objected to because of the following informalities:
In Claims 1-13, the article "A" should be added before "Packaged".
In Claims 1, 3-5, 12, 14, 16, the parentheses should be removed around the phrase "weight % based on total packaged concentrate" or "weight % based on water content

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REPÚBLICA DE COLOMBIA
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

RESOLUCIÓN No: 61180

Por la cual se deniega una patente de invención

Rad. PCT/ 07-58654

EL SUPERINTENDENTE DE INDUSTRIA Y COMERCIO
en ejercicio de sus facultades legales, en especial de las conferidas en el numeral 26 del artículo 3° del Decreto 4886 de 2011, y

CONSIDERANDO:

PRIMERO: Que mediante escrito radicado en esta Superintendencia el día 8 de junio de 2007 con el N° 07-058654-00000-0000, por la sociedad BAYER SCHERING PHARMA AKTIENGESELLSCHAFT, a través de apoderado, entró a fase nacional la solicitud de patente de invención titulada "VIRUS RECOMBINANTE DE LA ENFERMEDAD DE NEWCASTLE", que corresponde a la solicitud internacional PCT/EP2005/012186 del 10 de noviembre de 2005.

SEGUNDO: Que de conformidad con lo dispuesto en el artículo 27 del Tratado de Cooperación en Materia de Patentes (PCT) y la regla 51bis del Reglamento, que facultan a la Superintendencia sobre la aplicación de la Decisión 486 de la Comisión de la Comunidad Andina, se efectuó el examen de forma a la solicitud, de acuerdo con lo previsto en el artículo 38 de la mencionada Decisión andina.

TERCERO: Que efectuado el examen de forma en cuanto a los requisitos que debe llenar la solicitud y cumplidas las exigencias legales, se ordenó publicar el extracto sin que se hubieran presentado oposiciones por parte de terceros.

CUARTO: Que mediante escrito radicado en esta Superintendencia el día 14 de julio de 2008, el solicitante presentó petición de examen de patentabilidad, en los términos establecidos en el artículo 44 de la Decisión 486 de la Comisión de la Comunidad Andina.

QUINTO: Que el artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina establece que: *"Si la oficina nacional competente encontrara que la invención no es patentable o que no cumple con alguno de los requisitos establecidos en esta Decisión para la concesión de la patente, lo notificará al solicitante. Este deberá responder a la notificación dentro del plazo de sesenta días contados a partir de la fecha de la notificación. Este plazo podrá ser prorrogado por una sola vez por un período de treinta días adicionales. (...) Si el solicitante no respondiera a la notificación dentro del plazo señalado, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, la oficina nacional competente denegará la patente".*

SEXTO: Que de conformidad con lo dispuesto en el artículo 45 antes mencionado, se realizó el examen de fondo y se requirió al solicitante mediante Oficio No. 10210, notificado el 13 de junio de 2012, para que se pronunciara sobre las objeciones efectuadas a la solicitud en cuanto a que no es una invención, excepciones a la patentabilidad, falta de claridad, concisión y sustento de las reivindicaciones, carencia de unidad de invención y nivel inventivo, de acuerdo con las exigencias de los artículos 15 b), 18, 20 d), 25 y 30 de la Decisión 486.

Transcurrido el plazo establecido no se dio respuesta al requerimiento y sugerencias arriba indicados.

Industria y Comercio
SUPERINTENDENCIA

REPÚBLICA DE COLOMBIA
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

RESOLUCIÓN No: 61180

Por la cual se deniega una patente de invención

Rad. PCT/ 07-58654

RESUELVE:

ARTÍCULO PRIMERO: Denegar la patente de invención titulada "VIRUS RECOMBINANTE DE LA ENFERMEDAD DE NEWCASTLE", que entró en fase nacional en virtud del Tratado de Cooperación en Materia de Patentes (PCT).

ARTÍCULO SEGUNDO: Notificar el contenido de la presente resolución al doctor Ian Raisbeck Llinas, en su calidad de apoderado de BAYER SCHERING PHARMA AKTIENGESELLSCHAFT, o a quien haga sus veces, entregándole copia de la misma y advirtiéndole que contra ella procede el recurso de reposición, ante el Superintendente de Industria y Comercio, el cual podrá ser interpuesto en el momento de la notificación o dentro de los cinco 5 días hábiles siguientes a ella.

NOTIFÍQUESE Y CÚMPLASE
Dada en Bogotá, D.C., el 19 Oct 2012

EL SUPERINTENDENTE DE INDUSTRIA Y COMERCIO,

PABLO FELIPE ROBLEDO DEL CASTILLO

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Revisó: Jose Luis Salazar Lopez
Aprobó: Jose Luis LondoÑo Fernandez

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
EL SECRETARIO GENERAL
Certifica que la Resolución 61180 de fecha 19/10/2012
fué notificado mediante pagina web PI número 422
fijado el 23/10/2012 y desfijado el 23/11/2012

Al contestar favor indique el número
de radicación que se indica a continuación:
Cod Verificación: 1854030845

S Sede Centro: Carrera 13 No. 27-00 pisos 2, 5, 7 y 10 PBX: 5870000
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