



Industria y Comercio
SUPERINTENDENCIA

P C T
SOLICITUD
PATENTE DE INVENCION

21 EXPEDIENTE No

06-120701

54 TITULO VACUNA CONTRA HPV16 Y HPV18 Y AL
MENOS OTRO TIPO DE VPH SELECCIONADO DE VPH
31, 45 O 52"

51 CLASIFICACION INTERNACIONAL A61K 39/12

71 SOLICITANTE GLAXOSMITHKLINE BIOLOGICALS
S A

DOMICILIO RIXENSART, BÉLGICA

74 REPRESENTANTE LEGAL ALICIA LLOREDA RICAURTE
T P 53 215

72 BOGOTA, D C

29 NOV 2006

(FORMA P 10)

PETITORIO



SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
Rad: 06-120701- -00000-0000
Fec: 2005-11-29 18:08:54 Dep. 3020 NUECREACIONES
Tra: 11 PATENTEIYE
Eve: 379 FASENACIONALI
Act: 411 PRESENTACION Folios: 51

FORMULARIO UNICO DE SOLICITUD DE PATENTE PCT ENTRADA EN FASE NACIONAL

①

SOLICITUD DE:

☒ Patente de Invención

☐ Patente de Modelo de Utilidad

☐ Capítulo I.

☒ Capítulo II.

②

SOLICITANTE (71)

Nombre: **GLAXOSMITHKLINE BIOLOGICALS S.A.**

Dirección: Rue de l'Institut 89, B-1330 Rixensart, Bélgica.

Nacionalidad o Domicilio: Rixensart, Bélgica.

Lugar de Constitución: Bélgica

Teléfono: 3284270 Fax: 8069701

E-mail: jllcco@lloredacamacho.com

IDENTIFICACION

C.C.

☐

NIT

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C.E.

☐

Otro

☐

Cual _____

Número _____

③

REPRESENTANTE O APODERADO

Nombre: **ALICIA LLOREDA RICAURTE**

Dirección: Calle 72 No. 5-83 Piso 5º, Bogotá, Colombia

Teléfono: 3284270 Fax: 8069701

E-mail: jllcco@lloredacamacho.com

C.C.

☒

NIT

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C.E.

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Otro

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Cual _____

Número 39.690.713 TP 53.215

④

INVENTOR(ES) (72)

Nombre: **DEBRUS, Serge; MARTIN, Marie-Therese; STEPHEN, Robert John; STEPHENNE, Jean y WETTENDORFF, Martine, Anne, Cécile.**

Dirección: GlaxoSmithKline Biologicals S.A., Rue de l'Institut 89, B-1330 Rixensart y GlaxoSmithKline, 980 Great West Road, Brentford Middlesex TW8 9GS.

Nacionalidad o Domicilio: Bélgica y Gran Bretaña

⑤

PCT (86)

Solicitud Internacional No. PCT/EP2005/006461

Fecha: 14/06/05

Publicación Internacional No. WO 2005/123125 A1

Fecha: 29/12/05

⑥

Título (54)

"VACUNA CONTRA HPV16 Y HPV18 Y AL MENOS OTRO TIPO DE VPH SELECCIONADO DE VPH 31, 45 O 52"

⑦

Clasificación Internacional (51) A61K 39/12

⑧

Prioridad

SI ☒ NO ☐

(33) País de Origen

GB

US

(32) Fecha

18/06/04

26/04/05

(31) Número de Solicitud

0413510.9

11/114,301

⑨

Comprobante de Pago No. 06 - 88,786

Fecha: 11/17/06

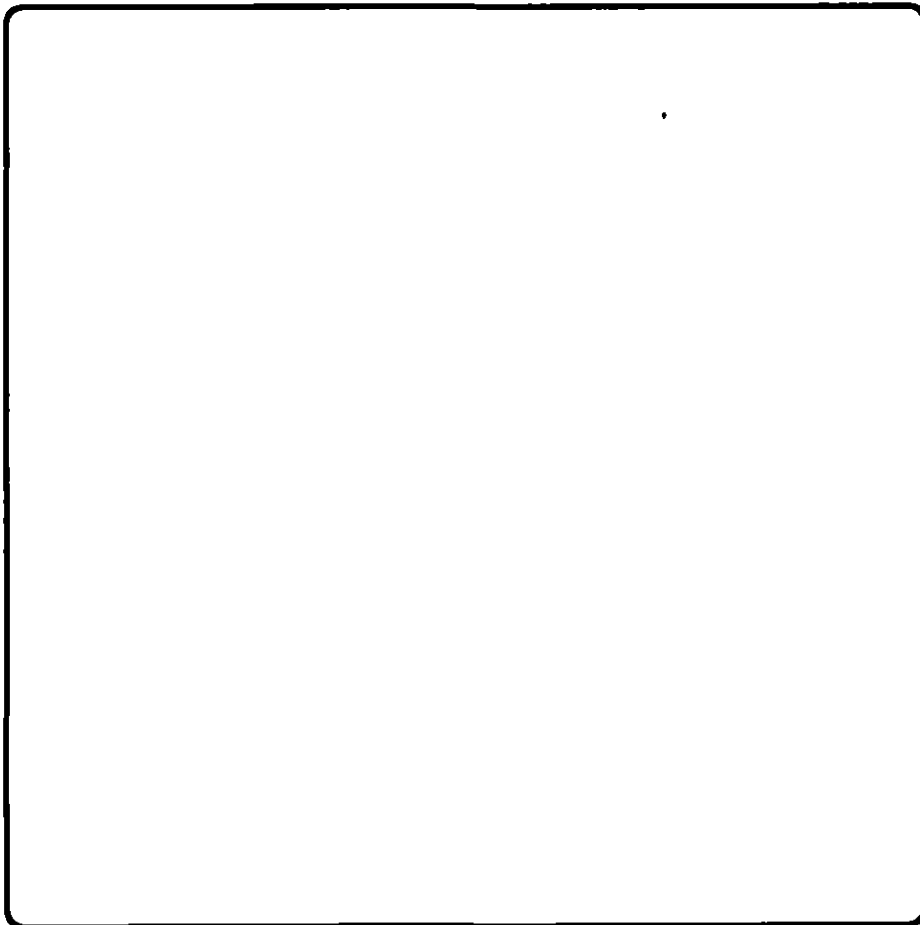
10

ANEXOS

- ☒ Comprobante de pago de la tasa de presentación de la solicitud.
- ☒ Documentos que acreditan la existencia y representación legal cuando los solicitantes sean personas jurídicas.
- ☒ Documentos de Representación Legal.
- ☐ Documento de cesión del inventor al solicitante o a su causante.
- ☐ Declaraciones
- ☐ Copia de la solicitud Internacional. (Resumen, descripción, reivindicaciones, dibujos)
- ☒ Traducción de la solicitud Internacional al castellano. (Resumen, descripción, reivindicaciones)
- ☐ Copia del examen preliminar Internacional efectuado por la Autoridad Internacional de Examen Preliminar (IPEA).
- ☐ Traducción del examen preliminar Internacional efectuado por la IPEA.
- ☐ De ser el caso, copia del contrato de acceso.
- ☐ De ser el caso, documento que acredite la licencia o autorización de uso de conocimientos tradicionales de las comunidades indígenas.
- ☐ Arte Final 12X12.
- ☐ De ser el caso, información sobre otras solicitudes de patente o títulos obtenidos en el extranjero por el mismo titular o su causante, relacionados parcial o totalmente con la invención de esta solicitud.
- ☐ De ser el caso, modificaciones efectuadas a la solicitud Internacional.
- ☒ Otros: Reporte de búsqueda Internacional con su traducción, anexos del reporte de búsqueda, publicación con su traducción y opinión escrita con su traducción.

11

FIGURA CARACTERISTICA:



12

Solicito la concesión de la patente,

NOMBRE: ALICIA LLORED RICAURTE

FIRMA:

C.C. 39.690.713

T.P. 53.215

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
Rad: 06-120701- -00000-0000
Fec: 2006-11-29 16:08:54 Dep. 2020 NUECREACIONES
Tr. 11 PATENTEYII
Eve: 379 FASENACIONALI
Act 411 PRESENTACION Folios: 51

NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 06 - 88,786
FECHA : NOVIEMBRE 17 DE 2006

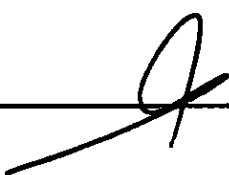
***** CONSIGNACION *****

DEPOSITANTE	TIPO PASO	BANCO	CUENTA	No. PAGO	FECHA PAGO	Vr. PAGO
LLOREDA Y CIA S. A	CONSIGNACION	BANCO POPULAR	050-00110-6	55443021	17/11/2006	4,837,000.00

***** CONCEPTO *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-01	SOLICITUDES	
1		TRAMITES DE SOL. DE PATENTE DE IN	463,000.00
		TOTAL :	\$ 463,000.00

CUATROCIENTOS SESENTA Y TRES MIL PESOS

RESPONSABLE : 

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____

ANEXO

El documento que acredita la calidad de REPRESENTANTES LEGALES en Bogotá de los doctores **ALICIA LLOREDA y/o GUSTAVO TAMAYO y/o IGNACIO SANTAMARIA y/o ENRIQUE ALVAREZ** para TODOS LOS ASUNTOS RELACIONADOS CON LA PROPIEDAD INDUSTRIAL de la sociedad peticionaria, que faculta a cada uno de dichos abogados para actuar individualmente, se está presentando con esta solicitud. En consecuencia, ruego a usted reconocer la personería de cada uno de dichos Representantes Legales para actuar dentro del presente expediente, a fin de que cualquiera de ellos pueda intervenir en cualquier tiempo respecto de la solicitud en él contenida.


ALICIA LLOREDA RICAURTE

T.P. No. 53.215

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TRADUCCION OFICIAL No. 2004-09-09-466
DE LAS PARTES PERTINENTES DE UN DOCUMENTO
PRESENTADO EN INGLES

PODER

Legalización de un Poder de Representación Legal, documento escrito en inglés y en español.

Certificado Notarial (Sociedad) documento también escrito en inglés y en español.

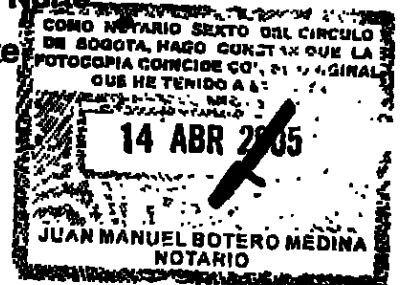
Notarización, documento escrito en español.

APOSTILLA

(Convención de La Haya del 5 de Octubre de 1961)

Reino Unido de la Gran Bretaña e Irlanda del Norte

1. País: Reino Unido de la Gran Bretaña e Irlanda del Norte
2. Este documento público
3. ha sido firmado James Kerr Milligan
4. actuando como Notario Público
5. lleva el sello/estampilla de dicho Notario Publico



CERTIFICADO

5. en Londres
6. el 01 de septiembre de 2004
7. por el Secretario de Estado Principal de Su Majestad para Asuntos Exteriores y del Commonwealth
8. Número: **G 749037**
9. Sello/Estampilla:
10. Firma: (ilegible)

TRADUCTOR OFICIAL
RESOLUCION 2177

ANITA ESCOBAR DE TAMAYO

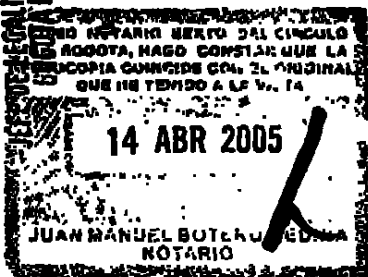
NO SE ASUME
RESPONSABILIDAD DEL TEXTO

2004 SET 10 A 10:47

ues

DE REGISTROS Y CATASTROS

DE BOGOTÁ D.C.



MINISTERIO DE
RELACIONES EXTERIORES

760567

Anita Escobar

CUYA FIRMA APARECE EN EL
DOCUMENTO DESEMPEÑA
LAS FUNCIONES INDICADAS

Sello de la Oficina de Asuntos
Exteriores y del Commonwealth

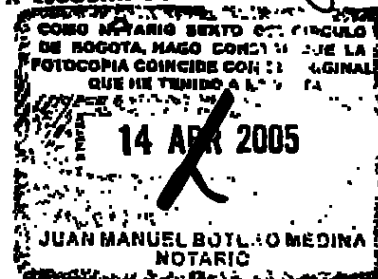
W. Glass
Por el Secretario de Estado

Si este documento se va a usar en un país que no es parte de la Convención de La Haya del 5 de octubre de 1961, debe presentarse a la sección consular de la misión que representa a ese país. Una apostilla o certificado de legalización únicamente confirma que la firma, sello o estampilla sobre el documento es auténtica. No significa que los contenidos del documento sean correctos o que la Oficina de Asuntos Exteriores y del Commonwealth apruebe los contenidos.

Traducido según mi mejor saber y entender, este día 9 de septiembre de 2004, en la ciudad de Bogotá. D.C. por Anita Escobar de Tamayo, Traductora Oficial, Res. 2177 de 1989, Ministerio de Justicia, República de Colombia.

TRADUCTOR OFICIAL
RESOLUCION 2177

Anita Escobar de Tamayo
ANITA ESCOBAR DE TAMAYO



Yo, el infrascrito, **JAMES KERR MILLIGAN**, Notario Público de la Ciudad de Londres, Inglaterra, por la Autoridad Real debidamente admitido y juramentado,

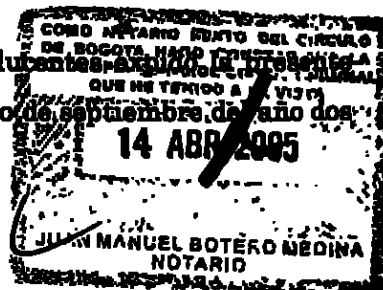
CERTIFICO:

QUE es auténtica la firma suscrita al pie del adjunto Poder, por ser del puño y letra del Señor Don **MARCUS JONATHAN WILLIAM DALTON**, cuya identidad me consta, Apoderado de la Sociedad denominada "**GLAXOSMITHKLINE BIOLOGICALS S.A.**" (anteriormente denominada "**Smithkline Beecham Biologicals s.a.**"), Sociedad legalmente constituida y existente de acuerdo con las leyes de Bélgica, con domicilio social en Rue de l'Institut 89, B-1330 Rixensart, Bélgica;

QUE el citado Señor Don **MARCUS JONATHAN WILLIAM DALTON** está debida y suficientemente autorizado para firmar documentos de esta clase, en virtud del Poder otorgado en su favor por la referida Sociedad fechado el 12 de junio de 2002, cuyo original he tenido a la vista.

Y QUE los objetos para los cuales se otorgó dicho Poder se encuentran dentro de los objetos o actividades de la citada Sociedad, de acuerdo con la Escritura Constitutiva y los Estatutos Sociales, los cuales he tenido a la vista.

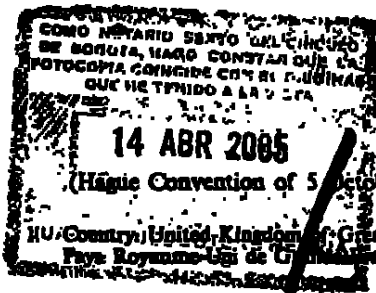
Y PARA QUE CONSTE y obre los efectos conducentes a partir de la presente, que firmo y sello en Londres, el día de hoy primero de septiembre de año dos mil cuatro.



2

JAMES KERR MILLIGAN
Notario Público de Londres, Inglaterra





APOSTILLE

(Hague Convention of 5 October 1961/Convention de La Haye du 5 octobre 1961)

HU/Country: United Kingdom of Great Britain and Northern Ireland
Pays: Royaume-Uni de Grande-Bretagne et d'Irlande du Nord

This public document/Le présent acte public

2. has been signed by / a été signé par James Keer Milligan
3. acting in the capacity of / agissant en qualité de Notary Public
4. bears the seal/stamp of / est revêtu du sceau/timbre de Notary Public

Certified/Attesté

5. at London/à Londres 6. the/le 01 SEP 2004
7. by Her Majesty's Principal Secretary of State for Foreign and Commonwealth Affairs/
par le Secrétaire d'Etat Principal de Sa Majesté aux Affaires Etrangères et du Commonwealth.

8. Number/sous No G 749037

9. Stamp/
timbre



10. Signature W GLASS

W Glass

For the Secretary of State / Pour le Secrétaire d'Etat

If this document is to be used in a country which is
not party to the Hague Convention of 5 October 1961,
it should be presented to the consular section of the
mission representing that country.

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Los abajo firmados, **GLAXOSMITHKLINE BIOLOGICALS S.A.**, una sociedad organizada y existente de conformidad con las leyes de Bélgica.

con domicilio en **rue de l'Institut 89,
B-1330 Rixensart,
Bélgica**

nombramos por el presente a **ALICIA LLOREDA y/o GUSTAVO TAMAYO y/o IGNACIO SANTAMARIA y/o ENRIQUE ALVAREZ**, domiciliados en Bogotá, Colombia, con oficinas en dicha ciudad, Calle 72 No. 5-83, piso 6o., nuestros representantes legales y verdaderos en Bogotá, con facultades para recibir notificaciones y nombrar apoderados judiciales o extrajudiciales, con el fin de dar cumplimiento al parágrafo 1o. del artículo 543 del decreto 410 de 1971.

Los doctores **ALICIA LLOREDA y/o GUSTAVO TAMAYO y/o IGNACIO SANTAMARIA y/o ENRIQUE ALVAREZ**, o los apoderados que cualquiera de ellos designe, quedan especialmente facultados para representarnos ante las autoridades colombianas, administrativas, judiciales o de policía, en todos los trámites relativos a la propiedad industrial, a cuyo efecto les facultamos para dar ante dichas autoridades todos los pasos necesarios al objeto indicado; elevar solicitudes, declaraciones, reclamos, recursos y presentar desistimientos; formular descripciones, enmiendas, limitar el alcance de las solicitudes, presentar oposiciones y apelaciones; instaurar cancelaciones, renunciar total o parcialmente a los registros, abonar todos los impuestos y cuotas y efectuar todo otro pago determinado por la ley; recibir todos los documentos y valores, dando el descargo respectivo; llenar cualesquiera otros requisitos y tomar, en fin, todas las medidas que creyeran conducentes al resguardo de nuestros intereses; y en caso de producirse oposición, pasando los antecedentes a los tribunales, quedan facultados para tomar intervención como demandantes o demandados ante los jueces que sean competentes, pudiendo transar, someter a árbitros, desistir, percibir y apelar, con todas las demás facultades que resulten necesarias, y por el presente declaramos desde ahora válido y bueno cuanto dichos representantes y/o los apoderados que cualquiera de ellos nombre hicieran en nuestro beneficio.

Asimismo, facultamos a los doctores **ALICIA LLOREDA y/o GUSTAVO TAMAYO y/o IGNACIO SANTAMARIA y/o ENRIQUE ALVAREZ**, para solicitar en la Oficina de Propiedad Industrial la protocolización de este documento y de cualquier poder que cualquiera de ellos otorgue a nombre nuestro.

Dado y firmado

The undersigned, **GLAXOSMITHKLINE BIOLOGICALS S.A.**, a corporation organized and existing under the laws of Belgium.

domiciled at **rue de l'Institut 89,
B-1330 Rixensart,
Belgium**

hereby appoint, **ALICIA LLOREDA and/or GUSTAVO TAMAYO and/or IGNACIO SANTAMARIA and/or ENRIQUE ALVAREZ**, of Bogotá, Colombia, with offices in said town, Calle 72 No. 5-83, Sixth Floor, our true and legal representatives in Bogotá, with power to receive notifications and to appoint judicial or extrajudicial agents, so as to comply with paragraph 1o. of article 543 of decree 410 of 1971.

Doctors **ALICIA LLOREDA and/or GUSTAVO TAMAYO and/or IGNACIO SANTAMARIA and/or ENRIQUE ALVAREZ**, or the agents by any one of them appointed, are especially empowered to represent us before the administrative, judicial or police authorities of Colombia, in all proceedings pertaining to industrial property, for which purpose we authorize them to take before the said authorities all the necessary steps for the said end; to file petitions, declarations, claims, recourses and withdrawals, to draw up specifications; to file amendments, to limit the scope of applications, file oppositions; file cancellations total or partially waive registrations, to pay all installments, and effect all other payments prescribed by law; to receive proper receipts; to comply with all other requirements and, in short, to take all those measures which they may deem fit for the protection of our interests; and in case of opposition, where the proceedings be passed over to the courts, we authorize said agents to act on our behalf, as complainants as well as defendants; before the competent judges, with full powers to make arrangements, to submit to arbitrators, to desist, to collect, to appeal and, in short, with all such further powers as may be required, and we hereby declare from now on as valid and good all that said representatives and/or the agents by any one of them appointed may do for our benefit.

We also authorize doctors **ALICIA LLOREDA and/or GUSTAVO TAMAYO and/or IGNACIO SANTAMARIA and/or ENRIQUE ALVAREZ**, to apply for protocolization of this document and of any power of attorney by any one of them conferred in our name, before the Office of Industrial Property.

31 AUG 2004

Given and signed this


Marcus Jonathan Willant DALTON
Attorney and Authorised Official

NOTARY SHOULD EXECUTE OTHER SIDE

CERTIFICADO NOTARIAL (SOCIEDAD)

Bélgica

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Hoy de de 200 , compareció , a quien conozco y de quien me consta que firmó el anterior documento, y quien debidamente juramentado declaró y dijo que él es de GLAXOSMITHKLINE BIOLOGICALS S.A., la compañía nombrada en el documento que antecede, una sociedad debidamente organizada y existente conforme a las leyes de Bélgica, domiciliada en la ciudad de Rixensart, Bélgica, legalmente constituida para un período de duración que aún no ha terminado, que ejerce su objeto social; conforme a las leyes de que el declarante está autorizado por dicha Compañía para conferir este poder; que el sello estampado por él al pie de tal poder, en nombre y en representación de la expresada Compañía, es el sello oficial de ella; y que el objeto para el cual se confiere este instrumento está comprendido dentro del de la sociedad. El suscrito Notario ha visto los Estatutos de incorporación firmados en con fecha , y certifica que de conformidad con la legislación de dicho País, la anterior declaración juramentada es prueba suficiente de que los hechos sobre que ella versa son ciertos o verdaderos, de lo cual da fe.

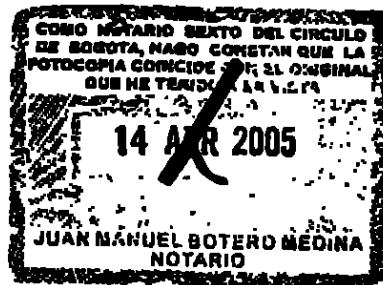
Notary Public (Notario Público).

NOTARIAL CERTIFICATE (CORPORATION)

Belgium

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On this day of 200 , personally came to me known, and known to me to be the person who signed the foregoing instrument, who being by me duly sworn did depose and say that he is the of GLAXOSMITHKLINE BIOLOGICALS S.A., the company named within, a Corporation organized and existing under the laws of the Belgium, domiciled at the City of Rixensart, Belgium, legally constituted for a period of tenure not yet expired, which exercises its corporate purposes according to the laws of , that the deponent is authorized by said Company to confer this power of attorney; that the seal affixed hereunto by the deponent in the name and on behalf of said Corporation is the corporate seal thereof; and that the purpose for which this instrument is granted is included within the corporate purposes of the Company. The undersigned Notary Public has seen the articles of Incorporation of said Corporation executed at , under date of , and hereby certifies that under the laws of said Country the foregoing acknowledgment is sufficient proof that the facts therein contained are true, which he attests.



8

		SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO DIVISION DE NUEVAS CREACIONES EXTRACTO PARA PUBLICACIÓN SOLICITUD DE PATENTE DE INVENCION PCT	
(21) N° de solicitud: 06 120 701.		(51) Int Cl: A61K 39/12	
(22) Fecha de solicitud: 29-11-06		(71) Solicitante(s) GLAXOSMITHKLINE BIOLOGICALS S.A.	
(30) Prioridad	(31) No. Prioridad	(32) Fecha	(33) País
	0413510.9	16-08-04	GB
	11/114,301	28-04-05	US
		(72) Inventor(es) DEBRUS, Serge; MARTIN, Marie-Therese; STEPHEN, Robert John; STEPHENNE, Jean y WETTENDORFF, Martine, Anne, Cécile.	
		(74) Apoderado: ALICIA LLOREDA RICAURTE	
(85)	Fecha límite inicio fase nacional: 18-01-07		(87) Publicación Internacional
(86)	Datos relativos a la presentación de la solicitud PCT		Fecha: 29-12-05
	Fecha de presentación de la solicitud: 14-08-05		N° publicación: WO 2005/123125 A1
	No. de solicitud PCT/EP2005/006461		
(54) Título: "VACUNA CONTRA HPV16 Y HPV18 Y AL MENOS OTRO TIPO DE VPH SELECCIONADO DE VPH 31, 45 O 52"			

Resumen - Reivindicación(es):

Reivindicación número 1.-

Una composición inmunogénica que comprende las PLV o cápsomeros de VPH 16 y VPH 18 y al menos otro tipo de cáncer de VPH, el otro tipo de cáncer seleccionándose entre la lista constituida por los tipos de VPH 31, 45 y 52, en la que la dosis de la PLV o capsómero de al menos otro tipo de cáncer se reduce con relación al del VPH 16 ó 18.

10

TRATADO DE COOPERACION DE PATENTES
PCT
REPORTE DE BUSQUEDA INTERNACIONAL

(PCT Artículo 18 y Reglas 43 y 44)

Registro de referencia del solicitante o agente RJS/VB60891	PARA FUTURAS ACCIONES ver Notificación o Transmisión del Reporte de Búsqueda Internacional (Forma PCT/ISA/220) así como, donde aplique, el ítem 5 a continuación.	
Solicitud internacional No. PCT/EP2005/006461	Fecha de registro internacional (día/mes/año) 14/06/2005	Fecha de Prioridad (Primera) (día/mes/año) 16/06/2004
Solicitante GLAXOSMITHKLINE BIOLOGICALS S.A.		

Este Reporte de Búsqueda Internacional ha sido preparado por esta Autoridad de Búsqueda Internacional y es transmitido al solicitante Según el Artículo 18. Una copia será transmitida a la Oficina Internacional.

Este Reporte de Búsqueda Internacional consta de un total de 6 hojas.

☒ También está acompañado de una copia de cada documento técnico anterior citado en este reporte.

1. Bases del reporte

a. Respecto al lenguaje, la búsqueda internacional se llevó a cabo según la solicitud internacional en el lenguaje en el cual se presentó, a menos que se indique de otro modo en este ítem.

☐ la búsqueda internacional se llevó a cabo según la traducción de la solicitud internacional proporcionada a esta Autoridad (Regla 23.1(b)).

b. ☐ Respecto a una secuencia de ácido nucleótico y/o amino revelado en la solicitud internacional, ver Recuadro No. I.

2. ☒ Ciertas reivindicaciones no pudieron localizarse (Ver Recuadro II).

3. ☐ Falta unidad de invención (ver Recuadro III).

4. Respecto al título,

☐ El texto es aprobado según lo presenta el solicitante.

☒ Esta Autoridad ha establecido que el texto es el siguiente:

VACUNA CONTRA HPV16 Y HPV18 Y AL MENOS OTRO TIPO DE VPH SELECCIONADO DE VPH 31, 45 O 52

5. Respecto al resumen,

☒ El texto es aprobado según lo presenta el solicitante.

☐ Esta Autoridad ha establecido el texto de acuerdo con la Norma 38.2(b), según aparece en el Recuadro III. El solicitante puede, un mes después de la fecha de haber enviado por correo este reporte de búsqueda internacional, presentar comentarios a esta Autoridad.

6. Respecto a los dibujos,

a. La figura de los dibujos que se publicará con el resumen es la Figura No. __

☐ como lo sugirió el solicitante.

☐ como lo seleccionó esta Autoridad, porque el solicitante no sugirió una figura.

☐ como lo seleccionó esta Autoridad, porque esta figura caracteriza mejor a la invención.

b. ☐ Ninguna de las figuras es para ser publicada con el resumen.

REPORTE DE BUSQUEDA INTERNACIONAL

Solicitud Internacional No.
PCT/EP2005/006461

A. CLASIFICACION DE MATERIA IPC 7 A61K39/12 A61K31/496 A61P31/00 A61P35/00 Según la Clasificación de Patentes Internacional (CPI) o según la clasificación nacional y la CPI	
B. CAMPOS BUSCADOS Documentación mínima buscada (sistema de clasificación seguido de símbolos de clasificación) IPC 7 A61K Documentación buscada fuera de documentación mínima hasta donde dichos documentos se incluyan en los campos buscados Base de datos electrónica consultada durante la búsqueda internacional (nombre de la base de datos y, donde sea práctico, buscar términos usados) EPO-Interno, BIOSIS, EMBASE, Dato WPI, PAJ	
C. DOCUMENTOS CONSIDERADOS RELEVANTES	
Categoría *	Citación de documento, con indicación, donde sea apropiado, de los textos relevantes Relevante a la reivindicación No.
P, X	GLAXOSMITHKLINE HPV VACCINE STUDY GROUP HARPER D M Y COLS: "Eficacia de una vacuna bivalente de partícula tipo virus L1 en la prevención de la infección con los tipos 16 y 18 del virus del papiloma humano en mujeres jóvenes: un ensayo aleatorio controlado" LANCET THE, LANCET LIMITED. LONDRES, GB, vol. 34, No. 9447, Noviembre 13, 2004 (2004-11-13), páginas 1757-1765, XP004746625 ISSN: 0140-6736 citado en la solicitud resumen 1-21
P, X	WO 2004/056389 A (GLAXOSMITHKLINE BIOLOGICALS SA; DUBIN, GARY; INNIS, BRUCE; SLAOU, MON) Julio 8 de 2004 (2004-07-08) tablas 1-3 1-21
☒ En el recuadro C se enumeran más documentos. ☒ En el anexo se enumeran miembros de la familia de patentes.	
* Categorías especiales de documentos citados: 'A' documento que define el estado general de la técnica el cual no se considera de relevancia particular 'E' documento anterior pero publicado en o después de la fecha de presentación internacional 'L' documento que puede arrojar dudas sobre reivindicaciones de prioridad o que se cita para establecer la fecha de publicación de otra citación u otra razón especial (según se especifica) 'O' documento que se refiere a una revelación oral, uso, exhibición u otros medios 'T' documento publicado antes de la fecha de presentación internacional pero después de la fecha de prioridad reivindicada 'T' documento posterior publicado después de la fecha de presentación internacional o fecha de prioridad y que no está en conflicto con la solicitud pero citado para entender el principio o teoría que sustenta la invención 'X' documento de relevancia particular; la invención reivindicada no puede considerarse nueva o no puede considerarse que comprende un paso inventivo cuando el documento se toma solo 'Y' documento de relevancia particular; la invención reivindicada no puede considerarse que comprende un paso inventivo cuando el documento se combina con uno o más documentos, siendo dicha combinación obvia para una persona experta en la técnica 'Z' documento miembro de la misma familia de patentes	
Fecha de la terminación real de la búsqueda internacional 8 de Noviembre de 2005	Fecha de envío por correo del reporte de búsqueda internacional 16/11/2005
Nombre y dirección del correo de ISA European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. No. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax No. (+31-70) 340-3016	Funcionario autorizado Renggli-Zulliger, N

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REPORTE DE BUSQUEDA INTERNACIONAL

Solicitud Internacional No.
PCT/EP2005/006461

C. DOCUMENTOS CONSIDERADOS RELEVANTES

Categoría *	Citación de documento, con indicación, donde sea apropiado, de los textos relevantes	Relevante a la reivindicación No.
P,X	VILLA L L Y COLS: "Vacuna de partícula tipo virus L1 del virus del papiloma humano (tipos 6, 11, 16 y 18), profiláctico tetravalente en mujeres jóvenes: un ensayo de eficacia multicentro fase II aleatorio doble-ciego placebo-controlado" LANCET ONCOLOGY, LANCET PUBLISHING GROUP, LONDRES, GB, vol. 6, No. 5, Mayo de 2005 (2005-05), páginas 271-278, XP004870902 ISSN: 1470-2045 resumen	1-21
A	BILICH A: "VACUNA VPH MEDIMMUNE/GLAXOSMITHKLINE" CURRENT OPINION IN INVESTIGATIONAL DRUGS, CURRENT DRUGS, LONDRES, GB, vol. 4, No. 2, Febrero de 2003 (2003-02), páginas 210-213, XP009029755 ISSN: 0967-8298 resumen	1-21
A	REINIS MILAN: "Evaluación de la tecnología: vacuna VPH (tetravalente), Aventis Pasteur MSD/CSL" CURRENT OPINION IN MOLECULAR THERAPEUTICS. ABR 2004, vol. 6, No. 2, Abril de 2004 (2004-04), páginas 206-211, XP009056594 ISSN: 1464-8431. el documento completo	1-21
A	WO 01/97840 A (MEDIMMUNE, INC) Diciembre 27 de 2001 (2001-12-27). resumen; figuras 1-7	1-21
A	EP 1 410 805 (GLAXOSMITHKLINE BIOLOGICALS S.A) Abril 21 de 2004 (2004-04-21) parrafos '0095! - '0104!; tabla 1	1-21
A	GIROGLOUT Y COLS: "Análisis inmunológicos de cápsidas del virus del papiloma humano" VACCINE, BUTTERWORTH SCIENTIFIC. GUILDFORD, GB, vol. 19, No. 13-14, 2001, páginas 1783-1793, XP002248440 ISSN: 0264-410X resumen	1-21
	— —/—	

REPORTE DE BUSQUEDA INTERNACIONALSolicitud Internacional No.
PCT/EP2005/006461

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C. DOCUMENTOS CONSIDERADOS RELEVANTES

Categoría *	Citación de documento, con indicación, donde sea apropiado, de los textos relevantes	Relevante a la reivindicación No.
A	RODEN R B Y COLS: "Evaluación de la relación serológica de los virus del papiloma humano genitales por inhibición de la hemoaglutinación" JOURNAL OF VIROLOGY THE AMERICAN SOCIETY FOR MICROBIOLOGY, US, vol. 70, No. 5, Mayo de 1996 (1996-05), páginas 3298-3301, XP002946729 ISSN: 0022-538X resumen —	1-21

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REPORTE DE BUSQUEDA INTERNACIONALSolicitud Internacional No.
PCT/ EP2005/006461**Casilla I Observaciones donde ciertas reivindicaciones fueron inascrutables (Continuación del punto 1 de la primera hoja)**

De conformidad con el Artículo 17.2)a), algunas reivindicaciones no fueron objeto de una búsqueda por las siguientes razones:

1. ☒ Reivindicaciones Nos.:
porque se relacionan con un asunto que no es necesario que esta Autoridad busque, a saber:

Aunque las reivindicaciones 19 y 21 están dirigidas a un método de tratamiento del cuerpo humano/animal, la búsqueda ha sido llevada a cabo y basada en los efectos alegados del compuesto/ la composición.
2. ☐ Reivindicaciones Nos.:
porque se relacionan con partes de la solicitud internacional que no cumplen con los requisitos prescritos hasta tal grado que no se puede llevar a cabo una búsqueda internacional significativa, específicamente:
3. ☐ Reivindicaciones No.:
porque son reivindicaciones dependientes y no están diseñadas de acuerdo con la segunda y tercera oración de la Norma 6.4(a).

Casilla III Observaciones donde falta unidad de invención (Continuación del punto 3 de la primera hoja)

Esta Autoridad de Búsqueda Internacional encontró invenciones múltiples en esta solicitud internacional, así:

1. ☐ Puesto que todos los derechos de búsqueda adicionales requeridos fueron pagados oportunamente por el solicitante, este informe de búsqueda internacional cubre todas las reivindicaciones investigables.
2. ☐ Puesto que todas las reivindicaciones investigables pudieron investigarse sin esfuerzo que justifique derechos adicionales, esta Autoridad no solicitó pago de derechos adicionales.
3. ☐ Puesto que sólo algunos de los derechos de búsqueda adicionales fueron pagados oportunamente por el solicitante, este informe de búsqueda internacional cubre únicamente aquellas reivindicaciones para las cuales se pagaron derechos, específicamente las reivindicaciones Nos.:
4. ☐ El solicitante no pagó oportunamente los derechos de búsqueda adicionales requeridos. Por consiguiente, este informe de búsqueda internacional se restringe a la primera invención mencionada en las reivindicaciones; está cubierto por las reivindicaciones Nos.:

Comentario sobre Protesta ☐ Los derechos de búsqueda adicionales estuvieron acompañados por la protesta del solicitante.

☐ El pago de los derechos de búsqueda adicionales no estuvo acompañado por una protesta.

REPORTE DE BUSQUEDA INTERNACIONAL
Informe sobre miembros de familia de patentes

Solicitud Internacional No.
PCT/ EP2005/006461

Documento de patente citado en el reporte de búsqueda	Fecha de publicación	Miembro(s) de la familia de patentes	Fecha de publicación
WO 2004056389. A	08-07-2004	AU 2003293942 A1 CA 2510457 A1 EP 1572233 A1	14-07-2004 08-07-2004 14-09-2005
WO 0197840 A	27-12-2001	AU 7545801 A CA 2411945 A1 EP 1292328 A1	02-01-2002 27-12-2001 19-03-2003
EP 1410805 A	21-04-2004	NONE	

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference RJS/VB60891	FOR FURTHER ACTION see Form PCT/ISA/220 as well as, where applicable, Item 5 below.	
International application No. PCT/EP2005/006461	International filing date (day/month/year) 14/06/2005	(Earliest) Priority Date (day/month/year) 16/06/2004
Applicant GLAXOSMITHKLINE BIOLOGICALS S.A.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ The international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, see Box No. I.

2. ☒ Certain claims were found unsearchable (See Box II).3. ☐ Unity of invention is lacking (see Box III).

4. With regard to the title,

☐ the text is approved as submitted by the applicant.

☒ the text has been established by this Authority to read as follows:

VACCINE AGAINST HPV16 AND HPV18 AND AT LEAST ANOTHER HPV TYPE SELECTED FROM HPV 31, 45 OR 52

5. With regard to the abstract,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the drawings,

- a. the figure of the drawings to be published with the abstract is Figure No. _____

☐ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

- b. ☐ none of the figures is to be published with the abstract.

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K39/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	GLAXOSMITHKLINE HPV VACCINE STUDY GROUP HARPER D M ET AL: "Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial" LANCET THE, LANCET LIMITED. LONDON, GB, vol. 364, no. 9447, 13 November 2004 (2004-11-13), pages 1757-1765, XP004746625 ISSN: 0140-6736 cited in the application abstract	1-21
P,X	WO 2004/056389 A (GLAXOSMITHKLINE BIOLOGICALS SA; DUBIN, GARY; INNIS, BRUCE; SLAQUI, MON) 8 July 2004 (2004-07-08) tables 1-3	1-21
-/-		

☒ Further documents are filed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document relating to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *Z* document member of the same patent family

Date of the actual completion of the international search

8 November 2005

Date of mailing of the international search report

16/11/2005

Name and mailing address of the ISA

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Fax (+31-70) 340-3016

Authorized officer

Renggli-Zulliger, N

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2005/006461

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	VILLA L L ET AL: "Prophylactic- quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial" LANCET ONCOLOGY, LANCET PUBLISHING GROUP, LONDON, GB, vol. 6, no. 5, May 2005 (2005-05), pages 271-278, XP004870902 ISSN: 1470-2045 abstract	1-21
A	BILICH A: "HPV VACCINE MEDIMUNE/GLAXOSMITHKLINE" CURRENT OPINION IN INVESTIGATIONAL DRUGS, CURRENT DRUGS, LONDON, GB, vol. 4, no. 2, February 2003 (2003-02), pages 210-213, XP009029755 ISSN: 0967-8298 abstract	1-21
A	REINIS MILAN: "Technology evaluation: HPV vaccine (quadrivalent), Aventis Pasteur MSD/CSL." CURRENT OPINION IN MOLECULAR THERAPEUTICS. APR 2004, vol. 6, no. 2, April 2004 (2004-04), pages 206-211, XP009056594 ISSN: 1464-8431 the whole document	1-21
A	WO 01/97840 A (MEDIMUNE, INC) 27 December 2001 (2001-12-27) abstract; figures 1-7	1-21
A	EP 1 410 805 A (GLAXOSMITHKLINE BIOLOGICALS S.A) 21 April 2004 (2004-04-21) paragraphs '00951 - '01041; table 1	1-21
A	GIROGLOU T ET AL: "Immunological analyses of human papillomavirus capsids" VACCINE, BUTTERWORTH SCIENTIFIC. GUILDFORD, GB, vol. 19, no. 13-14, 2001, pages 1783-1793, XP002248440 ISSN: 0264-410X abstract	1-21
	-/-	

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	RODEN R B S ET AL: "ASSESSMENT OF THE SEROLOGICAL RELATEDNESS OF GENITAL HUMAN PAPILLOMAVIRUSES BY HEMAGGLUTINATION INHIBITION" JOURNAL OF VIROLOGY, THE AMERICAN SOCIETY FOR MICROBIOLOGY, US, vol. 70, no. 5, May 1996 (1996-05), pages 3298-3301, XP002946729 ISSN: 0022-538X abstract	1-21

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2005/006461

Box I Observations where certain claims were found unsearchable (Continuation of Item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 19 and 21 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 3 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP2005/006461

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2004056389 A	08-07-2004	AU 2003293942 A1	14-07-2004
		CA 2510457 A1	08-07-2004
		EP 1572233 A1	14-09-2005
WO 0197840 A	27-12-2001	AU 7545801 A	02-01-2002
		CA 2411945 A1	27-12-2001
		EP 1292328 A1	19-03-2003
EP 1410805 A	21-04-2004	NONE	

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ANEXOS DEL REPORTE DE BUSQUEDA INTERNACIONAL

1. **Eficacia de una vacuna bivalente de partícula tipo virus L1 en la prevención de la infección con los tipos 16 y 18 del virus del papiloma humano en mujeres jóvenes: un ensayo aleatorio controlado**
2. **Vacuna de partícula tipo virus L1 del virus del papiloma humano (tipos 6, 11, 16 y 18), profiláctico tetravalente en mujeres jóvenes: un ensayo de eficacia multicentro fase II aleatorio doble-ciego placebo-controlado**
3. **Vacuna vph medimmune/glaxosmithkline**
4. **Evaluación de la tecnología: vacuna VPH (tetravalente), Aventis Pasteur MSD/CSL**
5. **Análisis inmunológicos de cápsidas del virus del papiloma humano**
6. **Evaluación de la relación serológica de los virus del papiloma humano genitales por inhibición de la hemoaglutinación**

Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial

Diane M Harper, Eduardo L Franco, Colette Wheeler, Doran G Ferris, David Jenkins, Anne Schulnd, Toufik Zahaf, Bruce Innis, Paul Naud, Newton S De Carvalho, Cr  ia M Pireli-Morins, Julia Teixeira, Mark M Blatter, Abner P Korn, Wim Quint, Gary Dubin, for the GlaxoSmithKline HPV Vaccine Study Group*

Summary

Background Vaccination against the most common oncogenic human papillomavirus (HPV) types, HPV-16 and HPV-18, could prevent development of up to 70% of cervical cancers worldwide. We did a randomised, double-blind, controlled trial to assess the efficacy, safety, and immunogenicity of a bivalent HPV-16/18 L1 virus-like particle vaccine for the prevention of incident and persistent infection with these two virus types, associated cervical cytological abnormalities, and precancerous lesions.

Methods We randomised 1113 women between 15–25 years of age to receive three doses of either the vaccine formulated with AS04 adjuvant or placebo on a 0 month, 1 month, and 6 month schedule in North America and Brazil. Women were assessed for HPV infection by cervical cytology and self-obtained cervicovaginal samples for up to 27 months, and for vaccine safety and immunogenicity.

Findings In the according-to-protocol analyses, vaccine efficacy was 91.6% (95% CI 64.5–98.0) against incident infection and 100% against persistent infection (47.0–100) with HPV-16/18. In the intention-to-treat analyses, vaccine efficacy was 95.1% (63.5–99.3) against persistent cervical infection with HPV-16/18 and 92.9% (70.0–98.3) against cytological abnormalities associated with HPV-16/18 infection. The vaccine was generally safe, well tolerated, and highly immunogenic.

Interpretation The bivalent HPV vaccine was efficacious in prevention of incident and persistent cervical infections with HPV-16 and HPV-18, and associated cytological abnormalities and lesions. Vaccination against such infections could substantially reduce incidence of cervical cancer.

Introduction

Cervical cancer is the most important manifestation of genital human papillomavirus (HPV) infection and is one of the leading causes of cancer mortality in women worldwide. The global disease burden of cervical cancer is estimated at 470 000 new cases and 230 000 deaths every year; almost 80% of the cases occur in developing countries, where in many regions it is the most common cancer among women.^{1,2} Cervical cancer is also the leading cause of years-of-life-lost in women in south central Asia, Latin America, and sub-Saharan Africa, results in a greater reduction of a woman's life expectancy compared with AIDS, tuberculosis, or maternal conditions in Latin America and Europe.³

The causal role of some high-risk HPV types in cervical carcinogenesis has now been clearly established by studies that take into account the many molecular, epidemiological, virological, cytological, and histological complexities of the disease's natural history. Molecular studies show high-risk HPV DNA has been detected in 99.7% of an international series of cervical cancers with highly sensitive PCR, and, in 100% of cases, confirmed by expert histological review.⁴

The odds ratio for cervical cancer associated with high-risk HPV infection has been estimated as greater than

150 in case-control studies.⁴ Findings from case-control studies and cohort studies together with laboratory evidence of HPV oncogenic expression, have established that persistent infection with high-risk HPV types is the necessary cause of cervical cancer.^{5,6} The most prevalent HPV types associated with cervical cancer are HPV-16 and HPV-18; HPV-16 accounts for more than 60% of cervical cancers, with HPV-18 adding about another 10%.^{1,7,8}

HPV vaccines based on L1 virus-like particles have shown promise in protecting against infection and development of lesions.^{9,10} Recently, a monovalent HPV-16 virus-like particle vaccine showed protection against persistent infection with HPV-16 and its associated cervical intraepithelial neoplasia (CIN).¹¹ These data suggest that L1 virus-like particle vaccines have the potential to reduce worldwide cervical cancer rates.

We did a double blind, multi-centre, randomised, placebo-controlled clinical trial to assess the efficacy of a bivalent HPV-16/18 virus-like particle vaccine against incident and persistent infections with HPV-16 and HPV-18. We also assessed vaccine efficacy against cytological abnormalities and CIN, and vaccine immunogenicity, safety, and tolerability.

Received 2004; 1564–1757–04

*Contributors listed at end of paper

Departments of Obstetrics and Gynecology and Community and Family Medicine, Norris Cotton Cancer Center, Dartmouth Medical School, Hanover, NH, USA (D M Harper MD); Department of Oncology and Epidemiology, McGill University, Montreal, Canada (E L Franco PhD); Departments of Molecular Genetics and Microbiology and Obstetrics and Gynecology, University of New Mexico Health Sciences Center, Albuquerque, NM, USA (C Wheeler PhD); Department of Family Medicine and Obstetrics and Gynecology, Medical College of Georgia, Augusta, GA, USA (D G Ferris MD); GlaxoSmithKline Biologicals, Macclesfield, Cheshire, UK (D Jenkins MD); GlaxoSmithKline Biologicals, King of Prussia, PA, USA (A Schulnd MD); GlaxoSmithKline Biologicals, G.D. Searle & Co., Kenilworth, NJ, USA (W Quint PhD); Department of Obstetrics and Gynecology, GlaxoSmithKline Biologicals, University of Pennsylvania, Philadelphia, PA, USA (N S De Carvalho MD); UN de Buenos Aires Hospital, San Paulo, Brazil (C M Pireli-Morins MD); Department of Gynecology, Oncology Division—CABIS, State University of Campinas, Campinas, Brazil (J Teixeira MD); Palmyra Physicians Research, Pittsburgh, PA, USA (M M Blatter MD); Department of Gynecology, University of California at San Francisco, San Francisco, CA, USA (A P Korn MD); and GlaxoSmithKline Biologicals, Delft, Netherlands (G Dubin PhD)

Correspondence to: Dr Diane M Harper, Norris Cotton Cancer Center, Dartmouth Medical School, Gynecologic Cancer Prevention Research Group, Room 503, One Medical Center Drive, Lebanon, NH 03756, USA (diane.m.harper@dartmouth.edu)

Methods

Study objectives and participants

The primary objective of this study was to assess vaccine efficacy in the prevention of infection with HPV-16, HPV-18, or both (HPV-16/18), between months 6 and 18 in participants who were initially shown to be seronegative for HPV-16/18 by ELISA and negative for HPV-16/18 DNA by PCR. Secondary objectives included: evaluation of vaccine efficacy in the prevention of persistent infection with HPV-16/18, and the evaluation of vaccine efficacy in the prevention of cytologically confirmed low-grade squamous intra-epithelial lesions (LSIL), high-grade squamous intra-epithelial lesions (HSIL), and histologically confirmed LSIL (CIN 1), HSIL (CIN 2 or 3) squamous cell cancer, or adenocarcinoma associated with HPV-16/18 infection between months 6 and 18, and months 6 and 27. The prevention of atypical squamous cells of undetermined significance (ASCUS) cytology associated with HPV-16/18 infection was added post-hoc to the outcome analyses.

We also did an exploratory analysis of the histopathological endpoints CIN 1 and 2 associated with HPV-16/18 DNA detected by PCR in lesional tissue. Other objectives included the assessment of vaccine immunogenicity, safety, and tolerability.

Investigators in North America (Canada and the USA) and Brazil recruited women for this efficacy study through advertisements or previous participation in an HPV cross-sectional epidemiology study that took place between July and December, 2000.

For each of the 32 study sites, an institutional review board approved the protocol, consent forms, and amendments. Women signed separate written consents for study participation and colposcopy. For those under 18 years, parental consent and assent from the participant were obligatory.

There were two study phases: an initial phase for vaccination and follow-up that concluded at month 18; and a blinded follow-up extension phase that concluded at month 27.

Women eligible for the initial phase (months 0–18) included healthy women aged 15–25 years, who had had no more than six sexual partners, no history of an abnormal Pap test or ablative or excisional treatment of the cervix, and no ongoing treatment for external condylomata; and who were cytologically negative, seronegative for HPV-16 and HPV-18 antibodies by ELISA, and HPV-DNA-negative by PCR for 14 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) no more than 90 days before study entry.

Women who completed the initial phase of the study earliest, and who did not have ablative or excisional therapy of the cervix, or hysterectomy after enrolment, were eligible to participate in the extension phase of the study (months 18–27).

Procedures

Each dose of the bivalent HPV-16/18 virus-like particle vaccine (GlaxoSmithKline Biologicals, Rixensart, Belgium) contained 20 µg of HPV-16 L1 virus-like particle and 20 µg of HPV-18 L1 virus-like particle. Each type of virus-like particle was produced on *Spodoptera frugiperda* Sf-9 and *Trichoplusia ni* Hi-5 cell substrate with AS04 adjuvant containing 500 µg aluminum hydroxide and 50 µg 3-deacylated monophosphoryl lipid A (MPL, Corixa, Montana, USA) provided in a monodose vial. The placebo contained 500 µg of aluminum hydroxide per dose, and was identical in appearance to the HPV-16/18 vaccine. Every study participant received a 0.5 mL dose of vaccine or placebo at 0 months, 1 month, and 6 months.

Health-care providers obtained cervical specimens with a cervical brush and spatula (washed in PreservCyt, Cytec Corporation, Boxborough, MA, USA) for cytology and HPV DNA testing at screening and months 6, 12, and 18. At months 0 and 6, and subsequently every 3 months, women self-obtained cervicovaginal samples with two sequential swabs (placed in PreservCyt) for HPV DNA testing.²⁰ A central laboratory (Quest Diagnostics, Teterboro, NJ, USA) reported cytology results (ThinPrep, Cytec Corporation) by use of the 1991 Bethesda classification system.

Protocol guidelines recommended colposcopy after two reports of ASCUS, or one report of atypical glandular cells of undetermined significance, LSIL, or HSIL, squamous cell carcinoma, adenocarcinoma in situ, or adenocarcinoma. These guidelines also recommended biopsy for any suspected lesions.

The central histology laboratory made an initial diagnosis from the formalin-fixed tissue specimens for clinical management. A panel of three pathologists made a subsequent consensus diagnosis for HPV-16 and HPV-18 associated lesions with the CIN system. This consensus diagnosis also included review of the sections taken at the time of microdissection for PCR detection of lesional HPV DNA.

HPV DNA isolated from the cytology specimen (MagNaPure Total Nucleic Acid system, Roche Diagnostics, Almere, Netherlands) and from the cervical biopsy specimen (proteinase K extraction) was amplified from an aliquot of purified total DNA with the SPF10 broad-spectrum primers that amplify a 65 bp region of the L1 gene.^{21,22} The amplification products were detected by a DNA enzyme immunoassay. A line probe assay (LIPA Kit HPV INNO LIPA HPV genotyping assay, SPF-10 system version 1, Innogenetics, Gent, Belgium, manufactured by Labo Bio-medical Products, Rijswijk, Netherlands) detected 25 HPV genotypes (6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, and 74).²³ Any specimen that was positive by DNA enzyme immunoassay was tested by type-specific HPV-16 and HPV-18 PCR. HPV-16 type-specific PCR primers amplified a 92 bp segment of the E6/E7 gene

and HPV-18 type-specific PCR primers amplified a 126 bp segment of the L1 gene.⁴⁴

We defined incident cervical infection with HPV-16/18 as at least one positive PCR result for HPV-16 or HPV-18 during the trial, and persistent infection with HPV-16/18 as at least two positive HPV-DNA PCR assays for the same viral genotype separated by at least 6 months.⁴⁴ HPV-DNA test results were concealed from investigators during the study and cytological and histological diagnoses were only revealed for clinical management purposes. Analyses included HPV-16/18 DNA results for cervical specimens and combined cervical and self-obtained cervicovaginal specimens.

We collected serum from study participants at months 0, 1, 6, 7, 12, and 18 for assessment of immunogenicity. Serological testing for antibodies to HPV-16 and HPV-18 virus-like particles was by ELISA. Recombinant HPV-16 or HPV-18 virus-like particles were used as coating antigens for antibody detection (see webappendix <http://image.thelancet.com/extras/04art10103webappendix.pdf>). Seropositivity was defined as a titre greater than or equal to the assay cut-off titre established at 8 ELISA units/mL for HPV-16 and 7 ELISA units/mL for HPV-18. Typical natural titres were determined by use of blood samples obtained from women in the preceding epidemiology study who were found to be seropositive for HPV-16 or HPV-18 by ELISA.

Women recorded symptoms experienced during the first 7 days after vaccination on diary cards with a three-grade scale of symptom intensity. Additionally, they reported to study personnel by interview all adverse events within the first 30 days after vaccination. Information on serious adverse events and pregnancies was collected throughout the study.

Statistical methods

Assuming a 6% cumulative incidence rate of both HPV-16 and HPV-18 type infections over 12 months, we estimated that 500 women per treatment group would provide 80% power to assess a lower limit of the 95% CI of the vaccine efficacy above zero. We assumed an 80% retention rate over 18 months. Interim analyses for efficacy, safety, and immunogenicity were done for future study planning purposes only; the O'Brien and Fleming method was used to adjust the α value for the final analysis after interim analyses occurred (overall $\alpha=0.05$; two-sided test).⁴⁵

Stratified, block randomisation according to validated algorithms was centralised with an internet randomisation system. Stratification was according to age (15–17, 18–21, and 22–25 years) and region (North America and Brazil). Each vaccine dose was attributed a randomly chosen number based on specific participant information entered into the computerised randomisation system by study personnel. Treatment allocation remains concealed from investigators and the women participating in a long-term follow-up study.

The intention-to-treat and according-to-protocol cohorts are shown in the figure, in which the reasons for exclusion from analyses are listed in rank order: women who met more than one exclusion criterion were only counted once according to the highest ranking criterion. We refer to the sets of participants entered in the intention-to-treat and according-to-protocol analyses as cohorts, although the information used to restrict subject inclusion in the according-to-protocol was only known after follow-up.

We did both according-to-protocol and intention-to-treat analyses for efficacy. Calculation of vaccine efficacy in the according-to-protocol 18-month analysis was based on the proportion of participants with HPV-16/18 infection in the vaccinated versus placebo groups. Vaccine efficacy was defined as 1 minus the ratio between these two proportions; 95% CIs measured the precision of the efficacy estimates. *p* values were calculated with the two-sided Fisher's exact test. Corresponding rates were expressed as the numbers of cases with the outcome divided by the numbers of participants at risk. The according-to-protocol 18-month cohort included enrolled women who received three scheduled doses of vaccine and complied with the protocol as described in the figure.

Calculation of vaccine efficacy in the intention-to-treat and according-to-protocol 27-month analyses was based on the Cox proportional hazard model using the time-to-occurrence of cases with HPV-16/18 infection in the vaccinated versus placebo groups. This allowed controlling for the accrued person-time data in each group. Vaccine efficacy was calculated using 1 minus the hazard ratio and *p* values calculated using the log rank test. Corresponding rates were expressed as the number of cases divided by the total person-time. All enrolled women who received at least one dose of vaccine or placebo, were negative for high-risk HPV-DNA at month 0, and had any data available for outcome measurement were included in the intention-to-treat cohort. The according-to-protocol 27-month cohort included outcome results from the according-to-protocol 18-month cohort and results that occurred during the extension phase (from 18 months to 27 months).

Calculation of *p* values for the safety analysis was performed using Fisher's exact test comparisons. The cohort for safety analysis included all enrolled women who received at least one dose of vaccine or placebo and complied with specified, minimal protocol requirements (figure).

Immunogenicity was assessed in a subset of the according-to-protocol safety cohort, which included women with serology results at months 0, 7, and 18, who received all three doses of study vaccine or placebo according to schedule, complied with the blood sampling schedule, and did not become positive for HPV-16/18-DNA during the trial (figure). Seropositivity rates between the vaccine and placebo groups were compared with Fisher's exact test

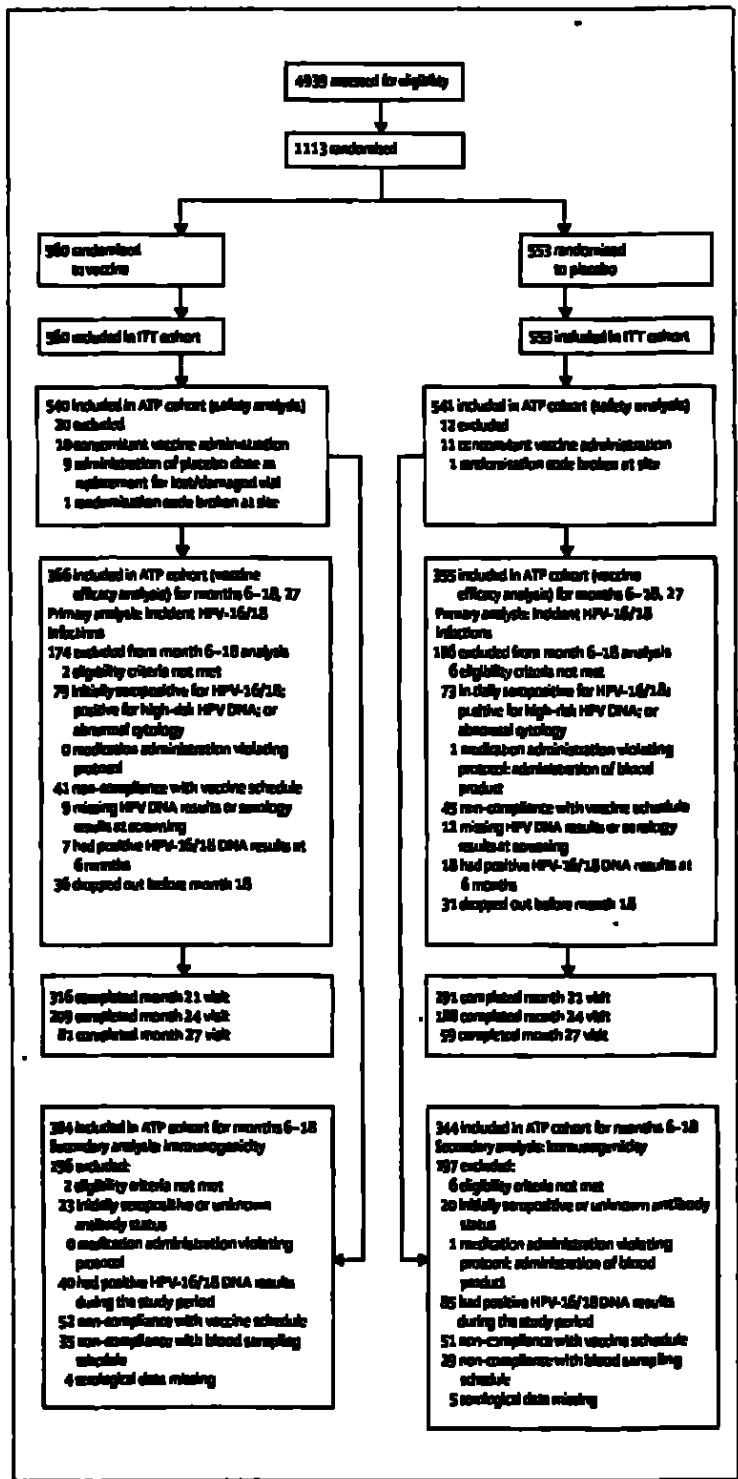


Figure 1: Trial profile

	ITT cohort*		ATP cohort*	
	Vaccine group (n=360)	Placebo group (n=553)	Vaccine group (n=366)	Placebo group (n=541)
Age (years)	20.4 (2.8)	20.5 (2.7)	20.1 (2.5)	20.5 (2.8)
Region				
North America†	303 (84.0%)	305 (55.2%)	181 (49.5%)	181 (51.3%)
South America†	258 (46.0%)	248 (44.8%)	185 (50.5%)	273 (49.7%)
Ethnic group				
White	389 (89.5%)	384 (69.4%)	244 (66.7%)	249 (70.1%)
Black	43 (7.7%)	41 (7.4%)	30 (8.2%)	31 (6.3%)
Asian	9 (2.4%)	4 (0.7%)	9 (2.5%)	4 (1.1%)
Other	119 (31.3%)	124 (22.4%)	83 (22.7%)	80 (23.5%)

Data are mean (SD) for age and number (N) for other values. Percentages may not add up to 100% exactly due to rounding. ITT = Intention-to-Treat; ATP = according to protocol (18 month cohort for efficacy). *The demographic distribution between groups within cohorts or between cohorts (Fisher's exact test). †North America and the USA.

Table 1: Demographic characteristics by analysis cohort and treatment group

($p < 0.001$ judged significant). Geometric mean titres were compared with ANOVA and Kruskal-Wallis test. Block randomisation and statistical analyses were done with SAS version 8.2 (SAS Institute, Cary, North Carolina).

Role of the funding source

This study was conceived jointly by GlaxoSmithKline Biologicals and consultants, some of whom also served as investigators. GlaxoSmithKline Biologicals funded and coordinated this study. A publication steering committee was assembled to represent all members of the HPV Vaccine Study group who collected data for the study and cared for the study patients. GlaxoSmithKline Biologicals did all HPV serological testing. Quest Diagnostics processed all cytology and histology specimens, and Delft Diagnostic Laboratory did PCR for HPV types. An independent external statistician worked separately on data analyses to maintain allocation concealment and the sponsor drafted the clinical study report for regulatory purposes with these data provided. The corresponding author had full access to the data and had final responsibility for submission for publication.

	Vaccine group (n=366)	Placebo group (n=553)
Compliance with vaccinations*		
Dose 1	360 (100.0%)	553 (100.0%)
Dose 2	349 (95.4%)	536 (96.9%)
Dose 3	343 (93.4%)	513 (92.8%)
Study visit completion		
Complete in month 18	480 (85.7%)	478 (86.4%)
Dropout†	80 (14.3%)	75 (13.6%)
Women lost to follow-up		
With complete vaccination course	18 (3.3%)	14 (4.3%)
With incomplete vaccination course	25 (2.7%)	11 (2.0%)

*Number of women receiving each dose (% doses received; other data on number (%)). †Women who did not come for the visit at month 18.

Table 2: Study compliance characteristics for women included in the Intention-to-Treat cohort

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	HPV type	Follow-up (months)	Vaccine group			Placebo group			Vaccine efficacy (95%CI)	p
			Cases	Episodes	Rate	Cases	Episodes	Rate		
ATP cohort (vaccine n=355, placebo n=355)										
Cervical samples	16	18	0	0	0.0%	18	25	5.1%	100% (79.4 to 100.0)	<0.0001
	18	18	1	1	0.6%	7	7	2.0%	73.3% (32.5 to 93.4)	0.102
	16/18	18	1	1	0.6%	23	30	6.5%	91.6% (64.5 to 98.0)	<0.0001
Combined cervicovaginal and cervical samples	16	27	6	6	0.9%	28	77	4.6%	81.3% (54.8 to 92.2)	<0.0001
	18	27	6	6	0.9%	16	27	2.3%	65.1% (30.8 to 85.2)	0.001
	16/18	27	12	12	1.8%	41	97	6.6%	73.6% (49.7 to 86.1)	<0.0001
ITT cohort (vaccine n=554, placebo n=553)										
Cervical samples	16	27	5	7	0.7%	30	50	4.6%	82.7% (55.2 to 93.3)	<0.0001
	18	27	3	3	0.4%	17	13	2.6%	82.1% (58.6 to 94.7)	0.102
	16/18	27	7	9	1.0%	42	65	6.5%	83.0% (62.0 to 92.4)	<0.0001
Combined cervicovaginal and cervical samples	16	27	14	14	1.7%	53	154	6.9%	75.3% (53.3 to 86.2)	<0.0001
	18	27	13	18	1.6%	31	67	3.9%	58.5% (29.7 to 78.8)	0.005
	16/18	27	25	39	3.1%	73	195	9.5%	62.6% (40.9 to 79.4)	<0.0001
See Methods for calculations of vaccine efficacy, corresponding rates, and p values. Cases=number of women; ITT=intention-to-treat; ATP=according-to-protocol; HPV-16/18 refers to single instances of HPV 16 or HPV 18 or HPV 16/18 infection or combined infections regardless of other HPV types over a minimum period of 6 months; NC=not cases reported; vaccine efficacy not estimated.										
Table 2: Vaccine efficacy for incident HPV-16/18 infections										

Results

Based on the estimate of appropriate study sample size, 1113 women were enrolled and randomized. We administered the study vaccine to 560 women and the placebo to 553 women (figure). The average age of enrolled women was 20 years (SD 3). The demographic characteristics were similar between the vaccine and placebo groups in North America and Brazil (table 1: webtable 1 at <http://image.thelancet.com/extras/04art10103webtable1.pdf>). We noted similar patterns of risk factors for HPV acquisition among women in each treatment group: about half of the women were current smokers, a large proportion had between two and five previous sexual partners, and most began sexual activity between 15 years and 19 years of age (see webtable 2 at <http://image.thelancet.com/extras/04art10103webtable2.pdf>).

Study compliance is shown in table 2. The major reasons for elimination from according-to-protocol

efficacy analysis were abnormal cytology, high-risk HPV DNA positivity, or seropositivity for HPV-16 or HPV-18 at enrolment; followed by non-compliance with the vaccine schedule and drop-out from the study up to month 18 (figure, table 2). 958 women (85%) completed the initial phase to month 18, with similar proportions of women from the vaccine and placebo dropping out of the study (table 2).

Analyses to assess our primary objective showed significant vaccine efficacy against incident HPV-16 and HPV-16/18 infections (table 3). In the according-to-protocol 18-month cohort, vaccine efficacy against incident HPV-18 infection was not statistically significant; however, in the intention-to-treat cohort, vaccine efficacy was significant (analysis of cervical samples only).

We noted 100% vaccine efficacy in the according-to-protocol cohorts against persistent HPV-16 and HPV-16/18 infections detected in both cervical and combined cervical and cervicovaginal samples (table 4). Although no

	HPV type	Follow-up (months)	Vaccine group		Placebo group		Vaccine efficacy (95% CI)	p
			Cases	Rate	Cases	Rate		
ATP cohort (vaccine n=355, placebo n=355)								
Cervical samples	16	18	0	0.0%	7	2.0%	100% (47.0-100)	0.007
	18	18	0	0.0%	0	0.0%	NC	-
	16/18	18	0	0.0%	7	2.0%	100% (47.0-100)	0.007
Combined cervicovaginal and cervical samples	16	27	0	0.0%	13	2.1%	100% (71.5-100)	0.0002
	18	27	0	0.0%	4	0.6%	100% (7.2-100)	0.000
	16/18	27	0	0.0%	16	2.6%	100% (76.8-100)	<0.0001
ITT cohort (vaccine n=554, placebo n=553)								
Cervical samples	16	27	1	0.1%	16	2.9%	93.9% (33.2-99.3)	0.0002
	18	27	0	0.0%	5	0.9%	100% (24.4-100)	0.025
	16/18	27	1	0.1%	20	3.1%	95.1% (63.5-99.3)	<0.0001
Combined cervicovaginal and cervical samples	16	27	4	0.5%	25	3.2%	84.5% (55.2-94.6)	<0.0001
	18	27	1	0.1%	11	1.9%	91.1% (31.0-98.6)	0.003
	16/18	27	4	0.5%	31	4.0%	87.5% (64.6-95.6)	<0.0001
See Methods for calculations of vaccine efficacy, corresponding rates, and p values. Cases=number of women; ITT=intention-to-treat; ATP=according-to-protocol; HPV-16/18 refers to two or more instances of HPV 16 or HPV 18 infection or combined infections regardless of other HPV types over a minimum period of 6 months; NC=not cases reported; vaccine efficacy not estimated.								
Table 4: Vaccine efficacy for persistent HPV16/18 infections								

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HPV type associated with ≥ASCUS	Vaccine group (n=564)			Placebo group (n=553)			Vaccine efficacy (95%CI)	P
	Cases	Episodes	Rate	Cases	Episodes	Rate		
16	1	1	0.2%	20	26	3.6%	95.2% (64.8-99.4)	<0.0001
18	1	1	0.2%	11	12	2.0%	91.2% (31.7-98.4)	0.002
16/18	2	3	0.4%	27	33	4.9%	92.9% (70.8-98.2)	<0.0001

See Methods for calculations of vaccine efficacy, corresponding rates, and p values. ITT = intention-to-treat. HPV 16/18 refers to instances of HPV-16 and/or HPV-18 as single or combined infections at the time of abnormal cervical smear. HPV-16 refers to the presence of HPV-16 irrespective of other HPV types present, and HPV-18 indicates the presence of HPV-18 irrespective of other HPV types present. ≥ASCUS includes women who had HPV-16/18-associated ASCUS, LSIL, or HSIL cytology reports. 1 provides number of times abnormal cytology was detected. Cases = number of women.

Table 5: Vaccine efficacy in preventing cytological abnormalities associated with HPV-16/18 infection (intention-to-treat cohort)

persistent HPV-18 infections were detected in cervical samples from the according-to-protocol 18-month cohort, significant vaccine efficacy against persistent HPV-18 infection was shown in the 27-month cohort for combined cervical and cervicovaginal samples. Additionally, significant vaccine efficacy against persistent HPV-16 and HPV-16/18 infections was noted in the intention-to-treat cohort in analyses of cervical samples only and of combined cervical and cervicovaginal samples.

As shown in table 5, 27 women in the placebo group and two in the vaccine group had HPV-16 and/or HPV-18 associated cytological abnormalities (vaccine group: one woman with ASCUS, and one with LSIL). 15 ASCUS,

14 LSIL, and one HSIL were reported in the placebo group and one ASCUS, two LSIL, and no HSIL in the vaccine group. Vaccine efficacy in the according-to-protocol cohort was 93.5% (95% CI 51.3-99.1; p=0.0002).

We also assessed women with histologically confirmed CIN 1 or 2 lesions, with HPV-16 or HPV-18 infection detected in the cytology specimen before colposcopy. Overall, seven women (six in the placebo group and one in the vaccine group), developed these lesions (table 6).

One woman in the vaccine group first tested positive for HPV-51 and HPV-56 at month 9. Her cytology specimen at month 12 tested positive for HPV types 18, 51, and 56. The CIN 1 lesion found in the colposcopically-directed

Findings by sample type		Sample collection time point (months)							
		6	9	12	15	18	21	24	27
1 (Vaccine)	Cytology/biopsy	-	-	LSIL/CIN 1	-	-	-	no	-
	HPV-16, 18 (c/c)	-	-	NR/18	-	-	-	-	-
	Other HR HPV (c/c)	-	51, 56/no	51, 56/51, 56	51/no	-	51/no	-	-
	Tissue (all HR HPV types)	-	-	51	-	-	-	-	-
2 (Placebo)	Cytology/biopsy	-	-	ASCUS	-	LSIL/CIN 1	-	-	no
	HPV-16, 18 (c/c)	-	-	16/16	16/no	16/16	16/no	16/no	-
	Other HR HPV (c/c)	-	-	-	-	-	-	-	-
	Tissue (all HR HPV types)	-	-	-	-	16	-	-	-
3 (Placebo)	Cytology/biopsy	-	-	-	-	LSIL	CIN 1	-	-
	HPV-16, 18 (c/c)	-	-	-	16/no	16/16	16/no	16/no	16/16
	Other HR HPV (c/c)	38/39	38/no	39/39	-	-	6/no	-	-
	Tissue (all HR HPV types)	-	-	-	-	16	-	-	-
4 (Placebo)	Cytology/biopsy	-	-	-	-	LSIL/CIN 1	no	no	no
	HPV-16, 18 (c/c)	-	-	16/16	16/no	16/16	-	-	-
	Other HR HPV (c/c)	-	-	58/58	38, 58/no	58/58	-	-	-
	Tissue (all HR HPV types)	-	-	-	-	16, 58	-	-	-
5 (Placebo)	Cytology/biopsy	-	-	-	-	LSIL/CIN 2	-	no	no
	HPV-16, 18 (c/c)	-	16/no	no/16	16/no	16	16/no	-	-
	Other HR HPV (c/c)	-	-	-	-	-	-	-	-
	Tissue (all HR HPV types)	-	-	-	-	16	-	-	-
6 (Placebo)	Cytology/biopsy	-	-	ASCUS	-	LSIL/CIN 1	-	-	-
	HPV-16, 18 (c/c)	16/16	16/no	16/16	16/no	16/16	16/no	-	-
	Other HR HPV (c/c)	-	-	-	39/no	58, 74/39	38, 58, 74/no	51, 74/no	51, 74/no
	Tissue (all HR HPV types)	-	-	-	-	16	-	-	-
7 (Placebo)	Cytology/biopsy	-	-	HSIL/CIN 2	-	-	-	-	no
	HPV-16, 18 (c/c)	16/16	16	16/16	16/no	-	-	-	-
	Other HR HPV (c/c)	66, 68/66	66/no	-	-	38, 66/38, 66	98/no	66/no	-
	Tissue (all HR HPV types)	-	-	16	-	-	-	-	-

Numbers are HPV types. Only positive HPV detection and abnormal cytology or histology results are shown. Months with no results indicate negative HPV results. Cytology groups (no) were obtained before the biopsy samples within the same time window. When the cytology and histology results are shown in the same time window, the cytology result preceded the biopsy. For cytology/biopsy findings, / separates cytology (left) from histology (right) results. For viral findings, / separates results based on self-obtained cervicovaginal samples (no) or cervical cytology specimens (y). Women with abnormal cytology at month 18 might have had cervical sample obtained by physician at a subsequent visit. Tissue refers to the colposcopically directed biopsy sample. HR HPV=high risk HPV. NR=no detected, no=not available or sample not obtained at timepoint. *HPV-59 was detected by a different detection method than that used for the other high risk HPV types (DNA sequencing).

Table 6: Timeline of virological, cytological, and histological findings among the seven women with HPV-16/18-associated abnormal cytology that was histologically confirmed as CIN (intention-to-treat cohort)

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biopsy contained only HPV-51, and not HPV-18. Subsequent HPV testing showed persistent HPV-51 through month 21; therefore, the lesion was finally judged to be associated with HPV-51.

Three women who received placebo developed CIN 1: all had ASCUS or LSIL cytology and a pattern of persistent HPV-16 infection. HPV-16 was detected in the lesions of all three women. Two women remained HPV-16 positive after biopsy and the third woman had a persistent HPV-58 infection in addition to persistent HPV-16 infection.

Consensus diagnosis confirmed CIN 2 diagnoses in three other women in the placebo group with preceding ASCUS, LSIL, and HSIL cytological abnormalities. In each case, HPV-16 was detected in all lesions and persistent HPV-16 infections preceded the lesions.

No serious adverse events related to vaccination occurred in either vaccine or placebo groups. The vaccine group had more injection site symptoms (pain, swelling, redness) than the placebo group (table 7; overall injection site symptoms 5.9% difference between proportions, 95% CI 2.1–10.1, Fisher's exact test), but these symptoms tended to be transient and mild. The difference in incidence of injection site reactions between the groups had no effect on compliance with completion of the vaccination course (table 2). The general symptoms of fatigue, gastrointestinal complaints, headache, itching, and rash were equally common in both the vaccine and placebo groups. Three women in the placebo group dropped out because of non-serious adverse events; one woman in the vaccine group dropped out because of a serious adverse event (spontaneous abortion) that was not related to vaccination (figure, table 7).

Among the vaccinated women in the according-to-protocol cohort from month 0 to month 7, 100% seroconverted to HPV-16-positive and 99.7% seroconverted to HPV-18-positive after three doses of vaccine (table 8). By 18 months, 100% of the women had seroconverted for both HPV-16 and HPV-18. Comparison of GMTs at month 7 between vaccine and placebo groups for each antibody type was significant ($p < 0.0001$).

Geometric mean titres for naturally occurring infections were 50 ELISA units/mL (SD 0.5, 95% CI 40.9–60.4) for antibodies against HPV-16 and 41 ELISA units/mL (0.5, 34.2–49.0) for antibodies against HPV-18. Geometric mean titres for vaccine-induced antibodies to HPV antibodies were over 80 and 100 times greater than those seen in natural infections with HPV-18 and HPV-16, respectively. Vaccine-induced titres remained substantially raised at 18 months, and were still 10–16 times higher than those seen in women with natural HPV-16 or HPV-18 infections, respectively.

Discussion

The results of this trial show that the bivalent HPV-16/18 virus-like particle vaccine was highly efficacious in preventing incident and persistent

	Vaccine group (n=531)	Placebo group (n=538)	p
Serious adverse events			
Related to vaccination	0	0	-
During study*	22 (4.0%)	19 (3.5%)	0.636
Injection site symptoms†			
Pain	405 (93.4%)	469 (87.2%)	0.0006
Swelling	187 (34.9%)	113 (21.0%)	<0.0001
Redness	189 (35.4%)	131 (24.3%)	0.0004
Overall‡	409 (94.0%)	472 (87.7%)	0.0004
General symptoms			
Fatigue	308 (56.0%)	289 (53.7%)	0.175
Gastrointestinal	178 (33.5%)	173 (32.0%)	0.602
Headache	331 (62.3%)	329 (61.2%)	0.706
Itching	130 (24.5%)	309 (57.3%)	0.106
Rash	60 (11.3%)	54 (10.0%)	0.352
Relaxed temperature§	88 (16.6%)	73 (13.6%)	0.172
Overall	458 (86.3%)	463 (86.3%)	0.860
Withdrawal from study			
Due to non-serious adverse event	0	3 (0.6%)	0.248
Due to serious adverse event	1 (0.1%)	0	0.487

Rate represent women in the according-to-protocol safety cohort who returned a diary card or reported an unsolicited symptom. Data are number (%) of participants. *Participants who reported a serious adverse event during the entire study period (months 0–77). †Participants who reported a specified symptom within 7 days of vaccine injection. ‡Sum of participants who presented with at least one type of symptom within 30 days of vaccine injection. §Shedding on oral temperature > 37.5°C; no temperature of > 39.0°C were reported.

Table 7: Adverse events after any vaccine dose in according-to-protocol cohort for safety

HPV-16/18 infection in fully vaccinated healthy young women. The vaccine was also highly efficacious in the broader group of women, including those who did not fully comply with the protocol.

The incidence of HPV-16 infection in the placebo group was sufficient to detect significant differences compared with the vaccine arm in all cohorts. However, the incidence of HPV-18 was much lower, as noted in other studies.¹⁶ Nonetheless, significant results for vaccine efficacy against HPV-18 were obtained in the intention-to-treat cohort, where there were sufficient numbers of events for analysis. We conclude that the bivalent vaccine shows high efficacy against both incident and persistent HPV-16 and HPV-18 infections.

Incident HPV infection was common in young women in this study, as noted in previous cohort studies.¹⁷ Because we used two PCR methods (broad-primer testing and type-specific testing) designed for maximum sensitivity to detect HPV-16/18, many incident infections were detected only on one occasion, particularly in cervicovaginal samples. It is likely that this single timepoint detection represents the presence of very small amounts of HPV DNA, possibly as a result of HPV presence not related to active infection or very low-grade transient infection.¹⁸

We provide evidence for the close relation between the development of persistent HPV infection and the concomitant development of cytological abnormalities, followed by the detection of CIN from biopsy. These results are consistent with those of previous epidemiological cohort studies of the natural history of HPV infection. Compared with the subjective readings of

	Time	n	Total number of immunopositive individuals (%; 95% CI)	Geometric mean titre in ELISA units/ml (95% CI)
Anti-HPV-16				
Vaccine group	Enrollment	357	0 (0%, 0.0-1.0)	40 (4.0-4.0)
	Month 7	351	351 (100.0%, 99.0-100.0)	53345 (4766.9-5969.6)
	Month 18	348	348 (100.0%, 99.9-100.0)	8024 (706.4-909.2)
Placebo group	Enrollment	350	0 (0%, 0.0-1.2)	40 (4.0-4.0)
	Month 7	350	10 (3.2%, 1.6-5.8)	43 (4.0-4.3)
	Month 18	350	10 (3.2%, 1.6-5.8)	42 (4.3-4.3)
Anti-HPV-18				
Vaccine group	Enrollment	357	0 (0%, 0.0-1.0)	35 (3.5-3.5)
	Month 7	351	339 (96.7%, 95.4-100.0)	13647 (3015.1-3754.9)
	Month 18	348	348 (100.0%, 99.9-100.0)	4805 (304.5-543.8)
Placebo group	Enrollment	350	0 (0%, 0.0-1.2)	15 (3.5-3.5)
	Month 7	350	4 (1.3%, 0.4-3.3)	34 (3.5-3.7)
	Month 18	350	1 (0.3%, 0.0-1.8)	35 (3.5-3.6)
n=total number of individuals.				

Table 8: Anti-HPV antibody response to HPV-16 and HPV-18 virus-like particles (according-to-protocol cohort)

n = total number of individuals.

Table 2: Anti-HPV antibody response to HPV-16 and HPV-18 virus-like particles (according to protocol cohort)

cytology and cervical histopathology, detection of persistent infection with a type-specific HPV is a reliable endpoint with high reproducibility.¹⁶ Previous studies have established the role of persistent HPV infection as the necessary cause of cervical cancer. Our data lend support to the conclusion that persistent type-specific infection with HPV over 6-12 months should be a recommended endpoint for vaccine efficacy trials.¹⁷

Taken together, our data provide compelling evidence that this HPV-16/18 vaccine is highly efficacious against persistent HPV-16/18 cervical infection, cytological abnormalities associated with HPV-16/18, and histological development of HPV-16/18-associated CIN. However, a limitation to our study was that it was not powered to estimate efficacy for histopathologically confirmed cervical lesions.

We have shown that the HPV-16/18 virus-like particle vaccine adjuvanted with AS04 induces a level of antibody production against HPV-16/18 that is much higher than that induced by natural infection. Previous work has shown that combinations of the adjuvants MPL and aluminum salts induce an enhanced immune response compared with antigen alone or adjuvanted with only aluminum, at both the humoral and cellular level.¹⁸⁻²⁰ These findings suggest that the immune responses induced in vaccinated women may provide a longer duration of protection than the protective effects induced by natural HPV infection; however, a protective antibody level has not been established nor is there sufficient data currently available to estimate the duration of vaccine-induced protection.

In this trial, the bivalent HPV-16/18 vaccine appeared to be safe and well tolerated. No serious vaccine-related adverse events were reported. Neither local nor general vaccine related symptoms affected overall subject compliance. Greater local reaction rates were observed in the vaccine group, but general symptom rates were equivalent to placebo. The AS04 adjuvant has been used in other vaccine studies and found to be generally safe and well tolerated.²¹

Our findings indicate that the vaccine could contribute substantially to reducing worldwide rates of cervical cancer. However, large-scale trials with long-term follow-up are needed to extend our findings and confirm that vaccination prevents cervical cancer.

Mathematical modelling predicts that a prophylactic vaccine programme, directed at young adolescent women, is likely to be cost-effective in both screened and unscreened populations, with important long-term implications for cervical cancer prevention, especially in countries where screening is limited or unavailable.²²⁻²⁴ Additional benefits could come from vaccination of older women. In countries with opportunistic or organised screening programmes, the high vaccine efficacy in preventing cytological abnormalities associated with HPV-16/18 shows the potential to reduce the number of women receiving additional cytology or colposcopy, thereby reducing the cost of medical treatments associated with cervical screening programmes. In the USA, these preventable costs are estimated at several billion dollars per year.²⁵

Further studies are in progress to provide additional information to enable the effective implementation of HPV vaccination as a public health measure aimed at reducing the global burden of cervical cancer.

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Conflict of interest statement

D Jenkins, A Schmidt, T Zahaf, B Janda, and G Dolin are employees of GlaxoSmithKline Biologicals. F Franco has served as consultant on HPV and cervical cancer epidemiology to GlaxoSmithKline and to other biotechnology or pharmaceutical companies (JM, Digene, Cytex) since 1998. D Ferris is a consultant for colonoscopy quality control for GlaxoSmithKline Biologicals. M Wirtgen is a participant on speaker bureau and is doing research with GlaxoSmithKline, Novartis, MSD, Wyeth, and MedImmune. J Tschirn received research funding from GlaxoSmithKline Biologicals during the study. W Quint is an employee of Delft Diagnostic Laboratory, which did the molecular biological testing for this study. No other authors reported any conflicts of interest.

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Pr phylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young w men: a randomised double-blind placebo-controlled multicentre phase II efficacy trial

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Summary

Background A randomised double-blind placebo-controlled phase II study was done to assess the efficacy of a prophylactic quadrivalent vaccine targeting the human papillomavirus (HPV) types associated with 70% of cervical cancers (types 16 and 18) and with 90% of genital warts (types 6 and 11).

Methods 277 young women (mean age 20.2 years [SD 1.7]) were randomly assigned to quadrivalent HPV (20 µg type 6, 40 µg type 11, 40 µg type 16, and 20 µg type 18) L1 virus-like-particle (VLP) vaccine and 275 (mean age 20.0 years [1.7]) to one of two placebo preparations at day 1, month 2, and month 6. For 36 months, participants underwent regular gynaecological examinations, cervicovaginal sampling for HPV DNA, testing for serum antibodies to HPV, and Pap testing. The primary endpoint was the combined incidence of infection with HPV 6, 11, 16, or 18, or cervical or external genital disease (ie, persistent HPV infection, HPV detection at the last recorded visit, cervical intraepithelial neoplasia, cervical cancer, or external genital lesions caused by the HPV types in the vaccine). Main analyses were done per protocol.

Findings Combined incidence of persistent infection or disease with HPV 6, 11, 16, or 18 fell by 90% (95% CI 71–97, $p < 0.0001$) in those assigned vaccine compared with those assigned placebo.

Interpretation A vaccine targeting HPV types 6, 11, 16, 18 could substantially reduce the acquisition of infection and clinical disease caused by common HPV types.

Introduction

Up to 70% of sexually active women will become infected with human papillomavirus (HPV) during their lifetime.¹ HPV infection causes about 470 000 cases of cervical cancer per year.² Although most cases of cervical cancer arise in the developing world where organised screening programmes with the Pap test have not been implemented, about 35 000 women die from this disease every year in the USA and Europe.³ Even though screening reduces the risk of cervical cancer, it does not prevent HPV infection or development of precancerous lesions,⁴ which need careful follow-up and often need excision.⁵ Moreover, HPV infections that manifest as genital warts arise in 1–2% of young adults,⁶ for which treatment is expensive and painful, and recurrences are common.⁷ A diagnosis of genital warts might also cause sexual dysfunction and emotional disruption.⁸

More than 35 types of HPV infect the genital tract.⁹ Of these, types 16 and 18 cause about 70% of cervical cancer and high-grade cervical intraepithelial neoplasia (CIN);¹⁰ HPV 6 and 11 cause 90% of anogenital warts.¹¹ A prophylactic vaccine that targets these types should thus substantially reduce the burden of HPV-associated clinical diseases.

HPV is a non-enveloped, encapsulated, double-stranded DNA virus.⁹ Expression of the L1 protein in heterologous systems (eg, yeast cells) generates non-infectious virus-like-particles (VLP) that resemble HPV virions.¹² In a placebo-controlled study,¹³ a yeast-produced HPV 16 L1 VLP vaccine was 100% efficacious in prevention of GIN caused by HPV 16 infection 17 months after vaccination in women who were HPV 16 naive at the time of vaccination, and results from studies^{14–16} have also shown HPV 11 or 18 L1 VLP vaccines to be highly immunogenic. These trials thus served as the basis for assessment of a quadrivalent HPV vaccine that targets HPV 6, 11, 16, and 18.

Methods

Study design

A phase II randomised, multicentre, double-blind placebo-controlled study of a quadrivalent HPV (type 6, 11, 16, and 18) L1 VLP vaccine was done in two parts. Part A was a sequential dose-escalation safety assessment, in which participants, investigators, and staff were blinded as to assignment of vaccine or placebo, but not to assignment of doses in the active-treatment group. Part B was a fully blinded dose-ranging assessment of



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endpoint of persistent infection associated with HPV 6, 11, 16, or 18, or cervical or external genital disease compared with placebo. Women with persistent infection were defined as those who had the same vaccine-HPV-type DNA in cervicovaginal samples obtained 7 months after vaccination as those obtained from two or more consecutive visits (required to be 4 months or longer apart unless at least one tissue sample was diagnosed as cervical disease by a panel of pathologists), or as those who had vaccine-HPV-type DNA detected in a sample recorded during the last visit before being lost to follow-up. HPV-associated disease was defined as a tissue sample diagnosed as CIN by a panel of pathologists 7 months after vaccination; vulval intraepithelial neoplasia; vaginal intraepithelial neoplasia; external genital warts; or cervical, vulval, or vaginal cancer with vaccine-HPV-type DNA detected in tissue from, or in a swab of, the same lesion and in cervicovaginal samples obtained at the visit before the biopsy visit.

Laboratory analyses

All Pap tests and histological assessment were done in the setting of the study. Pap tests were reported in accordance with the Bethesda 2001 System.²⁰ Women underwent colposcopy if they were diagnosed with

atypical squamous cells, in which high-grade squamous epithelial lesions could not be excluded; low-grade squamous intraepithelial lesions; high-grade squamous intraepithelial lesions; or atypical glandular cells. After the release of the American Society for Colposcopy and Cervical Pathology Biopsy Guidelines for management of atypical squamous cells of undetermined importance,²¹ the protocol was modified so that residual liquid from these Pap tests was analysed by Hybrid Capture IITM (Digene, Gaithersburg, MD, USA) testing. Patients with a positive result on either low-risk or high-risk HPV probes underwent colposcopy.

All participants had colposcopy at the end of the study. At colposcopy, biopsy samples of discrete abnormalities were taken with separate instruments and were processed separately for histopathological analysis to avoid HPV contamination. A sample of the same lesion or a sample adjacent to the biopsied lesion was submitted for HPV typing. Biopsy samples were processed and read by a central laboratory (Diagnostic Cytology Laboratories, Indianapolis, IN, USA) for medical management. Endpoint assignment was done by use of consensus diagnoses from a panel of pathologists (RJK, MHS, BMR, and AF) who were blinded to central-laboratory diagnoses, treatment group, and HPV status.²²

	Vaccine (n=277)	Placebo (n=275)	Population to which category applies		
			Per-protocol efficacy	Per-protocol harmonogeneity	Modified intention to treat efficacy
Reached D+1 injection*	276	275			
Excluded from per-protocol efficacy analyses					
HPV 6 or 11	59	61			
Positive to HPV 6 or 11†	28	39	•	•	•
Other‡	31	22			
HPV 16	75	72			
Positive to HPV 16†	49	51	•	•	•
Other‡	26	21			
HPV 18	49	46			
Positive to HPV 18†	17	21	•	•	•
Other‡	32	25			
Other reasons for exclusion§					
General protocol violation	31	25			
Vaccine temperature out of range	6	6	•	•	
Enrolled in another study	0	1	•	•	
Incomplete vaccination series	17	13	•	•	
Incorrect clinical recordal or data entry	1	0	•	•	
Received non-study vaccine	5	0	•	•	
Cervical ablation before day 1	3	0	•	•	
Month 7 visit out of acceptable day range	1	6	•	•	
Sexual intercourse within 48 h of visit at day 1 or month 7	0	1	•	•	
Second or third vaccination out of acceptable day range	12	14		•	
Missing month 7 pathology sample or results	2	3		•	
Month 7 return sample out of acceptable day range	4	15		•	
Excluded from modified intention-to-treat cohort¶					
HPV 6 or 11 (positive to HPV 6 or 11)	25	28			
HPV 16 (positive to HPV 16)	44	41			
HPV 18 (positive to HPV 18)	14	16			

*Participants may be included in more than one cohort. †Nonpositive at day 1, PCR positive to the relevant HPV type at or before month 7, or both. ‡See other reasons for exclusion. §Consolidated for all subtypes in per-protocol efficacy analysis. Women were consecutively once in each category, and may appear in more than one category. ¶Nonpositive or PCR positive, or both, for relevant HPV type at day 1.

Table 2: Summary of participants excluded from analysis

To define the serostatus cutoff (i.e. the lowest level of the assay's quantifiable range that can be reliably distinguished from negative samples), positivity rates for about 500 samples were assessed at 11 cutoffs ranging from 8 MU/L to 48 MU/L in increments of 4 MU/L. Before testing, serum samples were classified into panels according to their potential for being a true positive on the basis of clinical history and PCR results. Serostatus cutoff was then selected as the lowest time so that all, or nearly all, known PCR-negative samples and likely

Statistical analysis

Primary efficacy analyses were done in the HPV 6/11, 16, and 18 per-protocol efficacy cohorts, which consisted of women who were naive for the relevant HPV type at enrolment, remained free of infection with the same vaccine HPV type through completion of the vaccination regimen, had all three doses of vaccine or placebo, and did not violate the protocol. Efficacy cases were counted starting after month 7.

Overall, 20 women with the composite endpoint of persistent infection or diseases associated with the vaccine HPV types were needed for the study to have 89.8% power to declare the vaccine efficacious with a two-sided $\alpha=0.05$, assuming a true vaccine efficacy of 80%. Therefore, enrolment of about 254 participants in the placebo group and in the low-dose vaccine group was needed.

Secondary analyses were done for a modified intention-to-treat population that included all participants who were naïve (ie, seronegative and PCR negative) to the relevant HPV type at enrolment and who had had at least one vaccination. Efficacy cases in this population were counted from day 30. Immunogenicity was measured in a ser-

	Vaccine (n=377)	Placebo (n=375)
Age (years)		
Mean (SD)	20.3 (1.7)	20.0 (1.7)
Ethnic origin		
Asian	7 (2%)	11 (4%)
Black	25 (9%)	18 (7%)
Hispanic	14 (5%)	20 (7%)
White	235 (78%)	234 (78%)
Other	15 (5%)	12 (4%)
Country of origin		
USA	125 (46%)	125 (46%)
Brazil	94 (34%)	93 (34%)
Europe	58 (21%)	56 (20%)
Age at first sexual intercourse (years)		
Mean (SD)	16.7 (1.8)	16.7 (1.8)
Lifetime number of sexual partners		
0	17 (5%)	16 (5%)
1	80 (29%)	88 (32%)
2	72 (26%)	75 (27%)
3	62 (24%)	59 (22%)
4	41 (14%)	46 (17%)
Median (range)	1 (0-8)	2 (0-8)
Pap test		
Without Pap test at day 1	30 (9%)	6 (2%)
Unsatisfactory	3 (1%)	2 (>1%)
Negative for SIL	271 (87%)	238 (89%)
SIL Present	33 (12%)	28 (11%)
Atypical squamous cells of undetermined importance	16 (6%)	17 (6%)
Low-grade SIL	15 (5%)	10 (4%)
High-grade SIL	1 (>1%)	2 (>1%)
Atypical glandular cells	1 (>1%)	0
Contraceptive use*		
Male condom	63 (23%)	76 (28%)
Barrier/foam	48 (17%)	48 (17%)
Hormonal	161 (58%)	157 (57%)
Other	21 (8%)	17 (6%)

SIL, squamous intraepithelial lesion. Data are number (%) unless otherwise stated. *The same participant may appear in more than one category. Withdrawal, withdrawal, or rhythm method.

Table 2. Characteristics by vaccination group at enrollment

	Vaccine (n=276)				Placebo (n=275)				Efficacy difference (95% CI) ^a	
	n ^b	Events	Women-years at risk	Incidence per 100 women-years at risk	n ^b	Events	Women-years at risk	Incidence per 100 women-years at risk		p
Outcome: Infection or disease associated with HPV 6, 11, 16, or 18	233	4	566.8	0.7	233	36	536.5	6.7	90 (71-97)	<0.0001
Infection associated with HPV 6, 11, 16, or 18	233	4	566.5	0.7	233	35	536.9	6.5	89 (70-97)	<0.0001
Disease associated with HPV 6, 11, 16, or 18	235	0	568.8	0	233	6	563.0	1.1	100 (16-100)	0.0151
External genital lesions	235	0	566.9	0	233	3	561.1	0.5	NA	NA
CM	235	0	562.2	0	233	3	552.5	0.5	NA	NA
Outcome by HPV type										
HPV 6 associated	214	0	517.5	0	209	13	501.2	2.6	100 (88-100)	<0.0001
HPV 11 associated	214	0	517.5	0	209	3	503.7	0.6	NA	NA
HPV 16 associated	189	3	484.4	0.6	198	11	465.4	2.4	86 (54-97)	<0.0001
HPV 18 associated	224	1	541.6	0.2	224	9	536.9	1.7	89 (71-100)	0.0103

NA=Number of events too small for meaningful efficacy estimates. ^aNumber who had at least one follow-up visit. ^bA participant enters only once within each category, but may be in more than one category. ^cUnfused as compared to a urinate, vulvar intraepithelial neoplasia, or vaginal intraepithelial neoplasia.

Table 3: Efficacy of quadrivalent vaccine against persistent infection or disease associated with HPV 6, 11, 16, or 18 in per-protocol population

protocol immunogenicity cohort, defined as members of the per-protocol efficacy cohort who were vaccinated and who had had serum samples obtained during the protocol-specified time frames, irrespective of HPV infection or disease status after month 7. This study presents the efficacy, immunogenicity, and tolerability of this low-dose vaccine compared with the pooled placebo groups. This low-dose vaccine was chosen for phase III studies.

Role of the funding source

Sponsor staff and clinical-site investigators designed the study. Sponsor staff were responsible for field monitoring, data entry, data review (including integrity and consistency checks), testing of clinical samples for HPV DNA and HPV immune responses, and analysis of data. All authors provided input into interpretation of the data, and writing and revising of the report. The corresponding author had full access to the data and had final responsibility for the decision to submit for publication.

Results

277 women were randomly assigned to quadrivalent HPV (types 6, 11, 16, and 18) L1 VLP vaccine (20, 40, 40, 20 µg, respectively) and 275 to placebo (figure 1). 431 (78%) were included in the per-protocol efficacy analyses for HPV types 6/11, 404 (73%) for type 16, and 456 (83%) for type 18 (table 1, figure 1). 275 (99%) women assigned low-dose vaccine and 275 (100%) assigned placebo were included in the safety analyses. The main reasons for exclusion from the per-protocol cohort were seropositivity to a vaccine HPV type at day 1 or presence of HPV 6, 11, 16, or 18 DNA before completion of the vaccination regimen. The HPV 6/11, 16, and 18 modified intention-to-treat cohorts included 498, 465, and 521 women, respectively.

Table 2 shows participants' baseline characteristics of the HPV vaccine and placebo cohorts. Characteristics of the modified intention-to-treat and per-protocol efficacy populations were much the same as those of the overall study cohort (data not shown).

The main efficacy analyses were done in the per-protocol efficacy cohort (table 3). Over the 30 months' follow-up after vaccination, combined incidence of persistent HPV 6, 11, 16, or 18 infection or associated genital disease decreased by 90% (95% CI 71-97) in women assigned vaccine compared with those assigned placebo (table 3). Of the 40 primary HPV endpoint cases, 13 were from detection of HPV 6, 11, 16, or 18 DNA on samples obtained at the last visit of record (three in the vaccination group [all at month 36] and ten in the placebo group [eight of ten at month 36]). HPV 16 DNA was detected in three women in the vaccine group at the last recorded visit, and one woman had verified, persistent HPV 18 infection detected at months 12 and 18 only. In the modified intention-to-treat cohort, the efficacy of the vaccine with regard to the primary endpoint was 89% (95% CI 73-96, p<0.0001; table 4).

Findings from interim immunogenicity analysis showed the lowest dose vaccine induced serological antibody responses to HPV 6, 11, 16, or 18 that were much the same as those of the intermediate-dose and high-dose preparations.¹⁴ The combined incidence of persistent infection with HPV 6, 11, 16, or 18 or associated genital disease did not differ between the high-dose, intermediate-dose, or low-dose groups (incidence per 100 women-years at risk 0.7, 1.3, and 0.5, respectively). Vaccine efficacy with regard to the composite endpoint of infection or disease associated with HPV 6, 11, 16, or 18 for the three doses pooled (n=701) was 88% (95% CI 76-94).

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	Vaccine (n=276)				Placebo (n=273)				Efficacy difference (95% CI)	p
	n*	Events	Women-years at risk	Incidence per 100 women-years at risk	n*	Events	Women-years at risk	Incidence per 100 women-years at risk		
Infection or disease associated with HPV6, 11, 16, or 18†	268	6	713.6	0.8	268	48	667.1	7.2	89 (73-96)	<0.0001
Infection associated with HPV6, 11, 16, or 18‡	256	6	720.5	0.8	254	47	665.6	7.1	88 (71-96)	<0.0001
Disease associated with HPV6, 11, 16, or 18†	266	0	720.5	0	262	39	712.4	5.4	100 (96-100)	0.0029
External genital lesion (CN)	265	0	715.9	0	261	4	712.4	0.6	NA	NA
	254	0	716.9	0	256	7	709.7	1.0	100 (92-100)	0.0072

NA, number of events too small for meaningful efficacy estimation. *Number who had at least one follow-up visit. †At least one sero-response in each category, but may be in more than one category. ‡Defined as: condylomata acuminata, other intraepithelial neoplasia, or vaginal intraepithelial neoplasia.

Table 4. Efficacy of quadrivalent HPV vaccine against persistent infection or disease associated with HPV 6, 11, 16, or 18 in modified intention-to-treat population

Vaccine-induced immune responses were assessed in the HPV 6 and 11, HPV 16, and HPV 18 per-protocol immunogenicity cohorts, which included 208, 194, and 219 women assigned vaccine, respectively. Concentrations of antibodies to HPV associated with protection from infection have not been defined.²⁰ The immune response to the vaccine was observationally compared with the serum antibody concentrations to HPV in those assigned placebo who had been infected with vaccine HPV types, had subsequently mounted an immune response, and had apparently cleared infection before enrolment (ie, who were seropositive and PCR negative for the relevant HPV type at enrolment). In those assigned placebo, geometric mean titre of antibodies to HPV remained constant throughout the 3 years of the study.

All women assigned active vaccine in the per-protocol immunogenicity cohorts developed detectable antibody responses to HPV 6, 11, 16, and 18 at completion of the vaccine regimen (ie, month 7). Vaccine-induced geometric mean titres of antibodies were substantially higher in women assigned active vaccine than in those assigned placebo who had a previous history of natural HPV infection (table 5). Although mean antibody titres in those assigned quadrivalent HPV L1 VLP vaccine started to decline after month 7 (data not shown), at month 36 they remained at or above the titres recorded for women who had an immune response, presumably associated with clearance of HPV infection. Of women who had a valid immunoassay result at month 36, 173 (94%) of 184

were seropositive for HPV type 6, 176 (96%) of 184 for type 11, all 177 for type 16, and 149 (76%) of 196 for type 18. Furthermore, antibody titres for 174 (89%) of the 196 women positive for HPV 18 were above the assay's lower limit of quantification. Analyses of antibody titres in four women assigned quadrivalent vaccine who developed persistent HPV 18 infection or who were detected with HPV 16 DNA at one visit before loss to follow-up were non-informative because they developed robust antibody responses at month 7. However, antibody titres to HPV 18 after month 7 for the participant who had persistent infection with HPV 18 were slightly lower than for most of those who were allocated active vaccine.

The low-dose HPV vaccine was generally well tolerated (table 6). Adverse events at the injection site were higher in women allocated active vaccine than in those allocated placebo. Pain was the most common injection-site adverse event and headache the most common systemic adverse event. Most (94%) adverse events were of mild or moderate intensity. Only one patient (in the placebo group) discontinued treatment, because of hypo-aesthesia thought not to be caused by the placebo. There were no vaccine-related serious adverse events. Neither intermediate-dose nor the high-dose quadrivalent HPV vaccine was excluded from assessment in phase III trials on the basis of an unacceptable safety profile. However, a modest increase (from 3% to 6%) in injection-site adverse events was recorded with the higher doses (data not shown).

	Month 7				Month 36			
	Vaccine (n=276)		Placebo (n=273)		Vaccine (n=276)		Placebo (n=273)	
	n	GMT (MU/L, 95% CI)	n	GMT (MU/L, 95% CI)	n	GMT (MU/L, 95% CI)	n	GMT (MU/L, 95% CI)
HPV 6	208	582 (537-648)	17	53 (28-108)	184	93 (81-108)	16	61 (33-130)
HPV 11	208	697 (618-795)	4	84 (5-1638)	184	94 (81-110)	4	95 (19-498)
HPV 16	194	3893 (3334-8558)	15	37 (17-89)	177	308 (496-583)	25	29 (12-69)
HPV 18	219	811 (694-925)	12	42 (23-75)	196	60 (49-74)	20	28 (15-50)

GMT=geometric mean titre. †Serum seropositive and PCR negative for specific HPV type.

Table 5. Geometric mean titres of antibodies to HPV 6, 11, 16, and 18 by vaccination group in per-protocol immunogenicity population

	Vaccine (n=272) ^a	Placebo (n=274) ^b
Adverse events reported	250 (92%)	242 (88%)
Injection site	234 (86%)	212 (77%)
Symptomatic	187 (69%)	180 (66%)
Vaccine-associated adverse events	243 (89%)	225 (82%)
Injection site	234 (86%)	212 (77%)
Symptomatic	184 (68%)	180 (66%)
Serious adverse events	2 (1%)	2 (1%)

^aData on number (%). ^bExcludes three women without follow-up and one who had incorrect material or dose. ^cExcludes one woman without safety follow-up.

Table 6: Clinical adverse events

Discussion

We have shown that a multivalent vaccine is efficacious against HPV types that cause cancer and genital warts. Over 35 months' follow-up, incidence of persistent infection associated with HPV 6, 11, 16, or 18 decreased by 89% in women allocated active vaccine who had at least one dose (ie, the modified intention-to-treat population) compared with those allocated placebo. Vaccine efficacy was 90% in the per-protocol efficacy population, suggesting that the vaccine was protective even during the vaccination period. For example, during the course of vaccination (day 1 through month 7), three women assigned active vaccine and five women assigned placebo were detected with HPV 18 DNA. Of these, only one was verifiable persistent infection (in the placebo group). Thus, one woman allocated placebo and no women allocated active vaccine developed persistent HPV 18 infection during the vaccination period. Furthermore, efficacy with regard to clinical disease associated with HPV 6, 11, 16, or 18 was 100%.

This study was not originally powered to assess vaccine efficacy for the disease endpoints or for each HPV type separately. However, the fact that all three women with external genital lesions and all three with CIN were in the placebo group is encouraging in terms of protection against these disease endpoints. Analysis of the incidence of the main efficacy endpoint in the two higher-dose groups supported the decision to select the low dose of quadrivalent HPV L1 VLP vaccine as the final dose for assessment in the phase III programme.

The quadrivalent HPV vaccine was highly immunogenic. All women allocated active vaccine developed higher detectable antibody responses to HPV at month 7 than did those allocated placebo who had natural infection and who remained at higher or similar antibody titres throughout the study. Because women are at risk of HPV infection for as long as they are sexually active, protection induced by a HPV vaccine must be long-lived. At month 36, more than 94% of women were seropositive for HPV 6, 11, and 16; fewer (76%) women had antibody responses against HPV 18 at month 36, although 89% had antibody titres for HPV 18 above the assay's lower limit of quantification. Longer follow-up studies will be needed to assess the duration of

efficacy of this quadrivalent HPV vaccine, and to determine whether booster doses will be needed.

Results from a previous study¹⁸ found that a prototype yeast-produced HPV 16 L1 VLP vaccine given to women who were HPV 16-negative prevented acquisition of HPV 16 infection and associated CIN.¹⁸ A bivalent HPV 16/18 vaccine prepared with AS04 adjuvant was efficacious in preventing persistent infections and associated cervical abnormalities over a 27-month period.¹⁹

In the developed world, full implementation of cervical-cancer screening has substantially shifted the burden of HPV infection from cervical-cancer mortality to management of precancerous lesions. In these countries, in addition to further reduction in the incidence of cervical cancer, universal HPV vaccination might decrease the medical, psychological, and economic costs associated with management of abnormalities detected by Pap testing and CIN. Inclusion of HPV 6 and 11 in a vaccine could diminish the incidence of genital warts and low-grade CIN that are prognostically benign, costly,²⁰ and disruptive psychologically.⁷ In developing countries that have not implemented screening programmes for cervical cancer, the effect of a drop in rates of cervical cancer after implementation of universal HPV vaccination could be substantial. However, such implementation will need definition of the vaccine's effect on public health, cost-effectiveness, the optimum age for vaccination, and the duration of protection.

Universal HPV vaccination might be most effective when implemented in 10–13 year olds, who are likely to be HPV negative.^{21–23} The expectation that the vaccine will reduce cervical-cancer rates, the fact that HPV infection affects most women, and the lack of an effective means to prevent HPV infection in sexually active people lend support to the vaccination of preadolescents. The benefit of vaccination against oncogenic HPV types 16 and 18 is likely to be negligible in heterosexual men because they rarely develop HPV-associated genital cancers. However, a vaccine against the non-oncogenic HPV types 6 and 11 could be considered in the prophylaxis of genital warts in men and in women.

This study has shown that a candidate HPV 6, 11, 16, and 18 vaccine was generally well tolerated, induced high-titres of serum antibodies to HPV types, and effectively prevented acquisition of infection and clinical disease caused by common HPV types. Large-scale studies are under way.

Contributors

E Barr and G M Tsimms developed the study protocol based on a previous study designed by L A Koutsky and K U Jansen with input and support of K A Ault, C M Wheeler, D R Brown, A R Giuliano, D M Harper, F E Shkedy, M Leiman, and L L Villa. K U Jansen oversaw the Merck HPV vaccine research and development programme. E Barr, A J Sash, and G M Tsimms managed headquarters operations at Merck Research Laboratories. L L Villa, R L R Gade, C A Petta, R P Andrade, M Leiman, C Mehm, F E Shkedy, S-E Olsen, M Sainz, K A Ault, D R Brown, C M Wheeler, A R Giuliano, D M Harper, and L A Koutsky set up the study sites. K U Jansen and F J Taddeo developed the PCR-based HPV 6, 11, 16, and 18 detection assays and tested cervicovaginal samples using

for assays. E U Jansen and M T Eimer developed the mHPV 6, 11, 16, and 18 genotyping and tested study source samples. R J Korman, M H Stoler, B M Barnett, and A Ferenczy reviewed the histology slides. J Yu, I Ispahani, and R Rafter developed and implemented the data-analysis plan.

Main investigators

Brazil—C Gazi, G Andreotti, R Carneiro, E Piskareva, J Nerysila, F Corlito, and M Perotti.

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Conflicts of Interest

V Barr, M T Eimer, E U Jansen, F J Taddei, A J Smith, J Yu, L Luján, R Rafter, H L Sings, and G M Tarran are employees of Merck Research Laboratories, a division of Merck & Co, and potentially own stock and hold stock options in the company, or both. Merck is developing the quadrivalent HPV vaccine and funded this clinical trial. K A Ault, D B Brown, D M Harper, R J Korman, F E Skjeldestad, and L L Villa have received honoraria from Merck Research Laboratories given for consultation work or membership in the Phase III HPV Vaccine Steering and Regulatory Oversight Committees, or both, during the past 2 years. A Ferenczy, R J Korman, B M Barnett, and M H Stoler are members of the HPV Vaccine Program Pathology Panel. As such, they have been paid for developing the Panel's standard operating procedures and for histopathological readings of biopsy slides. R P Andrabi, K A Ault, D B Brown, H L Costa, A R Gidycz, L A Koutsky, R J Iversen, M Leiman, C Mahn, C A Petta, F E Skjeldestad, D M Harper, and C M Wheeler led clinical sites that participated in the study and were compensated for all activities related to execution of the study. They are also receiving similar funding for their work on Merck's HPV vaccine phase III program. L L Villa and A Ferenczy have been given honoraria for lectureships on behalf of Merck's HPV vaccine program. D B Brown, A R Gidycz, L L Villa, and C M Wheeler have been paid for consultations regarding the HPV vaccine program in Peru. K A Ault is a member of Merck's HPV Vaccine Obstetrics and Gynecology Advisory Board and as such receives honoraria for consultative work. D M Harper is a member of Merck's HPV Vaccine Young Adult Primary Care Advisory Board. F E Skjeldestad has received funding from Merck Research Laboratories in support of a national history study of HPV disease in young Norwegian women.

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HPV vaccine MedImmune/GlaxoSmithKline

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MedImmune, in collaboration with GlaxoSmithKline, is developing a vaccine consisting of self-assembling, virus-like particles for the prevention of human papillomavirus (HPV) infection of the genital tract and associated cervical cancer. The vaccine candidates include the prototype vaccine, MEDI-501 (a vaccine directed against HPV-11), MEDI-503 (directed against HPV-16) and MEDI-504 (directed against HPV-18). Following phase I studies, the HPV-16 and HPV-18 components were combined into a multivalent vaccine for subsequent trials; this vaccine is known as MEDI-517. Phase II trials of MEDI-517 were initiated by January 2000 and are currently ongoing.

Introduction

Cancer of the uterine cervix is the third most common cancer among women worldwide, with approximately 450,000 newly diagnosed cases each year and a 50% mortality rate [474255], [474259]. Certain types of human papillomaviruses (HPVs) are said to be major causative factors in the development of cervical cancer. In particular, HPV-16 is strongly associated with the development of the disease, being present in 50% of tumor specimens worldwide [318665]. Besides HPV-16, other HPV types such as HPV-18, -31, -33 and -45 are also tumorigenic. In addition to cervical cancer, these HPV types are involved in the development of other, less common cancers of the mucosal tissues (oropharyngeal, esophageal, penile and anal cancer). Other types of HPV, in particular HPV-6 and -11, are associated with the production of benign anogenital warts [318665]; these types of HPV are not, in general, considered to be precancerous.

Papillomaviruses are small, non-enveloped DNA viruses. Their genomes contain a so-called early region encoding for E6 and E7 oncoproteins and a late region encoding two capsid structural proteins (L1 major and L2 minor capsid proteins) [318696]. A number of vaccines against HPV are currently under investigation [439436], including those for both prophylactic and therapeutic approaches, by companies such as Merck [474935] and Xenova [427159] and by the National Cancer Institute [474953]. Two vaccines for the prevention of HPV infection by systemic immunization are currently under development by MedImmune and GlaxoSmithKline: MEDI-517, a vaccine eliciting antibody responses against HPV-16 and -18 L1 capsids for the prevention of cervical cancer [426984] and MEDI-501, a vaccine directed against HPV-11 for the prevention of anogenital warts [299654].

Synthesis and SAR

The native conformation of papillomavirus L1 protein is required for the elicitation of an effective humoral response following vaccination with this viral capsid component [318696], [439436]. Native L1 is capable of self-assembly into

Originator MedImmune Inc

Licensee GlaxoSmithKline

Status Phase II Clinical

Indication Papillomavirus infection

Actions Antiviral, Vaccine

Synonyms & Analogs Human papillomavirus vaccine, MEDI-501, MEDI-503, MEDI-504, MEDI-517

virus-like particles (VLPs) that can be purified, for example, by centrifugation in density gradients. MEDI-517 consists of a combination of VLPs composed of HPV-16 and -18 L1 capsid proteins [474265], while MEDI-501 contains HPV-11 L1 VLPs [189175]. Native L1 protein can be produced in eukaryotic cells. MedImmune developed production cell lines for its HPV vaccine candidates using an insect cell line (High Five), which has been shown to yield 25-fold higher amounts than standard cell lines (eg, Sf9 cells) [189175].

Pharmacology

Polyclonal rabbit antiserum raised against HPV-16 L1 VLPs blocked the infection of an immortalized human keratinocyte cell line with HPV-16, when the virus was preincubated with the antiserum for 1 h at 37°C [318665]. This demonstrates that VLP formulations can elicit neutralizing antibodies against papillomavirus. Responses against HPV VLP L1 vaccines appear to be generally virus-type specific. From a range of rabbit antisera directed against eight other HPV L1 VLPs, only one also blocked HPV-16 infection *in vitro* [318665]. Hence, the chances that MEDI-517 and MEDI-501 might protect against infection by viruses other than HPV-16/18 and HPV-11, respectively, appear to be low.

The feasibility of developing a vaccine to prevent human papillomavirus was indicated in a study of the systemic vaccination of dogs with VLPs consisting of baculovirus-expressed L1 protein of canine oral papillomavirus (COPV). Puppies were vaccinated at 8- to 10-weeks of age with two injections of the L1 protein (0.000125 to 20 µg) intradermally into the footpad. Within 2 weeks of challenge, the vaccinated animals developed circulating antibodies against COPV and became resistant to experimental challenge with COPV at a mucosal site. At low doses (0.000125 µg) three out of the five dogs in the study group still developed warts, but none of the animals developed warts at higher doses (≥ 0.05 µg) [318696].

Similar results were obtained in two other animal models, using bovine papillomavirus 4 (BPV-4) [474236] and cottontail rabbit papillomavirus (CRPV) [474257]. Of the 15 calves immunized with either BPV-4 L1 and L2 capsid proteins, or the L1 protein alone (50 µg VLPs/inoculation), only two developed warts after experimental challenge (with doses ranging from 2 to 20 × 10⁸ viral particles), compared with nine out of the ten control animals [474236]. Similarly, in rabbits

immunized with the same dose of either L1 alone or L1-L2 CRPV vaccine (50 µg VLPs/inoculation), 17 out of the 29 rabbits challenged (with 5×10^8 virus particles) did not develop lesions and the remainder only developed one to eight lesions. None of the animals developed cancer within one year of infection [474257]. Interestingly, the rabbits immunized with VLPs from BPV-1 or with denatured VLPs from CRPV did not display any protection when challenged with the same dose. Of the 30 rabbits tested, 27 developed extensive papillomas [474257].

In the case of CCRPV and CRPV infection, passive transfer of serum or immunoglobulin G from immunized to naive animals conferred protection from experimental infection [318696], [474257]. In the rabbit model ($n = 4$), 75% of animals were fully protected and one was partially protected [474257]. These results indicate that antibodies serve as a major protective component for protection in these models.

Metabolism

No data are currently available.

Toxicity

No data are currently available.

Clinical Development

Phase I

During 1997 and 1998, MedImmune conducted a phase I, double-blind, randomized, placebo-controlled, dose-escalating study with MEDI-501. The data showed that recipients developed dose-related antibody (IgG) responses to the vaccine and that MEDI-501 was safe and generally well tolerated [232788], [299654]. All of the healthy adult volunteers ($n = 65$) receiving one initial and two booster injections developed elevated levels of antibodies that showed the ability to neutralize HPV [299654], [474266]. The results of this study provided proof-of-principle of using VLP vaccines in humans [474265].

Further phase I studies were performed with vaccines containing L1 VLPs from either HPV-16 or -18, or a combination of both (MEDI-503, -504 and -517, respectively); the study arm for MEDI-517 involved healthy female volunteers ($n = 48$). The data demonstrated that the vaccine was safe and generally well tolerated. Furthermore, the study indicated that the vaccine has the potential to induce the desired immune response and to produce neutralizing antibodies [474265], [474266].

Phase II

Enrollment for phase II clinical trials of MEDI-517 was completed by the end of 2001 [474265]. Results from these trials have not yet been disclosed.

Currently, no information is available indicating that MedImmune is to promote MEDI-501 as a vaccine against genital warts into further clinical trials.

Side Effects and Contraindications

In the phase I trial of MEDI-501 involving 65 healthy adult volunteers, the most common adverse events described

within 4 days of injection were those commonly seen with alum-adjuvanted vaccines, including pain at the injection site, headache and fatigue [299654].

Current Opinion

In the US, the widespread use of cervical cytological screening has resulted in a 70% decline in mortality from cervical cancer in the last 50 years [474258]. In developing countries, however, cervical cancer remains the leading cause of death from cancer among women. Patients who are diagnosed with cervical cancer can be effectively treated by surgery, radiation therapy or chemotherapy to control local disease. Of all cervical cancer patients, however, 35% develop persistent or metastatic disease for which there is no consistent effective therapy. In this situation, development of a preventive vaccine offering protection against the major types of HPV which cause cervical cancer could be an important step to control the disease.

While proof-of-principle for anti-HPV vaccination has been obtained in animal models, the clinical development of MEDI-517 is still at an early stage, with only limited information available from a phase I trial performed in relatively few patients. Any speculation regarding the effectiveness of this vaccine, required doses, immunization schedule and duration of protection would be premature at this point.

A number of problems will be encountered during the development and clinical use of the vaccine; firstly, an outstanding question is whether anti-VLP antibodies can be detected in the vaginal secretions of immunized women and if the antibody response can prevent subsequent viral infection. While systemic immunization with L1 VLPs can elicit high titers of IgG in serum and (as demonstrated in monkeys) also in the mucosa, they do not elicit mucosal IgA responses [304766]. Whether IgG response is sufficient to prevent natural transmission of HPV in women remains to be seen. Nasal VLP formulations, known to elicit IgA in mice [474937], may be an alternative in the future. Secondly, MEDI-517 is expected to prevent infection caused by HPV-16 and -18, together covering over 90% of cervical carcinomas. The vaccine will not, however, cover all relevant HPV types and may have to be extended to a multivalent vaccine at a later stage. In addition, the cost of MEDI-517 containing recombinant proteins will be high and most likely not appropriate for mass immunization in developing countries. Finally, there are a number of social and behavioral issues related to the introduction of an HPV vaccine: (i) since the vaccine will not offer protection against all high-risk HPV types and because protection may not be life-long, the current practice of cytological screening (Pap smear) in the developed world will need to be maintained once the vaccine becomes available, although intervals may have to be revised. Women will have to be educated about these changes to prevent inadequate use of cervical cancer screening. The consequences with regards to healthcare economics are uncertain; (ii) education of the general public will be needed so that vaccination of children is accepted. False security amongst teenagers could lead to more promiscuity and greater exposure to different HPV types and other sexually transmitted diseases; (iii) effective control of genital HPV will probably require immunization

of both men and women, however, it is unclear whether men will accept a vaccine for a disease they will never develop, namely cervical cancer.

The events between the time of infection with HPV to progression to cancer can take decades and it will, therefore, be years before it is known if vaccination with MEDI-517 can effectively prevent HPV infection and the development of cervical cancer. As for MEDI-501, it is unclear whether MedImmune and GlaxoSmithKline intend to further develop this vaccine. While 1% of sexually active

individuals in the US have visible genital warts, preventive vaccination for this condition, which only rarely causes significant morbidity or mortality, does not appear a viable goal. In addition, a bivalent vaccine against both HPV-6 and -11, the two major genotypes causing genital warts, would have to be developed for this indication.

Commercial Opinion

In October 2002, Merrill Lynch reported that phase II trials were ongoing and predicted that the HPV vaccine was not likely to be approved before 2008 [477603].

Licensing

GlaxoSmithKline Inc

In June 1996, MedImmune in-licensed rights to key intellectual properties, patents and patent applications for HPV vaccines, from the Deutsches Krebsforschungszentrum (German Cancer Research Centre) [212925]. MedImmune also has a royalty-bearing worldwide exclusive license to commercialize HPV vaccine technology from the University of Rochester and Georgetown University. This technology is the subject of patent applications filed in the US and overseas and now exclusively licensed to MedImmune. MedImmune is to make certain license and research payments to the University [189175]. SmithKline Beecham (SB, now GlaxoSmithKline) and MedImmune signed a research and commercialization agreement in December 1997. In exchange for exclusive worldwide rights to MedImmune's HPV vaccine technology, SB agreed to provide MedImmune with an upfront payment, future funding and potential developmental and sales milestones which together could total over US \$85 million, as well as royalties on any product sales [272068]. The terms of the alliance were finalized in January 1998 [275041].

Development history

Phase II trials were initiated by January 2000 [352634] and were fully enrolled by the end of 2001 [473895]. The trials, which consist of four arms, are apparently being conducted with MEDI-517 rather than MEDI-501 [467734]; no information is available indicating that MEDI-501 is being further investigated in clinical trials. In January 2003, MedImmune announced that GSK was to present the phase II data in April 2003; at this time, MedImmune expected phase III trials to commence in the fourth quarter of the year [476563]. As of October 2001, MedImmune expected to finalize the phase III trial endpoints for HPV vaccines following an FDA meeting scheduled for November 28 [425924].

Developer	Country	Status	Indication	Date	Reference
GlaxoSmithKline plc	UK	Phase II	Papillomavirus infection	27-DEC-00	384716
MedImmune Inc	US	Phase II	Papillomavirus infection	18-JAN-00	352634

Literature classification

Biology

Study Type	Effect Studied	Experimental Model	Result	Reference
<i>In vitro</i>	Expression of HPV-11 L1 VLPs	High Five insect cell lines	High Five insect cell lines yielded at least 25-fold higher amounts of HPV-11 L1 VLPs than standard cell lines.	189175
<i>In vitro</i>	Neutralization of HPV-16	An immortalized human keratinocyte cell line infected with HPV-16	Antisera against HPV-16 L1 VLPs demonstrated neutralizing activity, inferring type-specific protection.	318885
<i>In vivo</i>	Antibody titres and protection	Rabbits were immunized with either L1 alone, L1-L8 CRPV vaccine, BPV-1 VLPs or denatured VLPs from CRPV then challenged with 5×10^{10} viral particles.	Vaccines consisting of L1 VLPs from CRPV were found to be immunogenic (17 out of the 29 animals challenged did not develop lesions), but VLPs from BPV-1 and denatured VLPs from CRPV did not convey any protection (27 out of the 30 animals tested developed extensive papillomas).	474257

Clinical

Effect Studied	Model Used	Result	Reference
Safety and immunogenicity of MEDI-501	Phase I, double-blind, randomized, adjuvant-controlled clinical study with MEDI-501, involving healthy volunteers (n = 65).	MEDI-501 was safe, well-tolerated and immunogenic. The adverse events described were associated with alum-adjuvanted vaccines, including headache, fatigue and pain at the injection site.	298854

Clinical (continued)

Effect Studied	Model Used	Result	Reference
Safety and immunogenicity of MEDI-503, -504 and -517.	Phase I clinical study with MEDI-503, -504, and -517 in healthy adult HPV-negative women (n = 48).	The vaccines appeared to be safe, well tolerated and immunogenic.	474288

Associated patent

Title: Production of human papillomavirus capsid protein and virus-like particles.

Assignee: University of Rochester

Publication: WO-09429137 15-SEP-04

Priority: US-0022517 09-MAR-03

Inventors: Ross RC, Bonnez W, Reichman RC.

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Technology evaluation: HPV vaccine (quadrivalent), Aventis Pasteur MSD/CSL

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CSL, licensee of UniQuest's HPV technology, and Aventis Pasteur MSD (a joint venture between Merck & Co and Aventis) are jointly developing a vaccine for the potential prophylaxis of genital warts and cervical cancer caused by human papilloma virus infection. Enrollment for a phase III trial has been completed.

Introduction

According to the World Health Organization, over 470,000 women are diagnosed with cervical cancer every year, making it the second most common cancer among women worldwide. Particularly in developing countries, it is a leading cause of cancer-related mortality [<http://www.who.int/cancer/publications/en>], [304507]. Most cervical neoplasias, whether intraepithelial or invasive, can be attributed to the human papillomavirus (HPV) infection [510801]. Of over 130 HPV types identified so far, approximately 30 'high-risk' types are associated with genital tract lesions. The DNA of these high-risk types, namely HPV-16, -18, -31, -33, -35 and -45, in addition to some minor types, can be found in 100% of human cervical cancers and is also associated with other forms of genital neoplasias. On the other hand, HPV-6 and -11 are responsible for genital warts and do not usually cause cervical cancer. Therefore, prevention of persistent infection with HPVs should substantially reduce the occurrence of genital cancers [499436].

A number of prophylactic and therapeutic HPV vaccines are currently at various stages of development. While prophylactic vaccines are mainly designed to induce a virus-neutralization antibody response, therapeutic vaccines usually elicit a cell-mediated cytotoxic response, leading to elimination of cells expressing non-structural viral proteins [437961], [304507].

Papillomavirus-like particle vaccines are particularly attractive candidates for an effective HPV prophylactic vaccine. Virus-like particles (VLPs) are non-infectious empty viral capsids composed of the major viral capsid protein L1, either alone or combined with the minor capsid protein L2 [318643], [439436].

Neutralization antibodies induced by immunization with VLPs recognize immunodominant, conformational epitopes, which are highly type specific [519613]. Surface conformational and linear epitopes on HPV-6, -11, -16 and -18 have been mapped in detail with special attention to the inter-type differences and immunological cross-reactivity

Originator UniQuest Ltd

Licensees Aventis Pasteur MSD/CSL Ltd

Status Phase III Clinical

Actions Anticancer, Antiviral, Vaccine

Indication Papillomavirus infection

Biotechnology Recombinant protein

Synonym Human papillomavirus vaccine

between particular HPV types [510877], [510879], [510881], [519614].

The prophylactic recombinant HPV-16 VLP vaccine (Novavax Inc), produced in insect cells using recombinant baculovirus technology, is currently in phase II trials [474953], [486644], [517360]. This technology was also utilized in Cervarix, the GlaxoSmithKline plc/Medimmune Inc vaccine, also in phase II trials [479799], [495022].

Development of therapeutic vaccines for the treatment of HPV-related diseases is mainly focused on the induction of a cell-mediated cytotoxic immune response against the E6 and E7 viral oncoproteins expressed in HPV-associated tumors [437961], [439436]. TA-HPV, a live recombinant vaccinia virus expressing E6 and E7 proteins of HPV-16 and -18, and TA-CIN, based on L2, E6 and E7 recombinant proteins produced in *Escherichia coli*, were being developed by Xenova Group plc but were 'deprioritized' following the acquisition of KS Biomedix Ltd by Xenova in September 2003 [433547], [509159], [518288]. Another promising therapeutic vaccine is HspE7 (StressGen Biotechnologies Corp). This vaccine, based on chimeric molecules comprising mycobacterial heat shock proteins and HPV-16 E7 [418373], is currently in phase III trials for the treatment of anal dysplasia and HPV infection, and phase II trials for the treatment of uterine cervical tumors.

A prophylactic L1 VLP HPV vaccine has been jointly developed by Aventis Pasteur MSD and CSL Ltd [509200]. This quadrivalent vaccine against HPV is being tested in phase III trials [433342], [469807]. This vaccine is based on L1 HPV-6, -11, -16 and -18 VLPs produced with the use of recombinant technology in *Saccharomyces cerevisiae*.

Synthesis and SAR

A monovalent HPV-16 L1 vaccine comprising the L1 viral polypeptide was prepared from recombinant vectors using the yeast *S. cerevisiae*. Purified particles were mixed with the amorphous aluminum hydroxyphosphate sulfate adjuvant [469807], [471130]. The quadrivalent vaccine is composed of the HPV-6, -11, -16 and -18 L1 polypeptides [469807].

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HPV-16 VLPs were prepared from pLC201VV plasmid-infected CV-1 cells. The infected cells were harvested and lysed, and lysates clarified by centrifugation. Fractions were collected and tested for the presence of VLPs by electron microscopy [WO-09302184].

Preclinical Development

The HPV L1 protein alone, or together with the L2 minor capsid protein, has the ability to form VLPs when expressed by eukaryotic expression vectors [318643], [517396]. The immunogenicity of these VLPs is similar to the complete wild-type virions; presentation of the viral antigens, including conformational epitopes in the form of VLPs, resulted in a massive generation of virus-neutralization antibodies and elicited a specific cellular immune response [510439], [510875].

The immunogenicity of VLPs prepared using the yeast system has been demonstrated in the cotton-tail rabbit papillomavirus (CRPV) system. The immunization potential of recombinant CRPV L1 VLPs was determined after three immunizations when the vaccine was administered at 1- to 100- μ g doses. Sera of immunized rabbits contained a high titer of serum antibodies to CRPV L1 VLPs and neutralized CRPV *in vitro*, hence protecting the rabbits from challenge with CRPV [517397].

In studies in non-human primates, systemic immunization of African green monkeys with HPV-11 VLPs with aluminum adjuvant elicited massive HPV-11 VLP-specific serum antibody responses [304766]. Sera from immunized monkeys neutralized HPV-11 in the athymic mouse xenograft system and, moreover, significant levels of HPV-11-neutralizing antibodies were observed in cervicovaginal secretions. These data suggest that systemic immunization with HPV VLPs resulted in protection against HPV infection of the uterine cervix.

Monovalent or quadrivalent L1 VLP vaccines with aluminum hydroxyphosphate were administered intramuscularly to chimpanzees at weeks 0, 8 and 24 [510876]. Four weeks after the second and third immunizations, maximum levels of serum antibody titers and neutralizing antibodies were detected. The humoral immune response was accompanied by T-helper (Th)1 and Th2 cell cytokine responses to several HPV types while induction of cytotoxic T-lymphocytes (CTLs) was infrequent.

Metabolism and Pharmacokinetics

No data are currently available.

Clinical Development

The clinical program for Merck's HPV vaccines has been conducted in three stages: stage one incorporates a phase I and IIa investigation of the immunogenicity and tolerability of a broad range of doses of the monovalent HPV-16 L1 VLP vaccine; stage two is an immunogenicity and tolerability study on a range of quadrivalent (types 6, 11, 16 and 18) L1 LVP vaccines at various doses; and stage three, which began in 2003, will demonstrate whether the vaccines reduce the risk of acquisition of HPV infections and a broad spectrum of HPV-related diseases [469876].

Phase I

Phase I studies have established the immunogenicity and tolerability of a wide range of doses of monovalent vaccines. One clinical trial was conducted to determine the safety, tolerability and immunogenicity profiles of a series of research HPV-16 VLP vaccines [517417]. HPV-16-negative women aged 18 to 25 years ($n = 109$) received either placebo or one of three doses (10, 40 or 80 μ g) of vaccine. This HPV-16 VLP vaccine was generally well tolerated, highly immunogenic (with the 40- μ g dose as immunogenic as the 80- μ g dose) and capable of generating antibodies that would neutralize HPV-16. In the second trial, 460 women with a mean age of 21 years were enrolled [517421]. The incidence of detectable anti-HPV-16 titer at baseline was 28%. Again, preliminary results demonstrated that the HPV-16 VLP vaccine appeared to be highly immunogenic and generally well tolerated. The individuals were subsequently followed for up to three years, and the geometric mean titers were still higher than titers in women with a previous HPV-16 infection [517426].

Furthermore, the phase I study investigated the protective efficacy of the monovalent HPV-16 vaccine against HPV-16 infection [469876], [510878]. The study was divided into two protocols in which women received either HPV-11, HPV-16 or control at 0, 2 and 6 months. A total of 195 individuals were enrolled into the study, 129 of which received placebo or HPV-11 vaccine, and 66 of which received HPV-16 vaccine. There were no HPV-16 infections identified in the women who received the HPV-16 vaccine, while women who received placebo or HPV-11 vaccine suffered 14 incidences of HPV-16 infection (ten in women who had received the HPV-11 vaccine). Combined data from the HPV-11 and -16 trials demonstrated that the vaccines were well tolerated, highly immunogenic and protective. Notably, the neutralization antibodies were HPV-type specific, and immunization with L1 HPV-16 VLPs did not protect against infection with HPV-11 [510878].

Phase II

A double-blind, proof-of-principle phase IIa study enrolled 2392 women aged between 16 and 23 years with less than five lifetime sexual partners. The women were randomly assigned to receive either three doses of monovalent HPV-16 L1 VLP vaccine (40 μ g/dose with 225 μ g aluminum adjuvant; $n = 1194$) or placebo ($n = 1198$) [471029], [471130], [467437]. Doses were administered at day 0, month 2 and month 6. Of these, 1533 HPV-16-negative women in a primary study were randomly assigned to receive vaccine plus adjuvant ($n = 768$) or adjuvant as placebo ($n = 765$). These women tested HPV-16-DNA and HPV-16-antibody negative at enrollment, in addition to testing negative for HPV-16 DNA at 7 months. All women completed the full course of immunization, and after two years, 99.7% of individuals had seroconverted. Antibody titers were typically 5-fold greater than in those patients with previous HPV infection [501090]. The primary endpoint of the study was defined as the timepoint when at least 31 new HPV-16 infections were detected. This occurred after two years of the study; individuals were then followed for a median of approximately two further years. None of the women who

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received the HPV-16 vaccine developed persistent infection for an average of 17.4 months after final immunization. In contrast, there were 41 new cases of persistent HPV-16 infection in the control group, and nine cases of HPV-16-related cervical intra-epithelial neoplasia were detected among the placebo recipients. The incidence of persistent HPV-16 infection was 3.8/100 woman-years at risk in the placebo group and 0/100 woman-years at risk in the vaccine group (95% CI, in the range of 90 to 100; $p < 0.001$) [469807], [471029], [471130], [487437]. No data on the CTL response to HPV L1 VLP immunization have been published from this study, which was ongoing in June 2003 [501090].

A triple-blind, placebo-controlled, dose-ranging phase IIb study of the quadrivalent vaccine containing HPV-6, -11, -16 and -18 has been conducted in 1155 women aged 16 to 23 years with less than five lifetime sexual partners [469807], [487437]. The primary endpoint was to determine immunogenicity to the four HPV components at 7 months in individuals that were HPV naive at baseline. Another objective was to establish the appropriate dose for phase III trials. The study was conducted at different sites in Brazil, Northern Europe and the US. The HPV vaccine or aluminum placebo were administered on day 0, month 2 and month 6.

The interim analysis demonstrated that the vaccine was safe, well tolerated and induced a massive humoral immune response. The production of specific anti-HPV antibodies was measured by radioimmunoassay. The geometric mean titers after the third dose of anti-HPV-6, -11, -16 and -18 antibodies were 43-, 46-, 140- and 28-fold higher, respectively, in immunized women than in those with previous infection [487437] and were not dose dependent. There was no specific antibody response in the placebo group. At 18 months post administration, anti-HPV-16 antibody levels remained 5-fold higher than those observed in women with previous or ongoing infection [469807]. In the course of the study, HPV infection was determined on day 0 and month 7 by PCR.

Phase III

A large phase III trial for the quadrivalent vaccine has completed enrollment of 17,000 patients [516708]. No data are currently available.

Side Effects and Contraindications

Application of the vaccine has generally been well tolerated [469807], [471029] and no serious vaccine-related adverse effects have been observed. Local (mostly pain at the injection site) and systemic adverse events occurred at the same frequency in vaccinated and control groups of patients receiving aluminum placebo. In general, the nature and frequency of adverse events are comparable to other similar vaccines and can be attributed mainly to the effect of aluminum adjuvant.

Current Opinion

An efficient prophylactic vaccine should prevent the establishment of a long-term, persistent HPV infection and thus prevent the development of related diseases ranging from genital warts to cervical carcinoma. The phase I and II

trials of the L1 HPV-16 VLP vaccine revealed the potential of this vaccine to induce an immune response, with the results strongly suggesting that immunization provides good protection against infection with the corresponding HPV type. The mean titer of antibodies circulating in study individuals exceeded the levels considered to be sufficient for neutralization of infection several times over.

The vaccine has been demonstrated to be protective against HPV-16 infection within the studied population of HPV-negative women aged 16 to 24 years with less than five lifetime sexual partners. More data are required to determine the prophylactic effects of vaccination in women who do not meet the criteria of this study group. Special attention should be paid to the high-risk group of women who have had multiple sexual partners, to the HIV-positive individuals, and particularly to women who have an established HPV infection prior to vaccination [517708].

The protective efficacy of the HPV-6 and -11 components of the quadrivalent vaccine has not yet been proved. It is noteworthy that HPV-6 and -11 infections are cutaneous infections and not of the mucosal surface. It is not clear whether transudation of serum IgG, which is the major humoral protection of the cervix, would also protect the vulva, anal and perineal skin against HPV infection.

The major problem with developing a widely protective HPV vaccine based on eliciting neutralizing antibodies is the type specificity of the humoral immune response generated, and the lack of cross-reactivity of antibodies raised against one HPV type of L1 with the L1 polypeptides derived from the others; this phenomenon was demonstrated in the phase I trials. The quadrivalent vaccine covers the four clinically most important high-risk HPV types, namely HPV-6 and -11, which are responsible for most of the genital warts, and HPV-16 and -18, present in approximately 70% of cervical carcinomas. Interim analysis demonstrated that the vaccine induced an immune response against all four HPV types. An important question is whether incorporation of a further 30 known high-risk HPV types would increase the prophylactic potential of the vaccine and whether it would be feasible to manufacture such vaccines. Geographical variations of some less common HPV types were observed, for example, HPV-39 and -59 are confined to Central and South America, while the common types such as HPV-16 and -18, are present throughout the world [519618]. Thus, the vaccine comprising four to five of the most prevalent oncogenic HPV types would ensure sufficient protection in specific geographical regions. Another possible modality is the use of hybrid HPV L1 molecules in VLPs. Indeed, hybrid L1 molecules prepared using complementary regions of the L1 protein of different types can retain VLP-assembly properties and can trigger neutralizing antibodies against unrelated HPV types [519621].

Another task is the incorporation of E6 or E7 viral oncoprotein segments into the next generation of VLPs. This vaccine would potentially elicit T-cell responses leading to the elimination of infected cells. This modification could increase the therapeutic potential of VLP-based prophylactic vaccines. Indeed, such chimeric

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VLP vaccines are in preclinical studies [517405] and their benefit is to be evaluated.

HPV vaccine should be affordable and usable in developing countries.

The majority of HPV-related cancers occur in the developing world, while in developed countries their numbers tend to decrease; this is in part as a result of the implementation of cervical cytology screening. It is therefore important that an

Commercial Opinion
In December 2003, analysts at Morgan Stanley predicted that sales of the vaccine would reach US \$300 million in 2006, rising to US \$500 million in 2007 [519716].

Licensing

CSL Ltd
UniQuest, the technological division of the University of Queensland, licensed its HPV technology, involving genetic engineering, to CSL who acquired the rights to products arising from the collaboration [188304], [343576].

Merck & Co Ltd
Merck is co-developing the vaccine with CSL. CSL granted Merck a license for development and worldwide marketing excluding Australia and New Zealand [174582], [227624], [299837].

Aventis Pasteur MSD
By September 2003, the HPV vaccine was being developed by the Merck/Aventis joint venture Aventis Pasteur MSD [509200].

Development history

In December 2001, Merck reported that its HPV vaccine was in phase III trials [433342]; these trials were ongoing in November 2002 [469807], and by December 2003, enrollment in the pivotal phase III trial was complete, with 17,800 individuals recruited [518308]. Merck expect to file a PLA with the FDA in 2005 [516624], [518708]. In January 2004, Merck expected to launch the vaccine in 2006 [520117]. By June 2002, CSL was planning phase I studies of HPV vaccine formulated with its ISCOMATRIX adjuvant [455844], [490865].

Developer	Country	Status	Indication	Date	Reference
Aventis Pasteur MSD	US	Phase III	Papillomavirus infection	26-SEP-03	509200
CSL Ltd	US	Phase III	Papillomavirus infection	12-DEC-01	433342
Merck & Co Inc	US	Phase III	Papillomavirus infection	12-DEC-01	433342
CSL Ltd	Australia	Discovery	Papillomavirus infection	01-SEP-94	188304
UniQuest Ltd	Australia	Discontinued	Papillomavirus infection	01-SEP-94	188304

Literature classifications

Key references relating to the technology are classified according to a set of standard headings to provide a quick guide to the bibliography. These are as follows:

Biology: References which disclose aspects of the drug's pharmacology in animals.
Clinical: Reports of clinical phase studies in volunteers providing, where available, data on the following: whether the experiment is placebo-controlled or double- or single-blind; number of patients; dosage.

Biology					
Study Type	Effect Studied	Experimental Model	Result	Reference	
In vivo	Protection	Cotton-tail rabbits were immunised three times with L1 VLPs	The dose efficiently protected rabbits from challenge with CPV9. The sera of the immunised rabbits contained high-titred serum antibodies to CPV11 VLPs.	517597	
In vivo	Neutralising antibody titre	African green monkeys systemically immunised with HPV-11 VLPs	High-titres of HPV-11 VLP-specific serum antibodies were elicited. Sera from monkeys neutralized HPV-11 in an epithelial mouse xenograft model. Significant levels of HPV-11-neutralizing antibodies were observed in cervicovaginal secretions in the monkeys.	304785	

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Biology (continued)

Study Type	Effect Studied	Experimental Model	Result	Reference
In vivo	Antibody and cytokine responses.	Chimpanzees administered L1 VLP vaccines intramuscularly at weeks 0, 8 and 24.	Maximum levels of serum antibody titres and neutralizing antibodies were detected 4 weeks after the second and third immunizations. Th1 and Th2 cytokine responses to several HPV types were noted.	510578

Clinical

Study Type	Model Used	Result	Reference
Immunogenicity and tolerability.	Two-part phase I study of 108 women (aged 18 to 28 years) and 540 women (mean age 21 years) administered a wide range of doses of the monovalent HPV-16 VLP vaccine.	The vaccine appeared to be highly immunogenic and generally well tolerated. The 40-µg VLP dose was as immunogenic as the 80-µg dose. After three years follow-up, the geometric mean titres were higher in women with a previous HPV-16 infection.	517421
Tolerability and immunogenicity.	Phase I study, enrolling 129 women who received control (placebo or HPV-11 L1 VLP), and 68 who received HPV-16 L1 VLP vaccine.	The HPV-16 vaccine was well tolerated. There were no HPV-16 infections in the HPV-16 vaccine group. The HPV-11 vaccine did not protect against HPV-16 infection, with ten incidences of HPV-16 infection in women who received the HPV-11 vaccine.	510578
Proof-of-principle.	Phase IIa study of the monovalent HPV-16 L1 VLP vaccine in 1553 women aged from 18 to 23 years with < five lifetime sexual partners who were HPV-negative at the time of enrollment.	No HPV-16-vaccinated individuals (n = 788) developed HPV-16 infection, while there were 41 new cases in the control (alumination) group (n = 765). Of the women who finished the course, 88.7% were converted.	471130
Efficacy.	Phase IIb, triple-blind, placebo-controlled, dose-ranging trial of the quadrivalent L1 HPV-6, -11, -16 and -18 VLP vaccine in 1155 women aged 18 to 23 years who were HPV-negative at the time of enrollment and had < five lifetime sexual partners.	Vaccination with the quadrivalent vaccine induced production of neutralizing antibodies against all four HPV types.	468807

Associated patent

Title Papillomavirus vaccine.

Assignee University of Queensland/CSL LTD

Publication WO-09302184 04-FEB-03

Priority AU-00007322 18-JUL-91

Inventors Fraser I, Zhou J.

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Immunological analyses of human papillomavirus capsids

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Abstract

Recombinant human papillomavirus (HPV) virus-like particles (VLPs) are promising vaccine candidates for controlling anogenital HPV disease. Questions remain, however, concerning the extent of capsid antigenic similarity between closely related virus genotypes. To investigate this issue, we produced VLPs and corresponding polyclonal immune sera from several anogenital HPV types, and examined these reagents in enzyme-linked immunosorbent assays (ELISAs) and in cross-neutralization studies. Despite varying degrees of L1 genetic sequence relatedness, VLPs of each type examined induced high-titer serum polyclonal antibody responses that were entirely genotype-specific. In an *in vitro* infectivity assay, only cognate VLP antisera were able to neutralize pseudovirions of HPV-16, HPV-18 and HPV-33, with two exceptions: HPV-31 and HPV-45 VLP post-immune sera demonstrated low levels of neutralizing activity against pseudovirions of HPV-33 and HPV-18, respectively. In other experiments, epitopes shared between closely related types were found to be less immunogenic than, and antigenically distinct from, primary type-specific B-cell determinants of the viral capsid. In addition, results from epitope blocking experiments suggested a close correlation between primary type-specific capsid antigenic sites and virion neutralization. These findings support the view that papillomavirus genotypes denote unique viral serotypes, and suggest that a successful vaccine for these viruses will likely require the inclusion of VLPs of each serotype for which protection is desired. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

Papillomaviruses are host-restricted small DNA viruses that infect higher vertebrates, including humans [1]. More than 70 genetically distinct human papillomavirus (HPV) genotypes have been identified or characterized [2], and while most of these usually cause only benign epithelial lesions (e.g. cutaneous warts), others have been associated with more severe disease, such as premalignant cervical/penile intraepithelial neoplasia and/or invasive carcinoma [3-6]. Mucosal HPV infection is now recognized as the primary etiologic factor in an estimated 4.8% of world cancer [7], with the burden of disease primarily in developing regions [4]. The strength of the association between HPV infection and

invasive disease has generated great interest in vaccine development [8,9].

Certain viruses that are controlled efficiently by neutralizing antibodies are able to form serotypes, which have been defined as different viral strains that do not elicit cross-neutralizing antibodies [10]. The concept of serotype has been useful in characterizing other human viral pathogens (e.g. poliovirus) [11], and in designing protective vaccines against them [12,13]. Difficulties in propagating HPV virions in cultured cells have hindered efforts to identify and characterize viral immunologic variants. To circumvent this problem, several groups developed recombinant L1 virus-like particles (VLPs), which replicate the antigenicity of native virions, and can be generated from any papillomavirus genotype for which sequence data are available [14-16]. Importantly, genital HPV VLPs have been shown to induce virus-neutralizing antibodies [17,18], and VLPs of animal papillomaviruses have demonstrated immunoprophylactic efficacy in relevant infection models

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[19–21], thus providing a strong rationale for clinical evaluation of VLPs in humans.

Further study of the role of immunologically distinctive epitopes in the development of immunity to papillomaviruses is needed to develop broadly effective strategies for controlling genital HPV disease. The relationship between papillomavirus genotypes and serotypes remains an important issue for vaccine development. For example, antibody-mediated neutralization of infectious virions appears to be genotype-restricted, i.e. certain types have demonstrated relatively unique capsid antigenic features [22–24], while others have demonstrated an ability to participate in cross-neutralization [24,25]. It may be that certain genotypes denote unique serotypes, while others fall within serogroups consisting of two or more related variants. If so, then it may be possible to use single antigens to induce protective responses against multiple genotypes. While such a concept may be attractive, the development of highly effective vaccines will require accurate information concerning genotypic/serotypic relationships, and precise knowledge of the relative immunogenicities of unique versus antigenically cross-reactive neutralization domains.

Results from previous studies have suggested a role for non-linear (i.e. conformationally dependent) B cell epitopes in antibody-mediated neutralization of genital HPV virions [17,18,24–29]. We undertook the present study to investigate capsid antigenic variation and the relative immunogenicities of non-linear B cell epitopes of several genital HPV types. We also sought evidence of an association between high-titer VLP polyclonal antibody specificities and virus-neutralizing activity, as such information might be consistent with the concept of serotype formation [10,30]. Genotypes in the present study were selected on the basis of genetic sequence relatedness, prevalence, and disease association, and included representative members of three phylogenetic categories (i.e. group A10 HPV-6 and HPV-11, group A9 HPV-16, HPV-31, HPV-33 and HPV-35; and group A7 HPV-18, HPV-39 and HPV-45). Results indicated that, independently of L1 sequence homology, VLPs of each genotype examined induced high-titer antibody specificities that were entirely genotype-specific. Previously characterized virus-neutralizing monoclonal antibodies were used in a VLP epitope-blocking assay to generate indirect evidence of a correlation between high-titer polyclonal antibody specificities and virus-neutralizing activity. To provide more direct evidence of virus-neutralizing activity, several groups have generated HPV pseudovirions [28,29,31,32]. In the present study, pseudovirions of HPV-16, HPV-18 and HPV-33 were produced and used to examine the potential for cross-neutralization among several clinically relevant HPV genotypes.

2. Materials and methods

2.1. Sources of L1 coding sequences and construction of recombinant baculoviruses

Construction of recombinant baculoviruses Ac11L1 and Ac18L1 has been described [15,22]. In the present study, HPV L1 sequences were amplified by PCR using oligonucleotide primers listed in Table 1. PCR products were purified, digested with Bgl II and Xba I, and inserted into baculovirus transfer vector pVL-1392 (kindly provided by V. Luckow). Recombinant baculoviruses were then constructed as described [33]. Ac16L1_{Roche} was generated from the HPV-16_{Roche} isolate [34]. Genomic clones of HPV-31 and HPV-35 were obtained from the American Type Culture Collection (Manassas, VA); genomic clones of HPV-33 and HPV-39 were kindly provided by M. Favre and G. Orth (Institut Pasteur, Paris), and the HPV-45 cloned genome was kindly provided by A. Lorincz (DiGene Diagnostics, Silver Spring, MD). HPV-16 L1 and L2 sequences used for the generation of recombinant vaccinia viruses were kindly provided by H.G. Köchel. Compared to the prototype L1 this isolate contains five nucleotide substitutions resulting in two amino acid exchanges at positions 202 (aspartate for histidine) and 266 (alanine for threonine), respectively.

Table 1
Oligonucleotides used for L1 cloning by PCR

HPV type		Oligonucleotide sequences ^a
31	Forward	5'-CATAGATCTTATAAATAATGTCTCTGTGGCGGCTAGC-3'
	Reverse	5'-CATACATTCTAGACTTACCTTTTATGTTTTTACGTTTTTGCTGG-3'
33	Forward	5'-CATAGATCTTATAAATAATGTCCGTGTGGCGGCTAGTG-3'
	Reverse	5'-CATACATTCTAGACTTATTTTATAACCTTTTGGCTTTTGACAGACG-3'
25	Forward	5'-CATAGATCTTATAAATAATGTCTCTGTGGCGGTCTAAC-3'
	Reverse	5'-CATACATTCTAGACTTAACTTTTTACTTTTCTACGTTTAGTG-3'
39	Forward	5'-CATAGATCTTATAAATAATGTGGCGGTCTAGTGACAGC-3'
	Reverse	5'-CATACATTCTAGACTTATTTAGACACACGTTTACG-3'
45	Forward	5'-CATAGATCTTATAAATAATGGCTTTGTGGCGGCTAGTGAC-3'
	Reverse	5'-CATACATTCTAGACTTATTTCTTACTACGTATACG-3'

^a L1 start/stop codons are underlined.

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Table 1
Oligonucleotides used for L1 cloning by PCR

HPV type		Oligonucleotide sequences ^a
31	Forward	5'-CATAGATCTTATAAATA <u>ATG</u> TCTCTGTGGCGGCTAGC-3'
	Reverse	5'-CATACATTCTAGAC <u>TTA</u> CCCTTTTATTTTTACGTTTTCGTTGG-3'
33	Forward	5'-CATAGATCTTATAAATA <u>ATG</u> TCCGTTGGCGGCTAGTG-3'
	Reverse	5'-CATACATTCTAGAC <u>TTA</u> TTTTTTTACCTTTTTCGTTTTCGACAGC-3'
35	Forward	5'-CATAGATCTTATAAATA <u>ATG</u> TCTCTGTGGCGGCTAAC-3'
	Reverse	5'-CATACATTCTAGAC <u>TTA</u> ACTTTTACTTTTCTACGTTTAGTG-3'
39	Forward	5'-CATAGATCTTATAAATA <u>ATG</u> TGCGCGTCTAGTGACAC-3'
	Reverse	5'-CATACATTCTAGAC <u>TTA</u> TTTAGACACACGTTTACG-3'
45	Forward	5'-CATAGATCTTATAAATA <u>ATG</u> GCTTTGTGGCGGCTAGTGAC-3'
	Reverse	5'-CATACATTCTAGAC <u>TTA</u> TTTCTTACTACGTATACG-3'

^a L1 start/stop codons are underlined.

Table 2
Titration of VLP polyclonal antisera

Antigens	ELISA titers of VLP polyclonal antibodies								
	Group A10		Group A9				Group A7		
	Anti-6	Anti-11	Anti-16	Anti-31	Anti-33	Anti-35	Anti-18	Anti-39	Anti-45
6 VLPs	>4500	>4500	<1500	<1500	<1500	<1500	<1500	<1500	<1500
11 VLPs	>1300	>121 500	<1500	<1500	<1500	<1500	<1500	<1500	<1500
16 VLPs	<1500	<1500	>4500	<1500	<1500	<1500	<1500	<1500	<1500
31 VLPs	<1500	<1500	<1500	>13 500	>1500	<1500	<1500	<1500	<1500
33 VLPs	<1500	<1500	<1500	>4500	>40 500	<1500	<1500	<1500	<1500
35 VLPs	<1500	<1500	<1500	<1500	<1500	>40 500	<1500	<1500	<1500
18 VLPs	<1500	<1500	<1500	<1500	<1500	<1500	>40 500	<1500	>4500
39 VLPs	<1500	<1500	<1500	<1500	<1500	<1500	<1500	>13 500	<1500
45 VLPs	<1500	<1500	<1500	<1500	<1500	<1500	>1500	<1500	>40 500

2.2. VLP production and purification

Trichophusia ni cells (Hi5, Invitrogen, San Diego, CA) were used for VLP production. Cells were propagated in 100-ml shake cultures in 1-l flasks (125 rpm, 27°C) in ExCell 400 serum-free medium (JRH BioSciences), and were infected at a multiplicity of infection of 3. Infected cell cultures were incubated with shaking for 72 h at 27°C. Following this, cells were pelleted (800 × g) and resuspended in 3 ml of Buffer N (phosphate-buffered saline (PBS); 0.5 M NaCl; phenylmethylsulfonyl fluoride (PMSF) (1 mM); Aprotinin (1 µg/ml); Leupeptin (5 µg/ml); Pefabloc (0.05 mM); Pepstatin A (1 µg/ml); protease inhibitors were obtained from Boehringer-Mannheim, Indianapolis, IN). Resuspended cells were sonicated on ice (four 5-s bursts), sonicates were diluted with PBS, and cesium chloride was added to a final concentration of 0.4 g/ml. Following ultracentrifugation (100 000 × g, 18 h, 15°C), bands at the expected buoyant density of empty capsids were removed by syringe and dialyzed against Buffer N (18 h, 4°C). Protein concentrations were determined using a commercial assay (MicroBCA, Pierce Biochemicals, Inc.). LI assembly into VLPs was verified by electron microscopy for each genotype, as previously described [22] (data not shown). VLPs of HPV-6 were kindly provided by S. Burke (MedImmune, Inc., Gaithersburg, MD), and were produced using similar methods.

2.3. VLP antibodies

For each HPV genotype, VLP polyclonal immune sera were prepared by immunizing a pair of New Zealand white rabbits as described [17,35]. Rabbit HPV-6 VLP polyclonal antiserum was the gift of R.B.S. Roden (NIH, Gaithersburg, MD, USA), and was produced using similar methods [23]. Methods used for the production and characterization of monoclonal antibodies included in this study have been described elsewhere [27,36].

2.4. Enzyme-linked immunosorbent assay (ELISA)

VLP ELISA methods have been described [37]. Briefly, purified VLPs were diluted in PBS to 0.01 mg/ml, and 0.1 ml aliquots were dispensed into wells of 96-well microtiter plates. In endpoint titration assays, serial dilutions of VLP immune sera were tested in duplicate against each of the VLP antigens. Specific absorbance values were calculated by subtracting values obtained in PBS wells from values obtained in antigen-containing wells, and replicate values were averaged. Replicate variation in these assays was minimal (i.e. less than 5%). For each antiserum, an endpoint titer was calculated by determining the highest dilution of serum that produced an absorbance value on the linear portion of the titration curve. Each antigen/antibody combination was evaluated by ELISA on at least three separate occasions with equivalent results.

2.5. Antigen denaturation

VLPs were denatured by boiling (10 min) in carbonate buffer (pH 9.5; 0.01 mg/ml final concentration). Non-denatured VLP antigens were diluted similarly in phosphate-buffered saline (PBS, pH 7.1) and kept on ice prior to analysis.

2.6. Antibody absorption

VLP post-immune sera were diluted using results from the endpoint titration assay as a guide (Table 2), and then tested in serum preabsorption ELISAs. Briefly, most of these antisera were evaluated at the relatively high dilution of 13 500. Due to a relatively lower titer, however, HPV-16 VLP antibodies were evaluated at the next lower dilution (i.e. 4500) (Table 2). Sera were diluted in phosphate-buffered saline and were either not preabsorbed, or preabsorbed overnight at 4°C with VLPs of either homotypic or heterotypic

(i.e. from the same phylogenetic group) HPV genotypes (0.1 mg/ml), as indicated (Table 4). Absorbed sera were centrifuged briefly prior to evaluation by ELISA (14 000 × g, 2 min). Residual optical density values following homotypic VLP absorption were subtracted from values obtained by heterotypic VLP absorption of the same antiserum. Negative values were adjusted to zero.

2.7. Epitope blocking ELISA

In this assay, a reference monoclonal antibody (MAb) is evaluated for the ability to inhibit VLP binding by polyclonal antibodies raised against VLPs of the same genotype. The ability of the MAb to cause greater than 50% reduction in polyclonal antibody binding is interpreted as evidence that the epitope defined by the MAb may represent a dominant specificity in the polyclonal antiserum. The assay requires that monoclonal antibodies (MAbs) and test polyclonal antisera be raised in different host species. To examine the ability of a MAb to interfere with VLP polyclonal antibody binding, VLP antigens were added to 96-well microtiter plates and allowed to attach for 2 h at room temperature. Plates were then washed (three times) and blocked (1 h, room temperature) with a solution containing bovine serum albumin (2% w/v in PBS). Next, serial dilutions of MAbs were added in parallel rows of the plate. Plates were incubated overnight at 4°C. Plates were washed as before and pre-titered VLP polyclonal antibodies were diluted to a pre-determined point (i.e. just below the level of antigen saturation) and added at constant dilution to all wells containing pre-formed VLP/MAb complexes. Bound immunoglobulins (Ig) (i.e. MAbs and rabbit polyclonal antibodies) were then detected by adding appropriate anti-Ig enzyme conjugates in alternative parallel rows of the microtiter plate. For HPV-11 and HPV-18, VLP polyclonal antisera were first pre-absorbed with VLPs of HPV-6 and HPV-45, respectively, to remove cross-reacting specificities.

2.8. Construction of vaccinia viruses recombinant for HPV L1 and L2

HPV-16 L1 was amplified by PCR and cloned into the NcoI and XhoI sites of pTM1 [38] to yield pTM16L1. L2 was cloned into the EcoRI and BamHI sites of pTM1 after amplification by PCR (pTM16L2). L1 and L2 of HPV-18 were amplified from pHVP-18 by PCR and cloned into the NcoI and BamHI sites of pTM1 to yield pTM18L1 and pTM18L2, respectively. Recombinant vaccinia viruses vac16L1, vac16L2, vac18L1, and vac18L2 were obtained after cotransfection of wild-type vaccinia virus DNA with pTM16L1, pTM16L2, pTM18L1, and pTM18L2, respectively, following published protocols [29]. The generation of

vac33L1 and vac33L2 recombinant for L1 and L2 of HPV-33, respectively, has previously been described [29].

2.9. Generation of pseudovirions

Pseudovirions of HPV-16, HPV-18, and HPV-33 were produced using published protocols with slight modifications [29]. Briefly, COS-7 cells were transfected with marker plasmid pEGFP-GFP-NLS. This plasmid codes for a dimer of the green fluorescent protein (GFP) to which the nuclear localization signal of HPV-33 L1 was fused in frame. Transfected cells were grown for 48 h and were subsequently infected with vaccinia viruses recombinant for HPV-16 L1 (vac16L1) and L2 (vac16L2), HPV-18 L1 (vac18L1) and L2 (vac18L2), or HPV-33 L1 (vac33L1) and L2 (vac33L2), respectively. Since the L1 and L2 genes were placed under the control of the phage T7 RNA polymerase promoter, the helper vaccinia virus VTF7-3 encoding the T7 RNA polymerase was used in addition. Pseudovirions were extracted from nuclei 40 h postinfection by sonication in hypotonic buffer supplemented with 0.5% NP40 and subsequently purified by buoyant caesium chloride density gradients and sucrose step gradients essentially as described [29].

2.10. Infectivity assay

Pseudovirions were preincubated for 1 h at 37°C with 100 µg/ml DNase I in a total volume of 25 µl PBS, pH 6.8, 10 mM MgCl₂. They were subsequently added to 2.6×10^5 COS-7 cells suspended in 300 µl PBS, pH 6.8, supplemented with 100 µg/ml bovine serum albumin and incubated for 1 h at 4°C as described [29]. Cells were then seeded into six-well plates, grown for 48–72 h and subsequently examined for GFP fluorescence. To avoid quenching of the fluorescence by the medium, cells were washed with PBS and fixed for 20 min with 2% formaldehyde in PBS. Cells were then covered with histogel.

3. Results

3.1. Recombinant expression of papillomavirus L1 proteins

We previously produced sub-open reading frame (ORF) HPV-6 L1 bacterial fusion proteins, and used them to localize the papillomavirus L1 (PVL1) common antigen region [35]. PVL1 antiserum was found to be broadly cross-reactive with L1 proteins of cutaneous and mucosal HPVs, and also BPV-1 [35]. In the present study, we used the PVL1 polyclonal antiserum (R5-409) [35] in a Western blot immunoassay to confirm expres-

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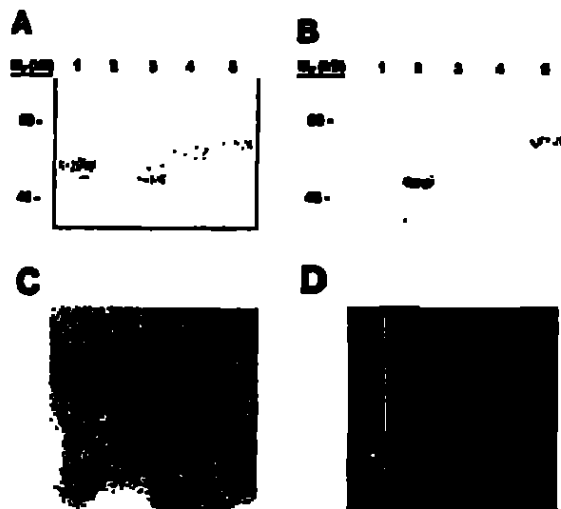


Fig. 1. SDS-PAGE/Western blot immunosay of recombinant VLP antigens. L1 VLPs of HPV-31 (lanes 1), HPV-33 (lanes 2), HPV-35 (lanes 3), HPV-39 (lanes 4) and HPV-45 (lanes 5) were purified and immunoblotted as described [15,35]. (A) The panel of HPV L1 proteins was probed with a rabbit polyclonal antiserum raised against the papillomavirus L1 (PVL1) common antigen region (R3-409) [35]. (B) The same panel was probed with a rabbit polyclonal antiserum raised against the amino-terminal region of HPV-6 L1 (R7-435) [35]. Analysis of pseudovirions: (C) Electron micrograph of pseudovirions of HPV-16 purified by cesium chloride buoyant density gradient followed by a sucrose step gradient. Pseudovirions were spotted onto carbon-coated copper grids and negatively stained with 2% phosphotungstic acid. Photographs were taken with a Zeiss EM900 transmission electron microscope at an instrumental magnification of $\times 50\,000$. The bar represents 100 nm. (D) COS-7 cells were incubated with HPV-16 pseudovirions in an infectivity assay, plated onto tissue culture plates, and subsequently grown for 72 h. GFP was visualized using a fluorescence microscope at an instrumental magnification of $200\times$.

sion of recombinant L1 proteins. While this antiserum was able to detect recombinant expression of most of the L1 proteins (i.e. types 31, 35, 39 and 45), it was relatively much less immunoreactive with HPV-33 L1 (Fig. 1A). In an equivalent assay conducted with a different HPV-6 L1 sub-ORF antiserum (R7-435, [35], HPV-33 L1 expression was confirmed by Western blot (Fig. 1B). These observations suggested that the L1

'common antigen' region may be relatively less well conserved in HPV-33, and that the L1 proteins of HPV-33 and HPV-45 were antigenically cross-reactive with the HPV-6 L1 amino-terminal region used to produce the antiserum [35] (Fig. 1B).

3.2. VLPs of nine HPV types induce high-titer type-specific polyclonal antibody specificities

VLP polyclonal immune sera were evaluated in an endpoint titration ELISA as described previously [37]. For each genotype evaluated, highest polyclonal antibody titers invariably were directed against VLPs of the same type used for immunization (i.e. homotypic VLPs) (Table 2). While significant cross-reactivity was not detected between members of different phylogenetic groups, variable levels of cross-reactivity were detected within groups, and these generally were consistent with variations in L1 sequence homology [23]. Strong reciprocal cross-reactivity was evident, for example, between closely related genotypes (i.e. group A10 types 6 and 11, and group A7 types 18 and 45), while group A9 (i.e. HPV-16-related viruses) cross-reactivities were relatively lower in magnitude (Table 2). These patterns of ELISA immunoreactivity were consistent with results reported previously by other investigators, that were generated using different methods [23].

3.3. Relative immunogenicities of linear versus non-linear capsid epitopes

Previous work has demonstrated conformational dependence of VLP antigenicity for several papillomavirus genotypes [14,15,22,23]. To evaluate the relative immunogenicities of linear versus non-linear epitopes of VLPs produced in the present study, polyclonal antisera were tested in an ELISA at high dilution (i.e. 1:500) against the same antigens used for immunization, in either native or denatured form. Results (Table 3) indicated that dominant VLP polyclonal antibody specificities for most of these genotypes were largely non-linear (i.e. conformationally dependent) in nature. HPV-35 presented an exception to this general

Table 3
Conformational dependence of VLP antigenicity

Antisera	VLP status	ELISA reactivity (A_{492})								
		6 VLPs	11 VLPs	16 VLPs	31 VLPs	33 VLPs	35 VLPs	18 VLPs	39 VLPs	45 VLPs
VLP Pab*	Native	2.680	2.771	0.809	1.622	2.749	2.165	2.204	1.957	1.389
	Denatured	0.118	0.646	0.175	0.223	0.870	1.567	0.221	0.167	0.190
PVL1	Native	0.279	0.119	0.203	0.148	0.107	0.121	0.105	0.131	0.102
	Denatured	1.778	1.054	0.779	1.099	0.311	1.067	1.034	0.946	1.003

* Cognate VLP polyclonal antisera.

Table 4
VLP serum preabsorption ELISAs

VLP antisera	Absorption antigens	ELISA seroreactivity (A_{492})			
Group A10		HPV-6 VLP	HPV-11 VLP		
α -HPV-6 VLP	None	1.738	0.958		
	HPV-6 VLP	0.000	0.000		
	HPV-11 VLP	1.567	0.000		
α -HPV-11 VLP	None	0.421	2.145		
	HPV-6 VLP	0.000	1.973		
	HPV-11 VLP	0.000	0.000		
Group A7		HPV-18 VLP	HPV-45 VLP		
α -HPV-18 VLP	None	1.542	0.357		
	HPV-18 VLP	0.000	0.000		
	HPV-45 VLP	1.625	0.000		
α -HPV-45 VLP	None	0.898	1.573		
	HPV-18 VLP	0.000	1.138		
	HPV-45 VLP	0.000	0.000		
Group A9		HPV-16 VLP	HPV-31 VLP	HPV-33 VLP	HPV-35 VLP
α -HPV-16 VLP	None	0.983	0.247	0.000	0.000
	HPV-16 VLP	0.000	0.000	0.000	0.000
	HPV-31 VLP	0.768	0.000	0.000	0.000
	HPV-33 VLP	0.965	0.228	0.000	0.000
	HPV-35 VLP	0.958	0.241	0.000	0.000
α -HPV-31 VLP	None	0.112	1.105	0.241	0.009
	HPV-16 VLP	0.000	1.025	0.228	0.000
	HPV-31 VLP	0.000	0.000	0.000	0.000
	HPV-33 VLP	0.009	0.881	0.000	0.025
	HPV-35 VLP	0.019	1.074	0.224	0.000
α -HPV-33 VLP	None	0.218	0.000	0.937	0.000
	HPV-16 VLP	0.000	0.000	1.030	0.000
	HPV-31 VLP	0.083	0.000	1.040	0.000
	HPV-33 VLP	0.000	0.000	0.000	0.000
	HPV-35 VLP	0.156	0.000	0.973	0.000
α -HPV-35 VLP	None	0.302	0.020	0.021	1.045
	HPV-16 VLP	0.000	0.000	0.012	1.140
	HPV-31 VLP	0.013	0.000	0.000	1.103
	HPV-33 VLP	0.104	0.000	0.000	1.058
	HPV-35 VLP	0.000	0.000	0.000	0.000

rule, however, as a significant level of immunoreactivity remained when HPV-35 antiserum was tested against denatured HPV-35 particles (Table 3). Native/denatured VLP antigens were evaluated within the same assay using PVL1 antiserum, and results confirmed that the common antigen region of HPV-35 L1 was masked in native particles, suggesting that the particles were largely intact [15]. Consistent with Western blot immunoassay results (Fig. 1), PVL1 was relatively less immunoreactive with denatured HPV-33 L1 than with denatured L1 proteins of other types (Table 3). These findings support the general conclusion that dominant antigenic specificities of papillomavirus capsids are primarily non-linear in nature.

3.4. Relative immunogenicities of unique versus cross-reactive capsid epitopes

VLPs and corresponding post-immune sera were next evaluated in a serum absorption ELISA. Polyclonal post-immune sera were diluted and absorbed as described (see Section 2 for details) and then reacted in an ELISA against VLPs as indicated (Table 4). Consistent with results in Table 2, sera raised against VLPs of closely related genotypes (i.e. types 6 and 11, and types 18 and 45) were immunoreactive with both homotypic and heterotypic related VLP antigens (Table 4). However, each of these sera demonstrated a higher level of immunoreactivity with the same antigen used for immu-

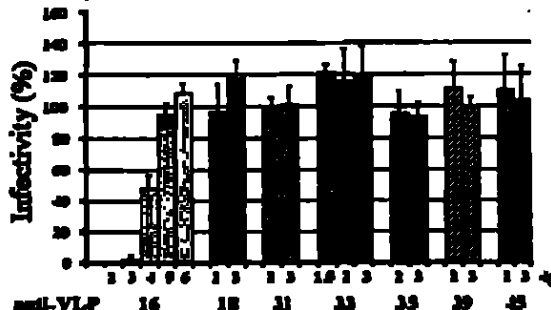
nization, and heterotypic VLP absorption of sera had only minor effects on these cognate interactions (Table 4), suggesting that the epitopes shared by HPV-6 and HPV-11, and by HPV-18 and HPV-45, were antigenically distinct from relatively more immunogenic type-specific domains. Similarly, high-titer antibody specificities within group A9 were affected only marginally by heterotypic VLP absorption, and observed antigenic cross-reactivities within group A9 were relatively much lower in magnitude than those within the other groups.

3.5. Cross-neutralization of pseudovirions

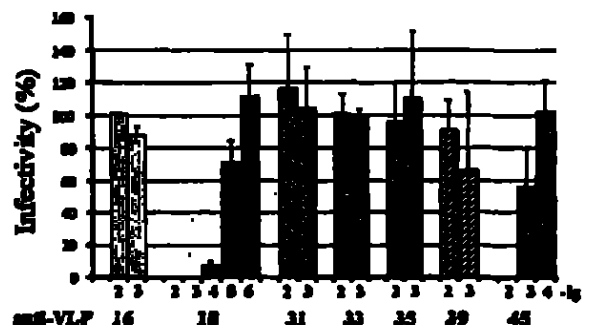
To test if ELISA cross-reactivity is due to cross-neutralizing antibodies, infectivity assays were carried out using HPV-16, HPV-18, and HPV-33 pseudovirions. These were generated by synthesis of capsid proteins in COS-7 cells harbouring the marker plasmid pEGF-PGFP-NLS, which codes for a dimeric green fluorescent protein (GFP). Generation of HPV-33 pseudovirions has been described previously [29]. Electron microscopy analysis of HPV-16 and HPV-18 pseudovirion preparations also revealed the presence of VLPs as shown for HPV-16 (Fig. 1C). Pseudoinfection

assays were carried out to prove that pseudovirions indeed contained the marker plasmid and were able to transfer it into COS-7 cells. To exclude the transfer of free DNA or DNA associated with outer surface structures of VLPs, pseudovirions were preincubated with DNase I. As shown in Fig. 1D for HPV-16, cells expressing GFP showed strong fluorescence mainly restricted to the nucleus, as expected. Similar results were obtained for HPV-18 and HPV-33. The number of infected cells was similar for all three HPV-types (data not shown). This suggests that HPV-16, HPV-18, and HPV-33 VLPs not only package the marker plasmid at similar yields but also transfer the marker plasmid into cells with comparable efficiency. For neutralization studies, pseudovirions were incubated with serial dilutions of VLP antisera and were added to COS-7 cells. Number of fluorescent cells served as a measure of infectivity. First we assayed the neutralization of pseudovirions by homologous VLP antisera. For HPV-33, complete neutralization was observed up to a dilution of $-4 \log_{10}$ (Fig. 2C) in agreement with our previous results [29]. For HPV-16 and HPV-18 the neutralization potential was slightly reduced at $-4 \log_{10}$ dilution. The residual infectivity amounted to 50% for HPV-16 and 10% for HPV-18, respectively (Fig. 2A and B). For

A. HPV-16 Pseudovirions



B. HPV-18 Pseudovirions



C. HPV-33 Pseudovirions

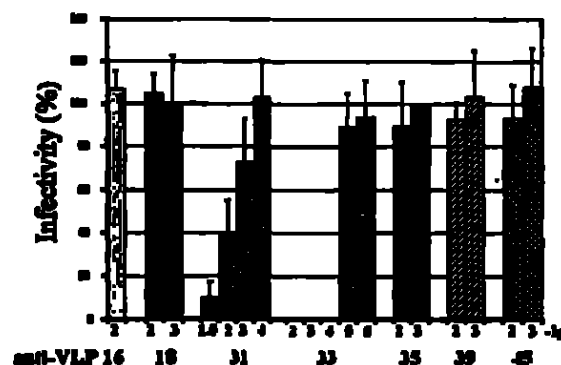


Fig. 2. Cross-neutralization analysis of pseudovirions. Pseudovirions of (A) HPV-16, (B) HPV-18 and (C) HPV-33 were preincubated with the indicated dilutions of the indicated VLP antisera for 1 h at 37°C and were then evaluated in an infectivity assay. An infectivity of 100% corresponds to 33 (A), 34 (B) and 35 (C) infected cells, respectively. Averages from three independent experiments are shown.

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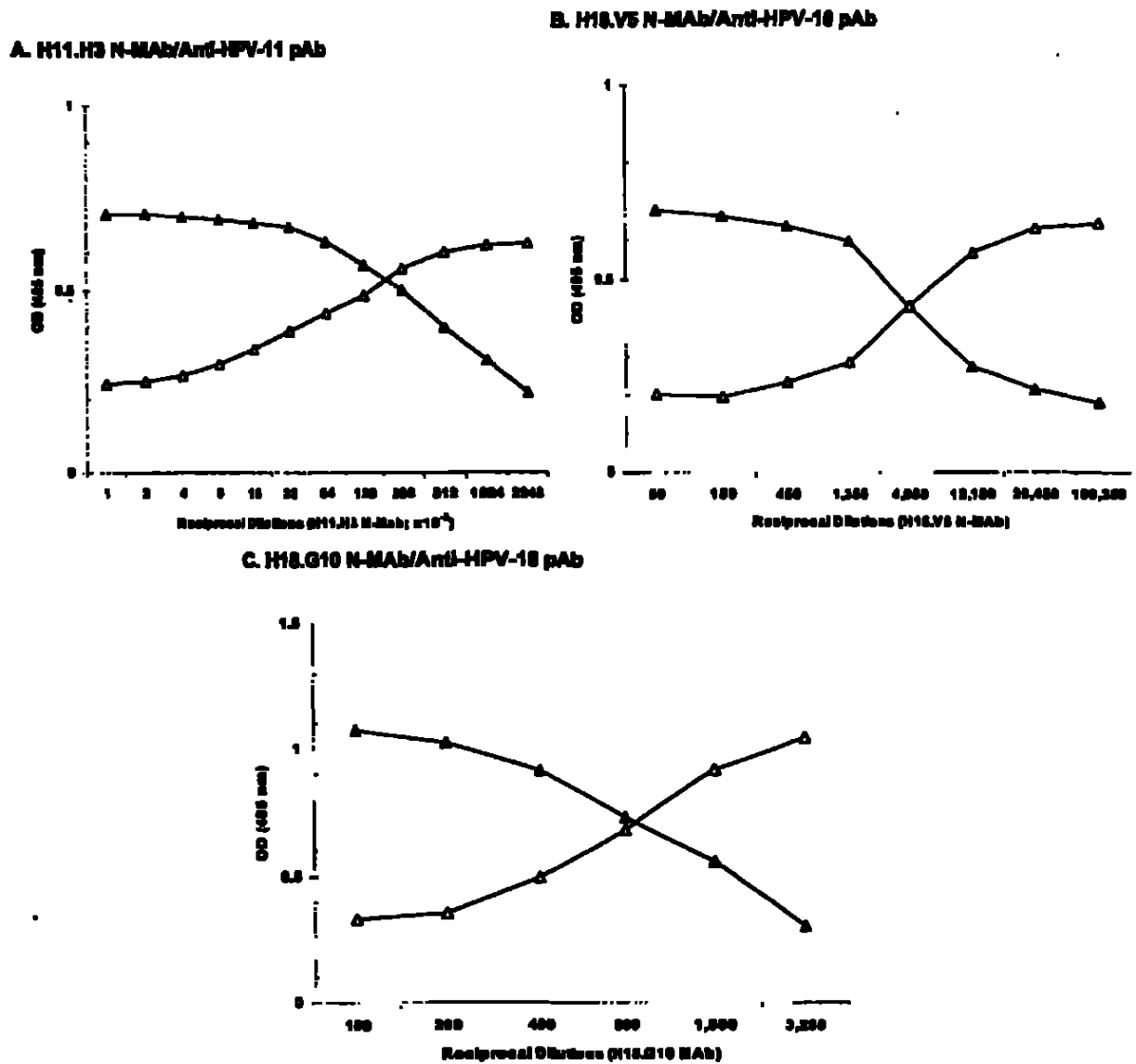


Fig. 3. Inhibition of reactivity of VLP polyclonal antisera with VLPs by type-specific neutralizing monoclonal antibodies (N-MAbs). (A) HPV-11 VLPs/H11.H3 N-MAb. Two-fold serial dilutions of H11.H3 N-MAb were reacted with HPV-11 VLPs in parallel rows of the microtiter plate, as indicated. Following this, rabbit HPV-11 VLP polyclonal antibodies were preabsorbed with HPV-6 VLPs (Section 2) and added at constant dilution (1:10 000) to VLP/N-MAb complexes. Bound H11.H3 N-MAb (filled symbols) was detected by adding anti-murine Ig enzyme conjugate in one row, and bound rabbit anti-HPV-11 VLP polyclonal antibodies (open symbols) were detected by adding anti-rabbit Ig enzyme conjugate in the second parallel row. (B) HPV-16 VLPs/H16.V5 N-MAb. Two-fold serial dilutions of H16.V5 were reacted with HPV-16 VLPs, as indicated. Next, HPV-16 VLP polyclonal antibodies were added at constant dilution (1:3200). Bound H16.V5 N-MAb (filled symbols) and bound rabbit anti-HPV-16 VLP polyclonal antibodies (open symbols) were detected as above. (C) HPV-18 VLPs/H18.G10 N-MAb. Two-fold serial dilutions of H18.G10 were reacted with HPV-18 VLPs as above. Rabbit HPV-18 VLP polyclonal antibodies were preabsorbed with HPV-45 VLPs and then added at constant dilution (1:13 300) to VLP/H18.G10 MAb complexes. Bound H18.G10 MAb (filled symbols) and bound rabbit HPV-18 VLP polyclonal antibodies (open symbols) were detected as above.

HPV-18 a notable reduction to approximately 80% was still observed at $-5 \log_{10}$ (Fig. 2B). The lower neutralization titer of the HPV-16 antiserum may possibly be due to the use of different HPV-16 isolates for the production of VLPs (114/K) and of pseudovirions. They differ at amino acid position 266 (T for A), which

has recently been shown to be crucial for binding of a neutralizing monoclonal antibody [39].

Next we assayed the cross-neutralization of pseudovirions by VLP antisera. None of the antisera tested cross-neutralized HPV-16 pseudovirions (Fig. 2A). HPV-31 VLP antiserum, which cross-reacts weakly

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with HPV-16 VLPs by ELISA, was unable to reduce the infectivity of HPV-16 pseudovirions. Interestingly, this was also found to be true for HPV-33 VLP antiserum, which previously demonstrated weak cross-neutralizing activity against HPV-16 virions [24]. By contrast, HPV-18 pseudovirions were completely neutralized by HPV-45 antiserum up to a dilution of $-2 \log_{10}$ (Fig. 2B), and reduction of infectivity to 50% occurred at $-3 \log_{10}$ dilution. None of the other antisera had any significant effect on HPV-18 pseudoinfection. This is consistent with our ELISA data. HPV-33 was cross-neutralized only by HPV-31 antiserum (Fig. 2C). Although cross-neutralization of HPV-33 pseudovirions was not complete even at low (1:30) dilution of this antiserum, a slight but significant reduction of infectivity to 75% was still observed at $-3 \log_{10}$ dilution. We conclude that weak cross-reactivity in ELISA may not always be due to cross-neutralizing antibodies.

3.6. Inhibition of reactivity of VLP polyclonal antisera with VLPs by virus-neutralizing monoclonal antibodies (N-MAbs)

A VLP epitope-blocking ELISA was developed to investigate whether highly immunogenic capsid epitopes recognized by VLP post-immune sera may be related to epitopes defined by genotype-specific N-MAbs. In this assay, VLPs and corresponding polyclonal sera were used along with previously characterized MAb H11.H3 [36]; H16.V5 [27,39,40]; H18.G10 [27]; H31.A6 [27] and H33.J3. The latter MAb has not been described previously; however, it also demonstrated conformational dependence and virus genotype-specificity by ELISA (data not shown). N-MAbs H11.H3 (Fig. 3A) and H16.V5 (Fig. 3B) efficiently inhibited most of the antibody activity in the corresponding VLP post-immune serum, suggesting that much of the activity in each antiserum may be directed at the same epitope recognized by the corresponding N-MAb. While we cannot rule out the possibility that these N-MAbs inhibited polyclonal antibody binding activity through steric hindrance, each antiserum has previously demonstrated strong neutralizing activity against cognate virions [17,18,24], which supports an interpretation involving direct competition for binding sites. High concentrations of N-MAbs were not able to completely block binding activity in post-immune sera. This may reflect the presence of other non-competing specificities in these antisera. A similar analysis of HPV-18 VLP antiserum and H18.G10 MAb produced comparable results (Fig. 3C), as did analyses of other VLP monoclonal/polyclonal combinations (i.e. H31.A6 MAb/HPV-31 VLP antibodies; and H33.J3 MAb/HPV-33 VLP antibodies; data not shown).

4. Discussion

Our central purpose was to demonstrate that, independently of L1 genetic sequence relatedness, genital HPV capsids generally demonstrate antigenic sites that are highly immunogenic and genotype-specific; that capsid antigenic cross-reactivity between closely related HPVs primarily involves epitopes that are less immunogenic and antigenically distinct from primary antigenic sites; and that predominant specificities involve virus-neutralizing domains. Cross-neutralization was first demonstrated between HPV-6 and HPV-11 [25], which are the most closely related of the known genital HPVs (92.4% identity of the L1 protein sequence) [23]. In that study, however, HPV-6 VLP polyclonal antibodies provided only partial neutralization of HPV-11, even at relatively low dilution (i.e. $-2 \log_{10}$) [25], whereas HPV-11 VLP antibodies demonstrated strong neutralizing activity at much greater dilution (i.e. $-4 \log_{10}$ and above) [25]. Similarly, while antibodies raised against VLPs of either HPV-16 or HPV-33 were able to neutralize infectious HPV-16 virions in vitro [24], results from that study indicated that HPV-33 cross-neutralizing activity was several orders of magnitude lower than that demonstrated by cognate (i.e. HPV-16) VLP antibodies [24]. We have now extended these observations in that HPV-31 and HPV-45 VLPs can induce low levels of HPV-33 and HPV-18 neutralizing antibodies, respectively, whereas the cognate pseudovirions were neutralized much more effectively. Taken together, these findings support the conclusion that cross-neutralization is relatively less efficient than direct neutralization mediated by homotypic VLP antibodies. In essence it seems that L1 genetic sequence differences of sufficient magnitude to define distinct genotypes may also be adequate to define unique viral serotypes.

Cross-neutralization was expected for HPV-18 and HPV-45 since these types display a relatively high level of L1 amino acid sequence homology (87.6% identity) [23]. However, the overall degree of sequence homology does not always seem to be a reliable indicator for cross-neutralization efficiency. Although HPV-31 is more closely related to HPV-16 (83.1% identity), HPV-31 polyclonal antibodies more efficiently cross-neutralize HPV-33 (78.4% identity) [23]. This indicates that specific amino acid residues probably located in the neutralization epitopes are critically important to define the capacity of inducing cross-neutralizing antibodies. Eight residues at positions 51, 58, 76, 105, 126, 139, 159, and 260 are identical in the L1 amino acid sequence of HPV-31 and HPV-33 but different in HPV-16 and/or HPV-35. This suggests that one or more of these amino acids may define the specificity of HPV-31 and HPV-33 neutralization, and their absence in HPV-16 may contribute to the lack of cross-neutralization of HPV-16 pseudovirions. Indeed, the residues at positions

51 and 58 as well as 126 and 139 are located within or close to two variable, hydrophilic regions of L1, which have recently been shown to influence the binding of neutralizing antibodies [39,41].

Not all antisera found to display cross-reactivity in ELISA were cross-neutralizing. This is most obvious for HPV-16. All types tested within group A9 were weakly cross-reactive with HPV-16 in ELISA but did not cross-neutralize. Interestingly, the observed cross-reactivity seems not to be type-specific with respect to the additional type recognized. That means that reactivity of, for example, HPV-31 VLP antiserum with HPV-16 VLPs was almost completely removed after preabsorption of the antiserum with HPV-33 and HPV-35 VLPs. Similarly, cross-reactivity of HPV-33 and HPV-35 VLP antisera with HPV-16 VLPs was strongly reduced after preabsorption with all group A9 VLPs tested. In contrast, cross-reactivity of anti-HPV-31 and HPV-16 with HPV-33 and HPV-31 VLPs, respectively, was hardly affected after preabsorption of the antisera with heterotypic VLPs. These results suggest that cross-neutralizing antibodies may be relatively type-specific with regard to the additional HPV type neutralized, whereas non-neutralizing cross-reactive antibodies may be more broadly cross-reactive.

Other investigators have shown that H16.V5 N-MAb strongly inhibits VLP binding activity in sera from HPV-16-infected individuals, and that such antibodies in turn could prevent H16.V5 N-MAb binding to VLPs [40]. These findings are consistent with the present results, and suggest that dominant polyclonal antibody specificities induced during natural infection may be highly similar, if not equivalent, to those induced by immunizations with recombinant VLPs.

It may be that results from the epitope-blocking assay reflect steric hindrance of antibody binding rather than direct blockade of equivalent binding specificities. However, with regard to two of the HPV types examined in this assay (i.e. HPV-11 and HPV-16), each of the polyclonal and monoclonal antibody preparations used has previously demonstrated high-titer neutralizing activity against cognate infectious virions [17,22,24,37]. Taken together, the present and previous results [17,24,36,42] suggest at least partial overlap between predominant antibody specificities present in HPV-11 and HPV-16 virion-neutralizing polyclonal antisera and specificities defined by N-MAbs H11.H3 and H16.V5, respectively.

Approaches to vaccination range from using killed or live attenuated organisms to recombinant proteins to subunit epitopes. While subunit vaccines may be attractive, data from this and other previous studies summarized above suggest that broad protection from genital HPV disease will require polyvalent vaccines that incorporate VLPs of each serotype for which protection is desired.

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Assessment of the Serological Relatedness of Genital Human Papillomaviruses by Hemagglutination Inhibition

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To assess the potential for cross-protection among genital human papillomavirus (HPV) types in virus-like particle (VLP)-based vaccinations, inhibition of HPV VLP-mediated hemagglutination by rabbit antisera raised against HPV type 6b (HPV-6b), HPV-11, HPV-16, HPV-18, HPV-31, HPV-33, and HPV-45 was analyzed. Only highly homologous types (HPV-6b and HPV-11, and HPV-18 and HPV-45) exhibited detectable serological cross-reaction for the class of antibodies that inhibit virion-to-cell surface binding. However, analysis of neutralizing monoclonal antibodies to several animal and human papillomaviruses indicated that over half of these antibodies do not prevent cell surface binding, but these latter antibodies do not appear to be more cross-reactive in enzyme-linked immunosorbent assays than those that mediate inhibition of hemagglutination. The data strongly suggest that while there may be limited cross-protection between highly (>85% L1 amino acid identity) homologous types, protection by HPV VLP-based vaccines will be predominantly type specific.

Experimental and epidemiologic studies support the concept that human papillomaviruses (HPVs) are important etiologic agents in cervical carcinogenesis (19, 31). Certain high-risk genital HPV types such as HPV type 16 (HPV-16), HPV-18, HPV-31, HPV-33, and HPV-45, but not low-risk HPV-6 and HPV-11, are associated with cervical and other anogenital cancers (1).

When expressed from a strong heterologous promoter in eukaryotic cells, the major papillomavirus capsid protein, L1, self-assembles into virus-like particles (VLPs) (13, 16, 25, 26). Immunization with L1 VLPs, but not unassembled L1, stimulates production of high titers of neutralizing antibodies (5, 16). This property and the absence of viral genomes suggest that VLPs could be used as a safe prophylactic vaccine to prevent papillomavirus infection (27). Studies of the efficacy of prophylactic VLP-based vaccines, which have been conducted with animal papillomaviruses, such as cottontail rabbit papillomavirus (CRPV), canine oral papillomavirus (COPV), and bovine papillomavirus type 4 (BPV-4), indicate that VLPs effectively prevent experimental papillomavirus infection (2, 17, 29). Protection can also be obtained by passive transfer of serum antibodies from vaccinated to naïve animals, suggesting that the protection is mediated by neutralizing antibodies (2, 29).

Although *in vivo* and *in vitro* data suggest that the neutralizing antibody response to papillomaviruses (or VLPs) is type specific when distantly related papillomaviruses have been examined (7, 15), the serological relatedness of more closely related HPVs is less clear. In particular, the L1 sequences of certain common genital HPVs share strong homology (Table 1) (3), but the degree to which antiviral antibodies cross-neutralize is unknown. Such information is important for understanding the evolution of papillomaviruses and for deter-

mining how many HPV types might be needed in a VLP-based vaccine that could protect against most genital HPV infections.

In vitro assays of cross-neutralization by antisera to heterologous HPV types represent the most relevant assays for evaluating the potential for cross-protection (20). However, for high-risk genital HPVs, the requisite assays are not yet available because of the lack of a source of infectious virus and/or of an appropriate infectivity assay (12). Therefore, several attempts have been made to develop surrogate assays for neutralization to determine the serological relatedness of the HPVs. Binding by antisera raised against VLPs of one HPV type to VLPs derived from another type in an enzyme-linked immunosorbent assay (ELISA) can demonstrate the presence (or absence) of cross-reactive epitopes (24). However, it is unclear what proportion of such cross-reactive antibodies is neutralizing, especially because VLP preparations usually contain significant quantities of improperly folded L1 that elicit and bind to nonneutralizing cross-reactive antibodies. In addition, contaminants common to VLP preparations used for immunization and antigen on the ELISA plate may give false-positive results.

Alternatively, the ability of antibodies to inhibit virion interaction with cell surfaces can be measured by inhibition of VLP-mediated agglutination of mouse erythrocytes (21). An advantage of this assay is that antibodies must bind to correctly assembled capsids to prevent cell surface binding. Therefore, it does not measure antibodies to internal or denatured epitopes. The induction of sera with high hemagglutination inhibition (HAI) titers to the homologous papillomavirus has been found to correlate with both *in vitro* neutralization of BPV-1 virions and protection of rabbits from experimental infection with CRPV (21). However, a preliminary analysis of monoclonal antibodies raised against BPV-1 virions suggested that cell surface binding inhibition assays may be more stringent than neutralization, in that a subset of the monoclonal neutralizing antibodies did not inhibit viral binding to the cell surface (21, 23).

In this study, we have examined the ability of polyclonal rabbit sera to VLPs derived from a number of common high-

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TABLE 1. L1 amino acid sequence identity comparison between common genital HPV types and a variant*

HPV type	L1 amino acid sequence identity (%) between HPV types							
	6b	11	16K	18	31	33	45	16Z
6b	100	92.4	69.6	64.3	69.8	69.6	65.5	69.4
11	92.4	100	69.1	62.7	70.3	68.8	64.0	68.9
16K	69.6	69.1	100	66.0	83.5	80.9	66.5	98.6
18	64.3	62.7	66.0	100	65.1	65.8	67.6	65.6
31	69.8	70.3	83.5	65.1	100	78.4	64.2	83.1
33	69.6	68.8	80.9	65.8	78.4	100	65.9	80.3
45	65.5	64.0	66.5	67.6	64.2	65.9	100	65.8
16Z	69.4	68.9	98.6	65.6	83.1	80.3	65.8	100

* Sequence comparison was performed by using the Genetic Computer Group (University of Wisconsin) computer program Dnash with a gap creation penalty of 1.00 and a gap extension penalty of 0.10 and is presented as maximum percent sequence identity.

and low-risk genital HPV types to inhibit hemagglutination mediated by VLPs derived from each of these HPV types. The ability of a number of neutralizing monoclonal antibodies to inhibit VLP-mediated hemagglutination was also explored to assess what proportion of neutralizing antibodies is measured in the assay.

Cross-inhibition of HPV VLP-mediated hemagglutination by antisera to different HPV types. Recombinant baculovirus expressed VLPs derived from seven of the most common genital HPV types (HPV-6b, -11, -16, -18, -31, -33, and -45), as well as VLPs of the L1 from a variant of HPV-16 isolated in Zaire (4, 14) and the assembly-deficient L1 of the reference strain of HPV-16 L1 (18, 28), were purified by sucrose gradient and cesium chloride gradient centrifugation as previously described (21). Polyclonal antisera to purified VLPs were generated by three immunizations of New Zealand White rabbits with 300 µg of L1 protein, the first immunization with Freund's complete adjuvant and subsequent booster immunizations with Freund's incomplete adjuvant at 2-week intervals. Sera were precleared of nonspecific hemagglutinating activity and heated for 30 min at 56°C (11) and threefold serially diluted in phosphate-buffered saline (PBS) containing 1 mg of bovine serum albumin per ml (21). The minimum concentration of VLPs necessary for complete agglutination of 50 µl of a 1% suspension of mouse erythrocytes was determined empirically for use in these assays. Different amounts of L1 protein were used for different types, because the efficiency of hemagglutination varied among types and preparations, presumably a result of differences in the percentage of L1 that was properly assembled.

Each serum was tested against every HPV VLP type and against rhesus papillomavirus (RhPV) type 1 VLPs (which exhibits 77.4% amino acid identity with HPV-16 L1) in an HAI assay to assess serological relatedness (Table 2). Certain HPV types consistently generated higher HAI titers against their homologous VLPs than did other HPV types, i.e., HAI titers from HPV-16 and HPV-18 were greater than those generated by HPV-6b, -11, -31, -33, and -45. The differences appeared to correspond to the efficiency of VLP assembly, since HAI titer was inversely correlated with the quantity of L1 used in the assays.

None of the HPV types showed consistent and reciprocal cross-inhibition of hemagglutination, except for the two most closely related pairs: HPV-6b with HPV-11 and HPV-18 with HPV-45 (Table 2). Even with these pairs, whose L1 proteins show 92.4 and 87.6% sequence identity, respectively (Table 1), cross-inhibition was relatively weak. Antisera to HPV-6b demonstrated 2- or 3-log₂-unit-greater reactivity with HPV-6b VLPs than HPV-11 VLPs and vice versa. This cross-reaction correlates with the partial neutralization of HPV-11 infectivity in the xenograft model exhibited by polyclonal antisera to HPV-6 VLPs and the significant reactivity of HPV-6 VLP antiserum with HPV-11 virions in ELISAs (6). Reactions between HPV-18 and HPV-45 showed 3- or 4-log₂-unit difference between the homologous and heterologous VLPs, consistent with their weaker sequence homology compared with HPV-6b and HPV-11. Weak cross-reactivity between HPV-16 and HPV-33 was observed in some assays but was not detected consistently, suggesting that any potential cross-reactivity is

TABLE 2. Type specificity of HAI

VLP	Titer of rabbit antiserum to VLP*										
	HPV-6b L1 + L2	HPV-11 L1 + L2	HPV-16K L1 + L2	HPV-18 L1 + L2	HPV-31 L1	HPV-33 L1 + L2	HPV-45 L1 + L2	HPV-16K L1	HPV-16Z L1	HPV-18F L1	HPV-6b, -11, -16, and -18
HPV-6b L1 + L2	24,300	900	•	•	•	•	•	•	•	•	8,100
HPV-11 L1 + L2	900	8,100	•	•	•	•	•	•	•	•	8,100
HPV-16K L1 + L2	•	•	24,300	•	•	300	•	24,300	8,100	•	8,100
HPV-18 L1 + L2	•	•	•	24,300	•	•	300	•	•	•	8,100
HPV-31 L1	•	•	•	•	2,700	•	•	•	•	•	•
HPV-33 L1 + L2	•	•	300	•	300	8,100	•	•	•	•	•
HPV-45 L1 + L2	•	•	•	300	•	•	8,100	•	•	•	300
HPV-16K L1	•	•	72,900	•	•	300	•	218,700	24,300	•	24,300
HPV-16Z L1	•	•	24,300	•	•	•	•	8,100	8,100	•	8,100
RhPV L1 + L2	•	•	•	•	•	•	•	•	•	•	•

* Titers are expressed as the median maximum antiserum dilution for HAI in three independent assays. Titers equivalent to those of the peak response are indicated by asterisks. HPV-16K, HPV-16Z, and HPV-18F correspond to the German 114/K, Zairen Z-1194, and nonassembling reference Asp-309-to-E6 mutant HPV-16 L1 isolates, respectively. HPV-6b L1+L2, HPV-11 L1+L2, HPV-16K L1+L2, HPV-18 L1+L2, HPV-31 L1, HPV-33 L1+L2, HPV-45 L1+L2, HPV-16K L1, HPV-16Z L1, and RhPV L1+L2 VLP preparations were diluted 256, 512, 2,000, 1,000, 256, 512, 512, 4,000, 256, and 256 times, respectively.

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possibility that significant numbers of cross-neutralizing antibodies of this class exist for the different HPV types that were not detected by the HAI assay. However, all of the neutralizing monoclonal antibodies in this class that have been tested to date are type specific in ELISAs for even closely related types: three monoclonal antibodies to HPV-11 (H11.H3, H11.G5, and H11.F1) do not recognize HPV-6b VLPs (6), monoclonal antibody SB6 binds HPV-1 but not HPV-4 (23), and the monoclonal antibodies to CRPV (CRPV-1A, -2C, -4B, and -5A) do not react with either HPV-1 or HPV-11 (8).

Taken together, the data suggest that the neutralizing antibodies that do not prevent virion binding to cells exhibit a type specificity that is similar to those antibodies that do inhibit hemagglutination. It therefore seems likely that most HPV genotypes represent separate serotypes. Definitive classification of serotypes and quantitation of cross-neutralization will require the ability to generate infectious virus for HPVs of any type and to develop quantitative infectivity assays for them. However, HAI is a simple assay that can monitor conformation-dependent and type-specific reactivity in individuals immunized with multiple HPV types.

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(71) Solicitante: (para todos los estados designados, excepto Estados Unidos de América): GLAXOSMITHKLINE BI LOGICALS S.A. [BE/BE]; Rue de l'Institut 89, B-1330 Rixensart (BE).

(72) Inventores: e

(75) Inventores/Solicitantes (para los Estados Unidos de América únicamente): DEBRUS, Serge [BE/BE]; GlaxoSmithKline Biologicals S.A., Rue de l'Institut 89, B-1330 Rixensart (BE). MARTIN, Marie-Therese [BE/BE]; GlaxoSmithKline Biologicals S.A., Rue de l'Institut 89, B-1330 Rixensart (BE). STEPHEN, Robert John [GB/GB]; GlaxoSmithKline, 980 Great West Road, Brentford Middlesex TW8 9GS (GB). STEPHENNE, Jean [BE/BE]; GlaxoSmithKline Biologicals S.A., Rue de l'Institut 89, B-1330 Rixensart (BE). WETTENDORFF, Martine, Anne, Cécile [BE/BE]; GlaxoSmithKline Biologicals S.A., Rue de l'Institut 89, B-1330 Rixensart (BE).

(74) Agente: DALTON, Marcus, Jonathan, William; GlaxoSmithKline, Corporate Intellectual Property (CN925.1), 980 Great West Road, Brentford, Middlesex TW8 9GS (GB).

(81) Estados Designados (salvo que de otro modo indicado para cada clase de la protección nacional disponible): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Estados Designados (salvo que de otro modo indicado para cada clase de la protección regional disponible): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), euroasiática (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM) europea (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR) OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaraciones bajo la Regla 4.17:

— relativo al derecho del solicitante de solicitar y obtener una patente (Regla 4.17(ii)) para las siguientes designaciones AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, patente ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), patente euroasiática (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM) patente europea (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR) patente OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

— relativo al derecho del solicitante de solicitar y obtener una patente (Regla 4.17(ii)) para las siguientes designaciones AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, patente ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), patente euroasiática (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM) patente europea (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR) patente OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

[continúa en la siguiente página]

(54) Título: "VACUNA CONTRA HPV16 Y HPV18 Y AL MENOS OTRO TIPO DE VPH SELECCIONADO DE VPH 31, 45 O 52"

(57) Resumen: Una composición inmunogénica y procedimientos para producir dicha composición, la composición comprendiendo las PLV de VPH 16 y 18 y al menos otro tipo de cáncer de VPH, el otro tipo de cáncer seleccionándose entre la lista constituida por los tipos 31, 45, y 52 de VPH, en la que la dosis de la PLV de al menos otros tipos de cánceres se reduce con relación a la de VPH 16 ó 18.

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— relativo al derecho del solicitante para reivindicar prioridad de la solicitud (Regla 4.17(iii)) para las siguientes designaciones AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, patente ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), patente euroasiática (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM) patente europea (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR) patente OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

— relativo al derecho del solicitante para reivindicar prioridad de la solicitud (Regla 4.17(iii)) para las siguientes designaciones AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG,

UZ, VC, VN, YU, ZA, ZM, ZW, patente ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), patente euroasiática (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM) patente europea (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR) patente OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
— de la calidad del inventor (Regla 4.17(iv))

Publicado:

— con reporte de búsqueda internacional
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(71) Applicant (for all designated States except US): GLAXO-SMITHKLINE BIOLOGICALS S.A. [BE/BE]; Rue de l'Institut 89, B-1330 Rixensart (BE).

(72) Inventors and

(73) Inventors/Applicants (for US only): DEBEUS, Serge [BE/BE]; GlaxoSmithKline Biologicals S.A., Rue de l'Institut 89, B-1330 Rixensart (BE). MARTIN, Marie-Therese [BE/BE]; GlaxoSmithKline Biologicals S.A., Rue de l'Institut 89, B-1330 Rixensart (BE). STEPHEN, Robert John [GB/GB]; GlaxoSmithKline, 980 Great West Road, Brentford Middlesex TW8 9GS (GB). STEPHENNE, Jean [BE/BE]; GlaxoSmithKline Biologicals S.A., Rue de l'Institut 89, B-1330 Rixensart (BE). WETTENDORFF, Martine, Anne, Cécile [BE/BE]; GlaxoSmithKline Biologicals S.A., Rue de l'Institut 89, B-1330 Rixensart (BE).

(74) Agents: DALTON, Marcus, Jonathan, William; GlaxoSmithKline, Corporate Intellectual Property (CN925.1), 980 Great West Road, Brentford Middlesex TW8 9GS (GB).

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

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH,

GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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— as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG,

[Continued on next page]

(54) Title: VACCINE AGAINST HPV16 AND HPV18 AND AT LEAST ANOTHER HPV TYPE SELECTED FROM HPV 31, 45 OR 52

(57) Abstract: An immunogenic composition and methods for producing said composition, the composition comprising VLPs from HPV 16 and 18 and at least one other HPV cancer type, the other cancer type being selected from the list consisting of HPV types 31, 45 and 52, wherein the dose of the VLP of the at least one other cancer type is reduced relative to that of HPV 16 or 18.

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SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patents (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI patents (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)

- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(III)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patents (BW, GH, GM, KE,

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- of inventorship (Rule 4.17(iv)) for US only

Published:

- with international search report
— before the expiration of the time limits for amending the claims and to be republished in the event of receipt of amendments

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

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De la
AUTORIDAD DE BUSQUEDA INTERNACIONAL

Para:

ver forma PCT/ISA/220

PCT

OPINION ESCRITA DE LA
AUTORIDAD DE BUSQUEDA INTERNACIONAL
(PCT Regla 43bis.1)

Registro de referencia del solicitante o agente ver forma PCT/ISA/220		Fecha de envío por correo (día/mes/año) ver forma PCT/ISA/210 (segunda página)																									
Solicitud Internacional No. PCT/EP2005/006461		Fecha de registro internacional (día/mes/año) 14.06.2005	Fecha de prioridad (día/mes/año) 16.06.2004																								
Clasificación Internacional de Patentes (IPC) o clasificación nacional e IPC A61K39/12																											
Solicitante GLAXOSMITHKLINE BIOLOGICALS S.A.																											
1. Esta opinión contiene indicaciones relacionadas con los siguientes ítems: <table><tr><td>☒</td><td>Casilla No. I</td><td>Bases del Reporte</td></tr><tr><td>☒</td><td>Casilla No. II</td><td>Prioridad</td></tr><tr><td>☒</td><td>Casilla No. III</td><td>No establecimiento de opinión respecto a novedad, nivel inventivo y aplicabilidad Industrial.</td></tr><tr><td>☐</td><td>Casilla No. IV</td><td>Falta de unidad de invención</td></tr><tr><td>☒</td><td>Casilla No. V</td><td>Afirmación razonada de conformidad con la Regla 43bis.1(a)(i) con respecto a novedad, nivel inventivo o aplicabilidad industrial; citas y explicaciones que sustentan dicha afirmación.</td></tr><tr><td>☐</td><td>Casilla No. VI</td><td>Ciertos documentos citados</td></tr><tr><td>☐</td><td>Casilla No. VII</td><td>Ciertos defectos en la solicitud internacional</td></tr><tr><td>☐</td><td>Casilla No. VIII</td><td>Ciertas observaciones sobre la solicitud internacional</td></tr></table> 2. ACCIONES FUTURAS Si se hace una demanda para examen preliminar internacional, esta opinión generalmente será considerada como una opinión escrita de la Autoridad Examinadora Preliminar ("IPEA"). Sin embargo, esto no aplica cuando el solicitante escoge una Autoridad diferente a ésta para que sea la IPEA y la IPEA escogida le ha notificado a la Oficina Internacional de conformidad con la Regla 66.1bis(b) que no se considerarán las opiniones escritas de esta Autoridad Examinadora Preliminar. Si esta opinión es, según se estipula anteriormente, considerada una opinión escrita de la IPEA, se invita al solicitante a presentar ante la IPEA una respuesta escrita junto, cuando sea apropiado, con modificaciones, antes de la fecha de expiración de tres meses desde la fecha de envío por correo de la Forma PCT/ISA/220 o antes de la fecha de expiración de 22 meses desde la fecha de prioridad, la que expire más tarde. Para opciones futuras, ver la Forma PCT/ISA/220 3. Para detalles adicionales, ver las notas a la Forma PCT/ISA/220				☒	Casilla No. I	Bases del Reporte	☒	Casilla No. II	Prioridad	☒	Casilla No. III	No establecimiento de opinión respecto a novedad, nivel inventivo y aplicabilidad Industrial.	☐	Casilla No. IV	Falta de unidad de invención	☒	Casilla No. V	Afirmación razonada de conformidad con la Regla 43bis.1(a)(i) con respecto a novedad, nivel inventivo o aplicabilidad industrial; citas y explicaciones que sustentan dicha afirmación.	☐	Casilla No. VI	Ciertos documentos citados	☐	Casilla No. VII	Ciertos defectos en la solicitud internacional	☐	Casilla No. VIII	Ciertas observaciones sobre la solicitud internacional
☒	Casilla No. I	Bases del Reporte																									
☒	Casilla No. II	Prioridad																									
☒	Casilla No. III	No establecimiento de opinión respecto a novedad, nivel inventivo y aplicabilidad Industrial.																									
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☒	Casilla No. V	Afirmación razonada de conformidad con la Regla 43bis.1(a)(i) con respecto a novedad, nivel inventivo o aplicabilidad industrial; citas y explicaciones que sustentan dicha afirmación.																									
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☐	Casilla No. VII	Ciertos defectos en la solicitud internacional																									
☐	Casilla No. VIII	Ciertas observaciones sobre la solicitud internacional																									
Nombre y dirección de correo de ISA: Oficina de Patentes Europea D-80298 Munich Tel .+49 89 2399 - 0 Tx: 523656 epmu d Fax:+49 89 2399 - 4465		Funcionario Autorizado Renggli-Zulliger, N Teléfono No. +49 89 2399-7482																									

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Casilla No. I. Bases de la opinión

1. Respecto al idioma, esta opinión se ha establecido según la solicitud internacional en el idioma en el cual se presentó, a menos que se indique de otro modo bajo este ítem.
☐ Esta opinión se ha establecido según una traducción del idioma original al siguiente idioma, que es el idioma de una traducción proporcionada para la búsqueda internacional (bajo las Reglas 12.3 y 23.1(b)).
2. Respecto a cualquier nucleótido y/o secuencia aminoácida revelada en la solicitud internacional y necesaria para la invención reivindicada, esta opinión se ha establecido según:
 - a. tipo de material:
☐ un listado de secuencia
☐ tabla(s) relacionada con el listado de secuencia
 - b. formato de material:
☐ en formato escrito
☐ en formato legible por computador
 - c. tiempo de presentación/suministro:
☐ contenido en la solicitud internacional según se registró
☐ registrada junto con la solicitud internacional en formato legible por computador
☐ proporcionada subsiguientemente a esta Autoridad para la búsqueda.
3. ☐ Además, en el caso en que más de una versión o copia de un listado de secuencia y/o tabla relacionada con ello se haya registrado o proporcionado, las declaraciones requeridas de que la información en las copias subsiguientes o adicionales es idéntica a aquella en la solicitud según se presentó o no va más allá de la solicitud según se registró, según lo apropiado, fueron suministradas.
4. Comentarios adicionales:

Casilla No. II Prioridad

1. Por consiguiente, no ha sido posible considerar la validez de la reivindicación de prioridad. Sin embargo, se ha establecido esta opinión sobre el supuesto de que la fecha relevante es la fecha de la prioridad reivindicada.
2. Se ha establecido esta opinión como si no se hubiese reivindicado ninguna prioridad debido al hecho de que se ha encontrado que la reivindicación de prioridad es inválida (Reglas 43bis.1 y 64.1). Por consiguiente, para los fines de esta opinión, se considera que la fecha de registro internacional indicada anteriormente es la fecha relevante.
3. Observaciones adicionales, si es necesario:

Casilla No. III No establecimiento de opinión respecto a novedad, nivel inventivo o aplicabilidad industrial

Las preguntas de si la invención reivindicada parece ser novedosa, comprender un paso inventivo (ser no obvia) o ser aplicable industrialmente no se han examinado respecto a:

- ☐ toda la solicitud internacional,
☒ reivindicaciones Nos. 19, 21

porque:

- ☒ dicha solicitud internacional, o dichas reivindicaciones Nos. 19, 21 se relacionan con la siguiente materia objeto que no requiere un examen preliminar internacional (*especifique*):

ver hoja separada

- ☐ la descripción, reivindicaciones o dibujos (*indique los elementos particulares a continuación*) de dichas reivindicaciones Nos. son tan poco claros que no se podría formar una opinión significativa (*especifique*):

- ☐ las reivindicaciones, o dichas reivindicaciones Nos. 1 (parcialmente) son sustentadas tan inadecuadamente por la descripción que no se podría formar una opinión significativa.

- ☐ no se ha establecido ningún reporte de búsqueda internacional para toda la solicitud o para dichas reivindicaciones Nos. 1-31 en parte.

- ☐ El nucleótido y/o lista de secuencia aminoácida no cumple con el estándar proporcionado en el Anexo C de las Instrucciones Administrativas en que:

la forma escrita

- ☐ no se ha proporcionado

- ☐ no cumple con el estándar

el formato legible por computador

- ☐ no se ha proporcionado

- ☐ no cumple con el estándar

- ☐ las tablas relacionadas con el nucleótido y/o lista de secuencia aminoácida, si están en formato legible por computador únicamente, no cumplen con los requerimientos técnicos proporcionados en el Anexo C-bis de las Instrucciones Administrativas.

- ☐ Ver la hoja separada para más detalles.

Casilla No. V Afirmación razonada bajo la Regla 43bis.1(a)(i) respecto a novedad, paso inventivo o aplicabilidad industrial; citaciones y explicaciones que sustentan dicha afirmación

1. Declaración

Novedad (N)	Sí: Reivindicaciones 1-21
	No: Reivindicaciones -
Nivel inventivo (IS)	Sí: Reivindicaciones -
	No: Reivindicaciones 1-21
Aplicabilidad industrial (IA)	Sí: Reivindicaciones 1-18, 20
	No: Reivindicaciones -

2. Citaciones y explicaciones

ver hoja separada

Re Item II
Prioridad

3. Observaciones adicionales:

Los documentos de prioridad pertenecientes a la presente solicitud no estaban disponibles al momento de establecer esta opinión preliminar. Por lo tanto, para la presente evaluación se asume que todas las reivindicaciones gozan de los derechos de prioridad a partir de la fecha de presentación del documento de prioridad (16.6.2004). Si posteriormente resulta que esto no es correcto, los siguientes documentos, citados en el Reporte Internacional de Búsqueda (ISR por sus siglas en inglés). Por sus siglas en inglés), podrían volverse relevantes para la evaluación de si la materia objeto de la presente solicitud satisface los criterios establecidos en el artículo 33(1) del PCT.

GLAXOSMITHKLINE HPV VACCINE STUDY GROUP HARPER D M Y COLS: "Eficacia de una vacuna bivalente de partícula tipo virus L1 en la prevención de la infección con los tipos 16 y 18 del virus del papiloma humano en mujeres jóvenes: un ensayo aleatorio controlado" LANCET THE, LANCET LIMITED. LONDRES, GB, vol. 34, no. 9447, Noviembre 13, 2004 (2004-11-13), páginas 1757-1765, XP004746625 ISSN: 0140-6736, citado en la solicitud.

WO 2004/056389 A (GLAXOSMITHKLINE BIOLOGICALS SA; DUBIN, GARY; INNIS, BRUCE; SLAOUI, MON) Julio 8 de 2004 (2004-07-08)

D3: VILLA L L Y COLS: "Vacuna de partícula tipo virus L1 del virus del papiloma humano (tipos 6, 11, 16 y 18), profiláctico tetravalente en mujeres jóvenes: un ensayo de eficacia multicentro fase II aleatorio doble-ciego placebo-controlado" LANCET ONCOLOGY, LANCET PUBLISHING GROUP, LONDRES, GB, vol. 6, no. 5, Mayo de 2005 (2005-05), páginas 271-278, XP004870902 ISSN: 1470-2045

Re Item III

No establecimiento de opinión con respecto a novedad, nivel inventivo y aplicabilidad industrial

Esta autoridad considera que la materia objeto relacionada con las reivindicaciones 19, 21, están cubiertas por las disposiciones de la Regla 67.1 (iv) del PCT. En consecuencia, no se formulará opinión alguna con respecto

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a la aplicabilidad industrial de la materia-objeto de estas reivindicaciones (Artículo 34(4)(a)(I) del PCT).

Re Item V

Declaración razonada bajo la Regla 66.2(a)(ii) con respecto a novedad, nivel inventivo y aplicabilidad industrial; referencias y explicaciones que sustentan dicha declaración.

Se hace referencia a los siguientes documentos:

- D1: BILLICH A: "VACUNA VPH MEDIMMUNE/GLAXOSMITHKLINE" CURRENT OPINION IN INVESTIGATIONAL DRUGS, CURRENT DRUGS, LONDRES, GB, vol. 4, no. 2, Febrero de 2003 (2003-02), páginas 210-213, XP009029755 ISSN: 0967-8298
- D2: REINIS MILAN: "Evaluación de la tecnología: vacuna VPH (tetravalente), Aventis Pasteur MSD/CSL" CURRENT OPINION IN MOLECULAR THERAPEUTICS. ABR 2004, vol. 6, no. 2, Abril de 2004 (2004-04), páginas 206-211, XP009056594 ISSN: 1464-8431.
- D3: WO 01/97840 A (MEDIMMUNE, INC) Diciembre 27 de 2001 (2001-12-27).
- D4: EP-A-1 410 805 (GLAXOSMITHKLINE BIOLOGICALS S.A) Abril 21 de 2004 (2004-04-21)

Novedad (Artículo 33(1) y (2) del PCT)

1) Ninguno de los documentos del arte previo citados revela una composición inmunogénica que comprenda VLPs o capsómeros de VPH 16 y 18 y al menos algún otro VPH seleccionado entre VPH 31, 45 y 52. Por lo tanto, la materia objeto de las reivindicaciones 1-21 es novedosa.

Nivel Inventivo (artículo 33(1) y (3) del PCT)

2) El documento del arte previo más cercano es D4, que revela una composición inmunogénica capaz de generar anticuerpos neutralizantes consistiendo de VLP VPH 16L y VLP VPH 18 L1 en combinación con hidróxido de aluminio y MPL 3D.

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La diferencia entre D4 y la presente solicitud es la provisión de al menos una vacuna trivalente que consiste de VLPs de VPH 16 y 18 y algún otro VPH seleccionado entre VPH 31, 45 y 52.

El problema puede ser visto entonces como la provisión de una vacuna trivalente contra VPH 16 y VPH 18 y algún otro tipo de VPH seleccionado entre VPH 31, 45 ó 52.

La solución propuesta es la provisión de una composición inmunogénica que comprende capsómeros de VPH16 y 18 así como VPH 31, 45 ó 52.

El resultado no es logrado en la presente solicitud, en la cual no se evalúa dicha composición trivalente.

En el campo de la inmunología no es suficiente que se revele una formulación de vacuna, también se requiere evidencia experimental de que se elicitaba una respuesta neutralizante, puesto que tal respuesta no puede predecirse.

Por lo tanto, la materia objeto de las reivindicaciones 1-18 no involucra nivel inventivo, dado que la vacuna hecha solamente de VPH16 y VPH18 (tal como se revela en los ejemplos de la presente solicitud) ya había sido revelada en el arte previo (D1 y D4 así como la composición tetravalente en D2).

La materia objeto de las reivindicaciones 19-21 será considerada inventiva solo si la composición allí usada es nueva e inventiva.

Aplicabilidad industrial (Artículo 33(1) y (4) del PCT)

3) Para la evaluación de si las presentes reivindicaciones 19, 21 tienen o no aplicabilidad industrial, no existe criterio unificado en los estados contratantes del PCT. La patentabilidad también puede depender de la formulación de las reivindicaciones. La EPO, por ejemplo, no reconoce como industrialmente aplicable la materia objeto de reivindicaciones de uso de un compuesto en tratamiento médico. Sin embargo, puede permitir reivindicaciones de primer uso en tratamiento médico de un compuesto conocido y el uso de tal compuesto para la fabricación de un medicamento para un nuevo tratamiento médico.

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**OPINION ESCRITA DE LA
AUTORIDAD DE BUSQUEDA INTERNACIONAL
(HOJA SEPARADA)**

**Solicitud Internacional No.
PCT/EP2005/006461**

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PATENT COOPERATION TREATY

5.
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From the INTERNATIONAL SEARCHING AUTHORITY

PCT

To:

GLAXOSMITHKLINE

Corp. Intel. Prop. (CN925.1)

Attn. Dalton, Marcus J

980 Great West Road

Brentford, Middlesex

TW8 9GS

UNITED KINGDOM

GlaxoSmithKline

Corporate IP

Received BRENTFORD

16 NOV 2005

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL SEARCH REPORT AND
THE WRITTEN OPINION OF THE INTERNATIONAL
SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

Applicant's or agent's file reference

RJB/VB60891

ATTY: R. J. Dalton

Date of mailing (day/month/year)

16/11/2005

ATTY CHECKED/FIL

FOR FURTHER ACTION

See paragraphs 1 and 4 below

International application No.

PCT/EP2005/006461

International filing date

(day/month/year)

14/06/2005

Applicant

GLAXOSMITHKLINE BIOLOGICALS S.A.

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

Filing of amendments and statement under Article 18:

The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 48):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes
1211 Geneva 20, Switzerland, Facsimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

- ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.
- ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. Reminders

Shortly after the expiration of 18 months from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90bis.1 and 90bis.3, respectively, before the completion of the technical preparations for international publication.

The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. These comments would also be made available to the public but not before the expiration of 30 months from the priority date.

Within 18 months from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later); otherwise, the applicant must, within 30 months from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

In respect of other designated Offices, the time limit of 30 months (or later) will apply even if no demand is filed within 18 months.

See the Annex to Form PCT/IB/301 and, for details about the applicable time limits, Office by Office, see the *PCT Applicant's Guide*, Volume II, National Chapters and the WIPO Internet site.

Name and mailing address of the International Searching Authority



European Patent Office, P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 851 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Mahjoubia Yassine

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PATENT COOPERATION TREATY

TME

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From the
INTERNATIONAL SEARCHING AUTHORITY

PCT

To:

see form PCT/ISA220

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43bis.1)

Date of mailing
(day/month/year) see form PCT/ISA210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA220

FOR FURTHER ACTION
See paragraph 2 below

International application No.
PCT/EP2005/008461

International filing date (day/month/year)
14.06.2005

Priority date (day/month/year)
16.06.2004

International Patent Classification (IPC) or both national classification and IPC
A61K39/12

Applicant
GLAXOSMITHKLINE BIOLOGICALS S.A.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA220.

3. For further details, see notes to Form PCT/ISA220.

Name and mailing address of the ISA:



European Patent Office
D-80399 Munich
Tel. +49 89 2369 - 0 Tx: 523699 epmu d
Fax: +49 89 2369 - 4465

Authorized Officer

Renggli-Zulliger, N
Telephone No. +49 89 2369-7462



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**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/EP2005/006461

Box No. I Basis of the opinion

1. With regard to the language, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).
2. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:
 - a. type of material:
☐ a sequence listing
☐ table(s) related to the sequence listing
 - b. format of material:
☐ in written format
☐ in computer readable form
 - c. time of filing/furnishing:
☐ contained in the international application as filed.
☐ filed together with the international application in computer readable form.
☐ furnished subsequently to this Authority for the purposes of search.
3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

Box No. II Priority

1. ☒ The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43bis.1 and 64.1) is the claimed priority date.
2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43bis.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.
3. Additional observations, if necessary:

see separate sheet

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Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application,
- ☒ claims Nos. 19, 21

because:

- ☒ the said international application, or the said claims Nos. 19, 21 relate to the following subject matter which does not require an international preliminary examination (specify):

see separate sheet

- ☐ the description, claims or drawings (indicate particular elements below) or said claims Nos. are so unclear that no meaningful opinion could be formed (specify):
- ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.
- ☐ no international search report has been established for the whole application or for said claims Nos.
- ☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:
 - the written form ☐ has not been furnished
 - ☐ does not comply with the standard
 - the computer readable form ☐ has not been furnished
 - ☐ does not comply with the standard
- ☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-bis of the Administrative Instructions.
- ☐ See separate sheet for further details

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**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/EP2005/006461

Box No. V Reasoned statement under Rule 43b/a.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	1-21
	No: Claims	-
Inventive step (IS)	Yes: Claims	-
	No: Claims	1-21
Industrial applicability (IA)	Yes: Claims	1-18, 20
	No: Claims	-

**2. Citations and explanations
see separate sheet**

Re Item II

Priority

3. Additional observations :

The priority documents pertaining to the present application were not available at the time of establishing this preliminary opinion. Hence, the current assessment is based on the assumption that all claims enjoy priority rights from the filing date of the priority document (16.6.2004). If it later turns out that this is not correct, the following document cited in the International Search Report (ISR) could become relevant to assess whether the claimed subject-matter of the present application satisfies the criteria set forth in Article 33(1) PCT.

GLAXOSMITHKLINE HPV VACCINE STUDY GROUP HARPER D M ET AL: "Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial" LANCET THE, LANCET LIMITED, LONDON, GB, vol. 364, no. 9447, 13 November 2004 (2004-11-13), pages 1757-1765, XP004746625 ISSN: 0140-6736 cited in the application

WO 2004/056389 A (GLAXOSMITHKLINE BIOLOGICALS SA; DUBIN, GARY; INNIS, BRUCE; SLAOUI, MON) 8 July 2004 (2004-07-08)

D3:VILLA L L ET AL: "Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial" LANCET ONCOLOGY, LANCET PUBLISHING GROUP, LONDON, GB, vol. 6, no. 5, May 2005 (2005-05), pages 271-278, XP004870902 ISSN: 1470-2045

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 19,21 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to

the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability, citations and explanations supporting such statement

Reference is made to the following documents:

- D1: BILLICH A: "HPV VACCINE MEDIMMUNE/GLAXOSMITHKLINE" CURRENT OPINION IN INVESTIGATIONAL DRUGS, CURRENT DRUGS, LONDON, GB, vol. 4, no. 2, February 2003 (2003-02), pages 210-213, XP009029755 ISSN: 0967-8298
- D2: REINIS MILAN: "Technology evaluation: HPV vaccine (quadrivalent), Aventis Pasteur MSD/CSL" CURRENT OPINION IN MOLECULAR THERAPEUTICS. APR 2004, vol. 6, no. 2, April 2004 (2004-04), pages 206-211, XP009056594 ISSN: 1464-8431
- D3: WO 01/97840 A (MEDIMMUNE, INC) 27 December 2001 (2001-12-27)
- D4: EP-A-1 410 805 (GLAXOSMITHKLINE BIOLOGICALS S.A) 21 April 2004 (2004-04-21)

Novelty (Article 33(1) and (2) PCT)

- 1) None of the cited prior art discloses an immunogenic composition comprising VLPs or capsomers from HPV 16 and 18 and at least one other HPV selected among HPV 31, 45 and 52. Therefore, the subject-matter of claims 1-21 is novel.

Inventive step (Article 33(1) and (3) PCT)

- 2) The closest prior art document is D4 disclosing an immunogenic composition able to generate neutralizing antibodies consisting of an HPV 16L1 VLP and HPV 18 L1 VLP in combination with aluminium hydroxide and 3D MPL.

The difference between D4 and the present application is the provision of at least a trivalent vaccine consisting of VLPs of HPV 16 and 18 and one other HPV selected from HPV 31, 45 and 52.

The problem can therefore be seen as the provision of a trivalent vaccine against HPV 16 and HPV 18 and one other HPV type selected from HPV 31, 45 or 52.

The solution proposed is the provision of an immunogenic composition comprising capsomers of HPV16 and 18 as well as HPV 31, 45 or 52.

The result is not achieved in the present application in that no such trivalent composition is tested.

→ In the immunology field it is not sufficient that a vaccine formulation is disclosed but experimental evidence that a neutralizing response is elicited is required, since such a response cannot be predicted.

Therefore, the subject-matter of claims 1-18 does not involve an inventive step, since vaccine made of only HPV16 and HPV18 (as disclosed in the examples of the present application) are disclosed in the prior art (D1 and D4 as well as a quadrivalent composition in D2).

The subject-matter of claims 19-21 will only be considered inventive if the composition used therein is novel and inventive.

Industrial applicability (Article 33(1) and (4) PCT)

3) For the assessment of the present claims 19, 21 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

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**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/EP2005/006461

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Registro de referencia del solicitante o agente RJS/VB60891	PARA FUTURAS ACCIONES Ver Forma PCT/IPEA/416	
Solicitud internacional No. PCT/EP2005/006461	Fecha de registro internacional (día/mes/año) 14.06.2005	Fecha de prioridad (día/mes/año) 16.06.2004
Clasificación Internacional de Patentes (IPC) o clasificación nacional e IPC INV. A61K39/12		
Solicitante GLAXOSMITHKLINE BIOLOGICALS S.A.		

1. Este reporte es el reporte de examen preliminar internacional, establecido por esta Autoridad Examinadora Preliminar Internacional bajo el Artículo 35 y transmitido al solicitante de conformidad con el Artículo 36. 2. Este REPORTE consta de un total de <u>9</u> hojas, incluyendo esta carátula. 3. Este reporte también es acompañado por ANEXOS, que comprenden: a. ☐ (enviado al solicitante y a la Oficina Internacional) un total de _ _ _ _ _ hojas, como sigue: ☐ hojas de la descripción, reivindicaciones y/o dibujos los cuales han sido modificados y son la base de este reporte y/o hojas que contienen rectificaciones autorizadas por esta Autoridad (ver Regla 70.16 y Sección 607 de las Instrucciones Administrativas). ☐ hojas que reemplazan hojas anteriores, pero las cuales esta Autoridad considera que contienen una modificación que va más allá de la revelación en la solicitud internacional como se presentó, como se indicó en el ítem 4 de la Casilla No. I y la Casilla Suplementaria. b. ☐ (enviado únicamente a la Oficina Internacional) un total de (indicar tipo y número de medio(s) electrónico(s)) _ _ , que contienen un listado de secuencias y/o tablas con ello, únicamente en una forma legible en computador, como se indicó en la Casilla Suplementaria Relacionada con el Listado de Secuencias (ver Sección 802 de las Instrucciones Administrativas).																	
4. Este reporte contiene indicaciones relacionadas con los siguientes ítems: <table border="0"> <tr> <td>☒ Casilla No. I</td> <td>Bases del reporte</td> </tr> <tr> <td>☒ Casilla No. II</td> <td>Prioridad</td> </tr> <tr> <td>☒ Casilla No. III</td> <td>No establecimiento de opinión respecto a novedad, nivel inventivo y aplicabilidad industrial</td> </tr> <tr> <td>☐ Casilla No. IV</td> <td>Falta de unidad de invención</td> </tr> <tr> <td>☒ Casilla No. V</td> <td>Afirmación razonada de conformidad con el Artículo 35(2) con respecto a novedad, nivel inventivo o aplicabilidad industrial; citaciones y explicaciones que sustentan dicha afirmación</td> </tr> <tr> <td>☐ Casilla No. VI</td> <td>Ciertos documentos citados</td> </tr> <tr> <td>☐ Casilla No. VII</td> <td>Ciertos defectos en la solicitud internacional</td> </tr> <tr> <td>☐ Casilla No. VIII</td> <td>Ciertas observaciones sobre la solicitud internacional</td> </tr> </table>		☒ Casilla No. I	Bases del reporte	☒ Casilla No. II	Prioridad	☒ Casilla No. III	No establecimiento de opinión respecto a novedad, nivel inventivo y aplicabilidad industrial	☐ Casilla No. IV	Falta de unidad de invención	☒ Casilla No. V	Afirmación razonada de conformidad con el Artículo 35(2) con respecto a novedad, nivel inventivo o aplicabilidad industrial; citaciones y explicaciones que sustentan dicha afirmación	☐ Casilla No. VI	Ciertos documentos citados	☐ Casilla No. VII	Ciertos defectos en la solicitud internacional	☐ Casilla No. VIII	Ciertas observaciones sobre la solicitud internacional
☒ Casilla No. I	Bases del reporte																
☒ Casilla No. II	Prioridad																
☒ Casilla No. III	No establecimiento de opinión respecto a novedad, nivel inventivo y aplicabilidad industrial																
☐ Casilla No. IV	Falta de unidad de invención																
☒ Casilla No. V	Afirmación razonada de conformidad con el Artículo 35(2) con respecto a novedad, nivel inventivo o aplicabilidad industrial; citaciones y explicaciones que sustentan dicha afirmación																
☐ Casilla No. VI	Ciertos documentos citados																
☐ Casilla No. VII	Ciertos defectos en la solicitud internacional																
☐ Casilla No. VIII	Ciertas observaciones sobre la solicitud internacional																
Fecha de presentación de la demanda 12.01.2006	Fecha de terminación de este reporte 12.10.2006																
Nombre y dirección de correo de la autoridad internacional de examen preliminar Oficina de Patentes Europea D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Funcionario autorizado RENGGLI- ZULLIGER, N Teléfono No. +49 89 2399-7482																

Casilla No. I Bases del reporte

1. Respecto al idioma, este reporte se basó en:

- ☒ La solicitud internacional en el idioma en el cual se presentó.
- ☐ Una traducción de la solicitud internacional al, que es el lenguaje de una traducción entregada para propósitos de:
- ☐ búsqueda internacional (según Reglas 12.3. y 23.1 (b)).
 - ☐ publicación de la solicitud internacional (según la Regla 12.4(a))
 - ☐ examen preliminar internacional (según las Reglas 55.2 (a) y/o 55.3(a)).

2. Respecto a los elementos de la solicitud internacional este Reporte se basó en (hojas reemplazadas que han sido suministradas a la oficina receptora en respuesta a una invitación según Artículo 14, son referidas en este reporte como "originalmente presentadas" y no son anexadas a este reporte):

Descripción, Páginas

1-45 como se presentaron originalmente

Reivindicaciones, Nos.

1-21 como se presentaron originalmente

☐ listado de secuencia y/o alguna tabla(s)-ver Casilla Suplementaria en relación al Listado de Secuencia

3. ☐ Las modificaciones resultaron en la cancelación de:

- ☐ la descripción, páginas
- ☐ las reivindicaciones, Nos.
- ☐ los dibujos, hojas/figuras
- ☐ listado de secuencia (*especificar*)
- ☐ cualquier tabla(s) relacionadas con la serie de listados (*especificar*)

4. ☐ Este reporte ha sido establecido como si (alguno de) los anexos corregidos a este reporte y numerado antes no ha sido hecho, desde que ellos han sido considerados a través de la presentación original, como esta indicado en la Casilla Suplementaria (Regla 70.2(c)).

- ☐ la descripción, páginas
- ☐ las reivindicaciones, Nos.
- ☐ los dibujos, hojas/figuras
- ☐ secuencia que enumera (*especificar*):
- ☐ alguna tabla(s) relatada al listado de secuencia (*especificar*):

* Si el Item 4 aplica, algunas o todas aquellas hojas pueden ser marcadas "reemplazadas".

Casilla No. II Prioridad

1. ☐ Se ha establecido este reporte como si no se hubiese reivindicado ninguna prioridad debido a la falta de suministro dentro del tiempo límite presentado para el requerimiento:

☐ copia de la solicitud anterior cuya prioridad se ha reivindicado (Regla 66.7(a)).

☐ traducción de la solicitud anterior cuya prioridad se ha reivindicado (Regla 66.7(b)).
2. ☐ Se ha establecido este reporte como si no se hubiese reivindicado ninguna prioridad debido al hecho de que se ha encontrado que la reivindicación de prioridad es inválida (Regla 64.1). Por consiguiente, para los fines de este reporte, se considera que la fecha de registro internacional indicada anteriormente es la fecha relevante.
3. Observaciones adicionales, si es necesario:

Ver hoja separada

**REPORTE PRELIMINAR INTERNACIONAL SOBRE
PATENTABILIDAD**Solicitud Internacional No.
PCT/EP2005/006461**Casilla No. III No establecimiento de opinión respecto a novedad, nivel inventivo y aplicabilidad industrial**

1. Las cuestiones de si la invención reivindicada parece ser novedosa, involucrar nivel inventivo (ser no obvia) o ser industrialmente aplicable no han sido examinadas respecto a:

☐ toda la solicitud internacional

☒ las reivindicaciones Nos. 19, 21

porque:

☒ dicha solicitud internacional, o dichas reivindicaciones Nos. 19, 21 se relacionan con la siguiente materia objeto las cuales no requieren examen preliminar internacional (*especificar*):
ver hoja separada

☐ la descripción, reivindicaciones o dibujos (*indique elementos particulares a continuación*) o dichas reivindicaciones Nos. son tan imprecisas que no pudo formarse una opinión significativa (*especifique*):

☐ las reivindicaciones o dichas reivindicaciones Nos. están sustentadas tan inadecuadamente por la descripción que no pudo formarse una opinión significativa.

☐ no se ha establecido un reporte de búsqueda internacional para dichas reivindicaciones Nos.

☐ el listado de secuencia de nucleótidos y/o aminoácidos no cumple con la norma proporcionada por el Anexo C de las Instrucciones Administrativas en que:

la forma escrita

☐ no ha sido suministrada

☐ no cumple con la norma

la forma legible en computador

☐ no ha sido suministrada

☐ no cumple con la norma

☐ las tablas relacionadas con el listado de secuencias de nucleótidos y/o aminoácidos, si únicamente en forma legible en computador, no cumple con los requerimientos técnicos proporcionados por el Anexo C-bis de las Instrucciones Administrativas.

☐ ver hoja separada para detalles adicionales.

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Re Item II

Prioridad

3. Observaciones adicionales:

Los documentos de prioridad pertenecientes a la presente solicitud no estaban disponibles al momento de establecer este reporte preliminar. Por lo tanto, para la presente evaluación se asume que todas las reivindicaciones gozan de los derechos de prioridad a partir de la fecha de presentación del documento de prioridad (16.6.2004). Si posteriormente resulta que esto no es correcto, los siguientes documentos, citados en el Reporte Internacional de Búsqueda (ISR por sus siglas en inglés), podrían volverse relevantes para la evaluación de si la materia objeto reclamada por la presente solicitud, satisface los criterios establecidos en el artículo 33(1) del PCT.

GLAXOSMITHKLINE HPV VACCINE STUDY GROUP HARPER D M Y COLS: "Eficacia de una vacuna bivalente de partícula tipo virus L1 en la prevención de la infección con los tipos 16 y 18 del virus del papiloma humano en mujeres jóvenes: un ensayo aleatorio controlado" LANCET THE, LANCET LIMITED. LONDRES, GB, vol. 364, no. 9447, Noviembre 13 de 2004 (2004-11-13), páginas 1757-1765, XP004746625 ISSN: 0140-6736, citado en la solicitud.

WO 2004/056389 A (GLAXOSMITHKLINE BIOLOGICALS SA; DUBIN, GARY; INNIS, BRUCE; SLAOU, MON) Julio 8 de 2004 (2004-07-08)

D3: VILLA L L Y COLS: "Vacuna de partícula tipo virus L1 del virus del papiloma humano (tipos 6, 11, 16 y 18), profiláctico tetravalente en mujeres jóvenes: un ensayo de eficacia multicentro fase II aleatorio doble-ciego placebo-controlado" LANCET ONCOLOGY, LANCET PUBLISHING GROUP, LONDRES, GB, vol. 6, no. 5, Mayo de 2005 (2005-05), páginas 271-278, XP004870902 ISSN: 1470-2045

Re Item III

No establecimiento de opinión con respecto a novedad, nivel inventivo y aplicabilidad industrial

Esta autoridad considera que la materia objeto relacionada con las reivindicaciones 19, 21, está cubierta por las disposiciones de la Regla 67.1 (iv) del PCT. En consecuencia, no se formulará opinión

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alguna con respecto a la aplicabilidad industrial de la materia-objeto de estas reivindicaciones (Artículo 34(4)(a)(I) del PCT).

Re Item V

Declaración razonada bajo la Regla 66.2(a)(ii) con respecto a novedad, nivel inventivo y aplicabilidad industrial; referencias y explicaciones que sustentan dicha declaración.

Se hace referencia a los siguientes documentos:

- D1: BILlich A: "VACUNA VPH MEDIMMUNE/GLAXOSMITHKLINE" CURRENT OPINION IN INVESTIGATIONAL DRUGS, CURRENT DRUGS, LONDRES, GB, vol. 4, no. 2, Febrero de 2003 (2003-02), páginas 210-213, XP009029755 ISSN: 0967-8298
- D2: REINIS MILAN: "Evaluación de la tecnología: vacuna VPH (tetraivalente), Aventis Pasteur MSD/CSL" CURRENT OPINION IN MOLECULAR THERAPEUTICS. ABR 2004, vol. 6, no. 2, Abril de 2004 (2004-04), páginas 206-211, XP009056594 ISSN: 1464-8431.
- D3: WO 01/97840 A (MEDIMMUNE, INC) Diciembre 27 de 2001 (2001-12-27).
- D4: EP-A-1 410 805 (GLAXOSMITHKLINE BIOLOGICALS S.A) Abril 21 de 2004 (2004-04-21)
- D5: WO 03/077942 A2 (Glaxosmithkline Biologicals SA) Septiembre 25 de 2003 (2003-09-25).

Novedad (Artículo 33(1) y (2) del PCT)

1) Ninguno de los documentos del arte previo citados revela una composición inmunogénica que comprenda VLPs o capsómeros de VPH 16 y 18 y al menos algún otro VPH seleccionado entre VPH 31, 45 y 52 en una cantidad reducida con respecto a VPH 16 y 18.

D5 revela una composición de vacuna compuesta por una mezcla en proporciones iguales de VPH 16, 18, 31 y 45 (ejemplo 3).

Por lo tanto, la materia objeto de las reivindicaciones 1-21 se considera novedosa sobre el arte previo citado.

Nivel Inventivo (artículo 33(1) y (3) del PCT)

2) El documento del arte previo más cercano es D5, que revela una composición de vacuna que comprende VLPs que contienen proteínas L1 o derivados funcionales de éstas, de genotipos VPH16, VPH18, VPH31 y VPH45. Se obtuvo una fuerte respuesta inmune contra cada genotipo usando una combinación de los cuatro antígenos en cantidades iguales (2 µg) (Figuras 4-7 y Ejemplo 3).

La diferencia entre D5 y la presente solicitud es la provisión de una composición inmunogénica que comprende VLPs o capsómeros de VPH16 y VPH18 y al menos otro tipo seleccionado entre 31, 45 y/o 52 en una cantidad reducida comparada con VPH16 ó VPH18.

Por lo tanto, el problema puede ser visto como la provisión de una vacuna alternativa contra VPH16, VPH18 y al menos otro tipo de VPH.

La solución propuesta es la provisión de una composición inmunogénica que comprende VLPs o capsómeros de VPH16 y 18 y al menos algún otro VPH seleccionado entre VPH31, 45 y 52 en una cantidad reducida con respecto a VPH 16 y 18.

El resultado no es logrado en la presente solicitud, en la cual no hay datos que muestren que una cantidad reducida de VPH31, 45 ó 52 en comparación con VPH16 ó 18 produzca una respuesta neutralizante por parte de los anticuerpos. Los únicos resultados revelados en la presente solicitud son datos de reacción cruzada usando una vacuna que comprende capsómeros de solo dos VPH, i.e., VPH16 y VPH18 con VPH31, 45 ó 52.

En el campo de la inmunología no es suficiente que se revele una formulación de vacuna, también se requiere evidencia experimental de que se elicitaba una respuesta neutralizante, puesto que tal respuesta no puede predecirse.

Por lo tanto, la materia objeto de las reivindicaciones 1-18 no cumple las provisiones del Artículo 33(1) y (3) del PCT.

La materia objeto de las reivindicaciones 19-21 será considerada inventiva solo si la composición allí usada es nueva e inventiva.

Aplicabilidad industrial (Artículo 33(1) y (4) del PCT)

3) Para la evaluación de si las presentes reivindicaciones 19, 21 tienen o no aplicabilidad industrial, no existe criterio unificado en los estados contratantes del PCT. La patentabilidad puede también ser dependiente de la formulación de las reivindicaciones. La EPO, por ejemplo, no reconoce como industrialmente aplicable la materia objeto de reivindicaciones de uso de un compuesto en tratamiento médico. Sin embargo, puede permitir reivindicaciones de primer uso en tratamiento médico de un compuesto conocido y el uso de tal compuesto para la fabricación de un medicamento para un nuevo tratamiento médico.

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To: STEPHEN, Robert, J. GlaxoSmithKline, CIP (CN825.1) 980 Great West Road Brentford, Middlesex TW8 9GS GRANDE BRETAGNE		GlaxoSmithKline Corporate IP Received BRENTFORD 16 OCT 2006 ATTY: KCP/UA...:CS IPM: /A ON UPDATION ATTY CHECKED: /LE (day/month/year)		NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (PCT Rule 71.1)	
				12.10.2006	
Applicant's or agent's file reference RJSVB60891			IMPORTANT NOTIFICATION		
International application No. PCT/EP2005/008481		International filing date (day/month/year) 14.06.2005		Priority date (day/month/year) 16.06.2004	
Applicant GLAXOSMITHKLINE BIOLOGICALS S.A. et al.					
- The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary report on patentability and its annexes, if any, established on the international application. - A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices. - Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices. REMINDER The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301). Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary report on patentability. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned. For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide. The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.					
Name and mailing address of the International preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399-0 Tx: 523656 epmu d Fax: +49 89 2399-4465			Authorized Officer Yassine, Mahjoub Tel. +49 89 2399-5762 		

Form PCT/PEA/416 (January 2004)

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PATENT COOPERATION TREATY
PCT
INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)
(PCT Article 36 and Rule 70)

Applicant's or agent's file reference RJSAVB60891	FOR FURTHER ACTION		See Form PCT/PEA/16
International application No. PCT/EP2005/008481	International filing date (day/month/year) 14.08.2005	Priority date (day/month/year) 16.08.2004	
International Patent Classification (IPC) or national classification and IPC INV. A61K39/12			
Applicant GLAXOSMITHKLINE BIOLOGICALS S.A. et al.			
1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36. 2. This REPORT consists of a total of 9 sheets, including this cover sheet. 3. This report is also accompanied by ANNEXES, comprising: a. ☐ sent to the applicant and to the International Bureau a total of sheets, as follows: ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 807 of the Administrative Instructions). ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box. b. ☐ (sent to the International Bureau only) a total of (Indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).			
4. This report contains indications relating to the following items: ☒ Box No. I Basis of the report ☒ Box No. II Priority ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability ☐ Box No. IV Lack of unity of invention ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement ☐ Box No. VI Certain documents cited ☐ Box No. VII Certain defects in the international application ☐ Box No. VIII Certain observations on the international application			
Date of submission of the demand 12.01.2006		Date of completion of this report 12.10.2006	
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80299 Munich Tel. +49 89 23399 - 0 Fax: +49 89 23399 - 4465		Authorized officer RENGGLI-ZULLIGER, N Telephone No. +49 89 23399-7482 	

**INTERNATIONAL PRELIMINARY REPORT
ON PATENTABILITY**

International application No.
PCT/EP2005/006461

Box No. I Basis of the report

1. With regard to the language, this report is based on

- ☒ the international application in the language in which it was filed
- ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of:
- ☐ international search (under Rules 12.3(a) and 23.1(b))
 - ☐ publication of the international application (under Rule 12.4(a))
 - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a))

2. With regard to the elements* of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report):*

Description, Pages

1-45 as originally filed

Claims, Numbers

1-21 as originally filed

- ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:

- ☐ the description, pages
- ☐ the claims, Nos.
- ☐ the drawings, sheets/figs
- ☐ the sequence listing *(specify):*
- ☐ any table(s) related to sequence listing *(specify):*

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

- ☐ the description, pages
- ☐ the claims, Nos.
- ☐ the drawings, sheets/figs
- ☐ the sequence listing *(specify):*
- ☐ any table(s) related to sequence listing *(specify):*

* If item 4 applies, some or all of these sheets may be marked "superseded."

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**INTERNATIONAL PRELIMINARY REPORT
ON PATENTABILITY**

International application No.
PCT/EP2005/006461

Box No.	II	Priority
1.	☐	This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested: ☐ copy of the earlier application whose priority has been claimed (Rule 66.7(a)). ☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).
2.	☐	This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.
3.	Additional observations, if necessary: see separate sheet	

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INTERNATIONAL PRELIMINARY REPORT
ON PATENTABILITY

International application No.
PCT/EP2005/006461

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application,
☒ claims Nos. 19,21

because:

- ☒ the said international application, or the said claims Nos. 19,21 relate to the following subject matter which does not require an international preliminary examination (*specify*):

see separate sheet

- ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):
- ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed (*specify*):
- ☐ no international search report has been established for the said claims Nos.
- ☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:
- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13ter.1(a) or (b) and 13ter.2.
- ☐ a meaningful opinion could not be formed without the tables related to the sequence listings; the applicant did not, within the prescribed time limit, furnish such tables in electronic form complying with the technical requirements provided for in Annex C-bis of the Administrative Instructions, and such tables were not available to the International Preliminary Examining Authority in a form and manner acceptable to it.
- ☐ the tables related to the nucleotide and/or amino acid sequence listing, if in electronic form only, do not comply with the technical requirements provided for in Annex C-bis of the Administrative Instructions.
- ☐ See separate sheet for further details

**INTERNATIONAL PRELIMINARY
REPORT ON PATENTABILITY
(SEPARATE SHEET)**

International application No.

PCT/EP2005/006461

Re Item II

Priority

3. Additional observations :

The priority documents pertaining to the present application were not available at the time of establishing this preliminary report. Hence, the current assessment is based on the assumption that all claims enjoy priority rights from the filing date of the priority document (16.6.2004). If it later turns out that this is not correct, the following document cited in the International Search Report (ISR) could become relevant to assess whether the claimed subject-matter of the present application satisfies the criteria set forth in Article 33(1) PCT.

GLAXOSMITHKLINE HPV VACCINE STUDY GROUP HARPER D M ET AL: "Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial" LANCET THE, LANCET LIMITED, LONDON, GB, vol. 364, no. 9447, 13 November 2004 (2004-11-13), pages 1757-1765, XP004746625 ISSN: 0140-6736 cited in the application

WO 2004/056389 A (GLAXOSMITHKLINE BIOLOGICALS SA; DUBIN, GARY; INNIS, BRUCE; SLAOU, MON) 8 July 2004 (2004-07-08)

D3:VILLA L L ET AL: "Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial" LANCET ONCOLOGY, LANCET PUBLISHING GROUP, LONDON, GB, vol. 6, no. 5, May 2005 (2005-05), pages 271-278, XP004870902 ISSN: 1470-2045

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 19,21 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to

the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Re Item V

Reasoned statement under Rule 66.2(a)(II) with regard to novelty, inventive step or Industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

- D1: BILLICH A: "HPV VACCINE MEDIMMUNE/GLAXOSMITHKLINE" CURRENT OPINION IN INVESTIGATIONAL DRUGS, CURRENT DRUGS, LONDON, GB, vol. 4, no. 2, February 2003 (2003-02), pages 210-213, XP009029755 ISSN: 0967-8298
- D2: REINIS MILAN: "Technology evaluation: HPV vaccine (quadrivalent), Aventis Pasteur MSD/CSL" CURRENT OPINION IN MOLECULAR THERAPEUTICS. APR 2004, vol. 6, no. 2, April 2004 (2004-04), pages 206-211, XP009056594 ISSN: 1464-8431
- D3: WO 01/97840 A (MEDIMMUNE, INC) 27 December 2001 (2001-12-27)
- D4: EP-A-1 410 805 (GLAXOSMITHKLINE BIOLOGICALS S.A) 21 April 2004 (2004-04-21)
- D5: WO 03/077942 A2 (Glaxosmithkline Biologicals SA) 25 September 2003 (2003-09-25).

Novelty (Article 33(1) and (2) PCT)

1) None of the cited prior art discloses an immunogenic composition comprising VLPs or capsomers from HPV 16 and 18 and at least one other HPV selected among HPV 31, 45 and 52 in reduced amount relative to HPV 16 and 18.

D5 discloses a vaccine composition composed of a mixture in equal proportion of HPV 16, 18, 31 and 45 (example 3).

Therefore, the subject-matter of claims 1-21 is considered novel over the cited prior art.

Inventive step (Article 33(1) and (3) PCT)

2) The closest prior art document is D5 disclosing a vaccine composition comprising VLPs containing L1 proteins or functional derivatives thereof from HPV16, HPV18, HPV31 and HPV45 genotypes. A strong immune response was obtained against each genotypes using a combination of the four antigens in equal amounts (2µg) (Figures 4-7 and Example 3).

The difference between D5 and the present application is the provision of an immunogenic composition that comprises VLPs or capsomers of HPV16 and HPV18 and at least a another type chosen between 31, 45 or/and 52 in a reduced amount compared to HPV16 or HPV18.

The problem can therefore be seen as the provision of an alternative vaccine against HPV16, HPV18 and at least another HPV type.

The solution proposed is the provision of an immunogenic composition comprising VLPs or capsomers from HPV 16 and 18 and at least one other HPV selected among HPV 31, 45 and 52 in reduced amount relative to HPV 16 and 18.

The result is not achieved in the present application in that no data showing that a reduced amount of HPV31, 45 or 52 compared to HPV16 or 18 raises an neutralizing antibody response. The only results disclosed in the present application are cross-reaction data using a vaccine comprising capsomers of only two HPVs i.e. HPV16 and HPV18 with HPV 31, 45 or 52.

In the Immunology field it is not sufficient that a vaccine formulation is disclosed but experimental evidence that a neutralizing response is elicited is required, since such a response cannot be predicted.

Therefore, the subject-matter of claims 1-18 does not fulfil the provisions of Article 33(1) and (3) PCT.

**INTERNATIONAL PRELIMINARY
REPORT ON PATENTABILITY
(SEPARATE SHEET)**

International application No.

PCT/EP2005/006461

The subject-matter of claims 19-21 will only be considered inventive if the composition used therein is novel and inventive.

Industrial applicability (Article 33(1) and (4) PCT)

3) For the assessment of the present claims 19,21 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

VACUNA CONTRA HPV16 Y HPV18 Y AL MENOS OTRO TIPO DE VPH

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SELECCIONADO DE VPH 31, 45 O 52**Resumen-Objeto y Finalidad de la Invención**

Una composición inmunogénica y procedimientos para producir dicha
10 composición, la composición comprendiendo las PLV de VPH 16 y 18 y al
menos otro tipo de cáncer de VPH, el otro tipo de cáncer seleccionándose
entre la lista constituida por los tipos 31, 45, y 52 de VPH, en la que la dosis de
la PLV de al menos otro tipos de cánceres se reduce con relación a la de VPH
16 ó 18.

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**VACUNA CONTRA HPV16 Y HPV18 Y AL MENOS OTRO TIPO DE VPH
SELECCIONADO DE VPH 31, 45 O 52**

La presente solicitud se refiere a vacunas contra papilomavirus.

5

Antecedentes de la invención

Los papilomavirus son pequeños virus de tumores de ADN, que son altamente específicos de especies. Hasta ahora, se han descrito por encima de 100 genotipos de papilomavirus humanos individuales (VPH). Los VPH son
10 generalmente específicos o bien para la piel (por ejemplo, VPH - 1 y - 2) o de superficies mucosas (por ejemplo, VPH - 6 y -11) y usualmente producen tumores benignos (verrugas) que persisten durante varios meses o años. Tales tumores benignos pueden ser preocupantes para los individuos a los que atañe pero no son amenazantes para la vida, con unas pocas excepciones.

15 Algunos VPH también están asociados a cánceres, también conocidos como tipos de VPH. La asociación positiva más fuerte entre un VPH y cáncer humano es la que existe entre VPH - 16 y VPH - 18 y carcinoma de cuello de útero. El cáncer de cuello de útero es la malignidad más común en los países desarrollados, con aproximadamente 500.000 nuevos casos que se producen
20 en el mundo cada año.

Otros VPH de particular interés con respecto a cáncer son los tipos 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82, 26, 53, y 66.

Las partículas que parecen virus VPH (PLV) se han sugerido como vacunas potenciales para tratamiento de VPH. Una vacuna bivalente que utiliza
25 las PLV se ha mostrado que es eficaz en la prevención de infección con los tipos 16 y 18 de VPH en mujeres jóvenes (Lancet, vol 364, publicación 9447,

noviembre de 2004, páginas 1757 - 1765).

Todavía existe una necesidad de una vacuna que proteja contra los tipos múltiples de VPH (por ejemplo > 2).

La presente invención se dirige a esta necesidad.

5

Resumen de la invención

En un primer aspecto la invención se refiere a una composición inmunogénica que comprende las PLV de VPH 16 y 18 y al menos otro tipo de cáncer de VPH, el otro tipo de cáncer se selecciona entre la lista constituida por los tipos 31, 45 y 52 de VPH, en los que la dosis de la PLV de al menos otro de los tipos de cáncer se reduce con relación a la del VPH 16 ó 18.

En un aspecto relacionado la invención se refiere a una composición inmunogénica que comprende las PLV del VPH 16 y al menos otro tipo de cáncer de VPH, el otro tipo de cáncer seleccionándose entre la lista constituida los por tipos 31 y 52 de VPH, en los que la dosis de la PLV de al menos otros tipos de cáncer se reduce con relación al VPH 16.

En un aspecto relacionado la invención se refiere a una composición inmunogénica que comprende las PLV del VPH 18 y VPH 45, en las que la dosis del VLP del VPH 45 se reduce con relación a la del VPH 18.

La invención además se refiere a una composición inmunogénica como se ha definido anteriormente en combinación con un adyuvante y/o vehículo.

La invención además se refiere a una vacuna que comprende una composición inmunogénica como se ha definido anteriormente con un excipiente farmacéuticamente aceptable.

La invención además se refiere a un procedimiento de prevención de

infección y/o enfermedad de VPH que comprende la administración a un individuo en necesidad del mismo de una composición o vacuna como se ha definido anteriormente.

La invención además se refiere a un procedimiento para preparar una
5 composición inmunogénica como se ha definido anteriormente que comprende la mezcla de las PLV de los VPH 16 y 18 con al menos otro tipo de cáncer de VPH, el otro tipo de cáncer seleccionándose entre la lista constituida por los tipos 31, 45, y 52 de VPH, en los que la dosis de la PLV de al menos otros tipos de cáncer se reduce con relación a la de los VPH 16 ó 18.

10 Los aspectos anteriores de la invención también pueden emplear capsómeros de VPH en lugar de las PLV.

Descripción detallada

Los inventores han determinado sorprendentemente que las PLV de los
15 VPH 16 y 18 pueden proporcionar protección cruzada contra infección y/o enfermedad por ciertos tipos de cáncer distintos de VPH, es decir, VPH 31, VPH 45 y VPH 52. Los datos se proporcionan en los ejemplos en esta memoria descriptiva.

La protección cruzada se puede considerar como la protección
20 proporcionada por una vacuna que contiene un tipo de VPH contra infección (incidente o persistente) y/o enfermedad producida por un tipo de VPH diferente. La infección incidente y persistente se definen como en la publicación de Lancet por Harper y col., vol. 364, publicación 9447, noviembre 2004, páginas 1757 - 1765. La protección cruzada se puede evaluar considerando la
25 eficacia de la vacuna (V. E.), en la que la V. E. es la % de mejora en protección

contra la infección por la vacuna comparada con un grupo placebo para un tipo dado.

De acuerdo a lo anterior, las vacunas de VPH que comprenden las PLV de VPH 16 y 18 se pueden formular usando una dosis inferior de otros tipo de PLV de cáncer (no - VPH 16/18) (31, 45 ó 52) que de otra manera se requerirían en ausencia de las PLV de VPH 16 y 18, mientras que todavía logran la misma respuesta protectora contra infección por VPH incidente y/o persistente para ese otro tipo. Puede ser ventajosa la reducción de la dosis de las PLV de otro tipo de cáncer en un escenario de vacuna multivalente, sin impacto significativo sobre la protección producida por aquellos otros tipos cuando la cantidad total de antígeno se puede limitar; por ejemplo, mediante restricciones físicas, químicas o reguladoras. También se permite que se produzcan más dosis de una vacuna para una cantidad dada de antígeno y puede potencialmente reducir el coste global de la vacuna.

La dosis de PLV en esta memoria descriptiva es convenientemente la cantidad de PLV, medida en peso.

En otras palabras, para lograr la misma respuesta protectora contra infección incidente y/o persistente, la dosis de otros PLV de tipo canceroso (no VPH 16/VPH 18) que necesitan usarse en combinación con las VLP de VPH 16 y/o VPH 18 se pueden reducir comparada con el nivel que se requiere en ausencia de tales tipos de PLV 16 y/o 18.

De este modo la invención se refiere a una composición inmunogénica que comprende las PLV de los VPH 16 y 18 y al menos otro tipo de cáncer de VPH, el otro tipo de cáncer seleccionándose entre la lista constituida los por tipos 31, 45 y 52 de VPH, en los que la dosis de la PLV de al menos otros tipos

de cáncer se reduce con relación al de VPH 16 ó 18.

En un aspecto alternativo la invención se refiere a una composición inmunogénica que comprende las PLV de los VPH 16 y 18 y al menos otro tipo de cáncer de VPH, el otro tipo de cáncer seleccionándose entre la lista constituida los por tipos 31, 45 y 52 de VPH, en los que la dosis de la PLV de al menos otros tipos de cáncer se reduce con relación a los que se requieren de otra manera, en ausencia de las PLV de VPH 16 y 18, que genera la misma protección contra infección por VPH incidente y/o persistente por ese tipo otro tipo de cáncer.

En un aspecto la dosis del otro PLV de tipo canceroso (no VPH 16, 18) es suficiente para proporcionar protección contra infección incidente y/o persistente por ese tipo, y en un aspecto de la invención la protección de la invención contra al menos infección incidente.

En un aspecto de la invención la composición de la invención es adecuada para protección de infección y /o enfermedad en sujetos humanos.

Se puede determinar una dosis adecuada mediante, por ejemplo, ensayos en seres humanos tales como los descritos en los ejemplos en esta memoria descriptiva.

La protección contra la infección incidente y/o persistente mediante un tipo de VPH dado, tal como por ejemplo VPH 16, 18, 31, 45 ó 52, la protección es convenientemente en el 50% de una población vacunada contra infección por ese tipo, y en un aspecto de la invención es 60% de protección, en un aspecto adicional 70% de protección, en un aspecto adicional 80% de protección, en un aspecto adicional 90% de protección, en un aspecto adicional 95% de protección, en un aspecto adicional 96% de protección, en un aspecto

adicional 97% de protección, en un aspecto adicional 98% de protección, en un aspecto adicional 99% de protección y todavía en un aspecto adicional 100% de protección.

Convenientemente esta protección se evalúa sobre un período de al menos 6 meses, tal como durante un período de al menos 9 meses, al menos 1 año, al menos 18 meses, convenientemente durante un período de 2 años o más de 2 años.

En un aspecto de la invención, la protección se ve contra infección o enfermedad producida por VPH 16 y/o VPH 18, y en un aspecto infección y/o enfermedad tanto por VPH 16 como VPH 18.

La prevención de la infección se puede evaluar mediante análisis de especies de VPH presentes en individuos vacunados, por ejemplo mediante análisis de PCR y/o técnicas de hibridación tales como las descritas en los documentos WO 03014402 y WO 9914377, incorporados en esta memoria descriptiva como referencia.

Cuando la composición inmunogénica de la invención comprende las PLV tanto de VPH 16 como 18 entonces las PLV de tipo canceroso no de VPH 16/18 es tipo 31, o tipo 45, o tipo 52, o una combinación de los mismos. En un aspecto la composición inmunogénica de la invención comprende las PLV de VPH 16, 18, 31 y 45. En un aspecto la composición inmunogénica de la invención comprende las PLV de VPH 16, 18, 31 y 52. En un aspecto la composición inmunogénica de la invención comprende las PLV de VPH 16, 18, 45 y 52. En un aspecto la composición inmunogénica de la invención comprende las PLV de VPH 16, 18, 31, 52 y 45. Cuando hay 2 o más otras PLV de tipo canceroso (por ejemplo, 31, 45, 31 y 52, 45 y 52), entonces al

menos uno de estos otros tipos de cáncer está presente a una dosis que se reduce al de VPH 16 o VPH 18.

En un aspecto la dosis de VPH 31 se reduce con relación al del VPH 16.

En un aspecto la dosis de VPH 52 se reduce con relación al del VPH 16.

5 En un aspecto la dosis de VPH 45 se reduce con relación al del VPH 18.

Convenientemente las composiciones inmunogénicas como se han definido anteriormente proporcionan protección contra infección incidente y/o infección persistente y/o enfermedad producida por VPH 16, VPH 18 y uno o más de los otros tipo de VPH (31, 45 ó 52) presentes en los grupos
10 enumerados anteriormente, tal como infección incidente.

Cuando una composición inmunogénica de la invención comprende las PLV de VPH 16 pero no las PLV de VPH 18 entonces convenientemente el tipo de cáncer de PLV no VPH 16/18 es el tipo 31 y/o tipo 52.

Cuando una composición Inmunogénica de la invención comprende las
15 PLV de VPH 18 pero no las PLV de VPH 16 entonces convenientemente el tipo de cáncer de PLV de VPH 16/18 es el tipo 45.

En un aspecto de la invención la composición comprende las PLV de VPH 16 y 18 en combinación con cualquiera o las PLV de tanto VPH 31 como VPH 45.

20 En un aspecto de la invención la composición comprende al menos las PLV de VPH 16 en combinación con las PLV de VPH 31.

La composición de la invención puede comprender, además de las PLV a dosis reducida, otras PLV de VPH a cualquier dosis adecuada. Por ejemplo, la composición de la invención puede comprender tipos de cáncer de "alto
25 riesgo" tal como uno o más de los VPH 33, 35, 39, 51, 56, 58, 59, 66, ó 68.

En un aspecto la composición puede comprender los también llamados tipos de "verrugas genitales" tales como VPH 6 y/u 11, o también llamados tipos "de piel" tales como VPH 5 y/u 8. En un aspecto las PLV adicionales están presentes a la misma dosis o mayor que VPH 16y/o VPH 18.

5 En un aspecto la composición puede comprender adicionalmente los también llamado tipos de "verrugas genitales" tales como VPH 6 y/u 11, o también llamados tipos de "piel" tales como VPH 5 y/u 8. En un aspecto las PLV adicionales están presentes en la misma dosis o mayor que VPH 16 y/o VPH 18.

10 En un aspecto de la invención la composición comprende las PLV de VPH 39 y VPH 51 y la dosis de al menos uno de éstos se reduce con relación a VPH 16 y/o VPH 18.

En un aspecto la cantidad de cualquier PLV adicional se selecciona de manera que proporcione algún grado de protección contra infección o
15 enfermedad contra el (los) tipo (s) adicionales.

Ciertas composiciones de la invención, individualizadas a continuación, comprenden la siguiente dosis de las PLV:

Número de composición	PLV de VPH 16 (μg)	PLV de VPH 18 (μg)	PLV de VPH 31 (μg)	PLV de VPH 45 (μg)
1	20	20	10	10
2	20	30	10	10
3	20	30	20	20
4	30	20	10	10

5	30	20	20	20
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- Convenientemente no existe interferencia significativa o biológicamente relevante entre las PLV de VPH en la composición de la invención, de manera que la vacuna de PLV combinada de la invención es capaz de ofrecer
- 5 protección eficaz contra la infección por cada tipo de PLV de VHP representada en la vacuna. Convenientemente la respuesta inmune contra un tipo de PLV dado en la combinación es al menos 50% de la respuesta inmune del mismo tipo de PLV cuando se mide individualmente, preferiblemente 100% o sustancialmente 100%. Para respuestas de los componentes de VHP 16 y
- 10 VHP 18, la vacuna combinada de la invención preferiblemente estimula una respuesta inmune que es al menos 50% de la proporcionada por una vacuna de PLV de VHP 16 / VHP 18. Convenientemente la respuesta inmune generada por la vacuna de la invención está a un nivel en el que el efecto protector de cada tipo de PLV se ve todavía. La respuesta inmune se puede medir
- 15 convenientemente, por ejemplo, mediante respuestas de anticuerpos que usan técnicas convencionales tales como ELISA, y mediante ensayos clínicos como se describen en esta memoria descriptiva.

En un aspecto la composición de la invención no comprende una proteína de choque por calor o fragmento de la misma.

- 20 En un aspecto la composición de la invención no comprende una proteína o péptido L2 de VHP. En otro aspecto la composición de la invención comprende una proteína o péptido L2 de VHP.

Las PLV de VHP y procedimientos para la producción de las PLV se conocen bien en la técnica. Las PLV típicamente se construyen a partir de las

proteínas estructurales L1 y L2 del virus, véanse por ejemplo, los documentos WO 9420137, WO 9629413 y WO 9405792. Cualquier PLV de VHP se puede usar en la presente invención tal como PLV de L1 o L1 + L2.

La formación de PLV se puede evaluar mediante técnicas
5 convencionales tales como, por ejemplo, microscopía electrónica y dispersión de luz láser dinámica.

En un aspecto de la invención la PLV es una PLV L1 solamente.

La PLV puede comprender la proteína L1 de longitud completa.

En un aspecto de la invención la proteína L1 usada para formar la PLV
10 es una proteína L1 truncada. Las proteínas L1 de VHP truncadas se describen en, por ejemplo, el documento US 6361778, incorporado en esta memoria descriptiva como referencia. En un aspecto adicional el truncamiento es un truncamiento C terminal. En un aspecto adicional el truncamiento C terminal elimina menos de 50 aminoácidos, convenientemente y preferiblemente menos
15 de 40 aminoácidos. Convenientemente la secuencia de L1 de VHP 16 comienza en el segundo codón de metionina, por ejemplo como se muestra en la secuencia más adelante, o posiciones análogas en los otros tipos de VHP. Cuando la PLV es una PLV de VHP 16 entonces en un aspecto el truncamiento C terminal elimina 34 aminoácidos de la secuencia de L1 de VHP 16. Cuando
20 la PLV es una PLV de VHP 18 entonces en un aspecto el truncamiento C terminal elimina 35 aminoácidos de la secuencia de L1 de VHP 18.

En un aspecto la secuencia de VHP 16 es la secuencia siguiente:

MSLWLPSEATVYLPPVPVSKVVSTDEYVARTNIYYHAGTBRLLAVGHPYFPIKKPNNKI	60
LVPKVSGLQYRVFRIHLPDPNKGFPDTSFY'NPDTRLVWACVQVBYGRQQPLQVGLSGH	120
PLLNKLDDTENASAYAANAGVDNRECISMDYKQTQLCLIGCKPPIGEHWGKGSPTNVAV	180
NPGDCPPELINNTVIQDGDMDVTGPGAMDFTTLQANKSEVPLDICTSICKYPPDYIKMVSE	240
PYGDSLFFYLRRBQMFVRHLFNRAAGAVGENVPDDLYIKGGSTANLASSNYFPTPSGSMV	300
TSDAQIFNKPYWLQRAQGHNNGICWGNQLFVTVVDTTRSTNMSLCAAISTSETTYKNTNF	360
KEYLRHGEEYDLQFIFQLCKITLTADVMTYIHSMNSTILEDWNFGLQPPPGCTLEDYRF	420
VTSQAIACQKHTPPAPKEDPLKKYTFWEVNLKEKFSADLDQFFLGRKFLQ	471

La secuencia de VHP 16 también puede ser la descrita en el documento WO 9405792 o US 6649167, por ejemplo, convenientemente truncada. Los truncamientos adecuados están truncados en una posición equivalente de
5 manera como se ha mostrado anteriormente, como se determina mediante comparación de secuencias.

En un aspecto la secuencia de VHP 18 es la siguiente secuencia:

MALWRPSDNTVYLPPPSVARV'VNTDDYVTRTSIFYHAGSSRLLTGPNPYFRVPAGGONKQ	60
DIPKVSAYQYRVFRVQLPDPNKGFLPDNSTYNPETQLVWACVQVBYGRQQPLGVGLSGH	120
PFY'NKLDDTESSHAATSNVSEDVRD'NVSVDYKQTQLCLIGCAPAIGEHWAEGTACKSRPL	180
SGQDCPPELKNVLEDDMDYDTGYGAMDFSTLQDTKCEVPLDIOQSICKYPPDYLOMSAD	240
PYGDSMFFCLRRQLFARHFWNRAQTMGDTVPPSLYIKGTGMRASPGSCVYSPSPSGSIV	300
TSDSQLFNKPYWLHKAQGHNNGVCWHNQLFVTVVDTTRSTNLTICASTQSPVPGQYDATK	360
FKQYSR'HVVEEYDLQFIFQLCTITLTADVMSYIHSMNSSILEDWNFGVPPPPFTSLVDYR	420
FVQSVAITCQKDAAPAENKDPYDKLKFWNVDLKEKFSADLDQYPLGRKFLVQ	472

10

Una secuencia de VHP 18 alternativa se describe en el documento WO 9629413, que puede estar convenientemente truncada. Los truncamientos adecuados están truncados en una posición equivalente a la mostrada anteriormente, como se determina por comparación de secuencias.

15 Otras secuencias de VHP 16 y VHP 18 se conocen en la técnica y

pueden ser adecuadas para uso en la presente invención.

Los truncamientos adecuados de VPH 31, VHP 45 y VHP 52 también se pueden realizar, adecuadamente retirando las porciones C terminales equivalentes de la proteína L1 a las descritas anteriormente como se determina
5 mediante alineación de secuencias.

Las proteínas L1 truncadas se describen en, por ejemplo, los documentos WO 9611272 y US 6066324, que se incorporan en esta memoria descriptiva como referencia.

En un aspecto las proteínas L1 truncadas son convenientemente
10 derivados de la proteína L1 funcional, capaces de inducir una respuesta inmune (si es necesario, cuando convenientemente se acompaña de adyuvante), dicha respuesta inmune siendo capaz de reconocer una PLV constituida por la proteína L1 de longitud completa y/o el tipo de VHP de la que se deriva la proteína L1.

15 Las PLV de la invención también pueden comprender otros tipos de derivados de proteínas funcionales, que incluyen mutantes de las proteínas L1 de VHP de longitud completa o truncadas tales como mutantes de supresión, sustitución, o inserción. La proteína L1 o derivada también puede ser una proteína de fusión, tal como la fusión de la proteína L1 con L2 o una proteína
20 temprana. La proteína L1 del derivado de proteína funcional es capaz de formar una PLV, y la formación de PLV se puede determinar mediante técnicas convencionales tales como, por ejemplo, microscopía electrónica y dispersión de luz de láser dinámica.

Las PLV también se pueden preparar en cualquier sustrato celular
25 adecuado tal como células de levaduras o células de insecto, por ejemplo,

células de baculovirus, y técnicas para la preparación de PLV se conocen bien en la técnica, tal como los documentos WO 9913056 y US 6245568, y las referencias en ellos, cuyos contenidos se incorporan en esta memoria descriptiva como referencia.

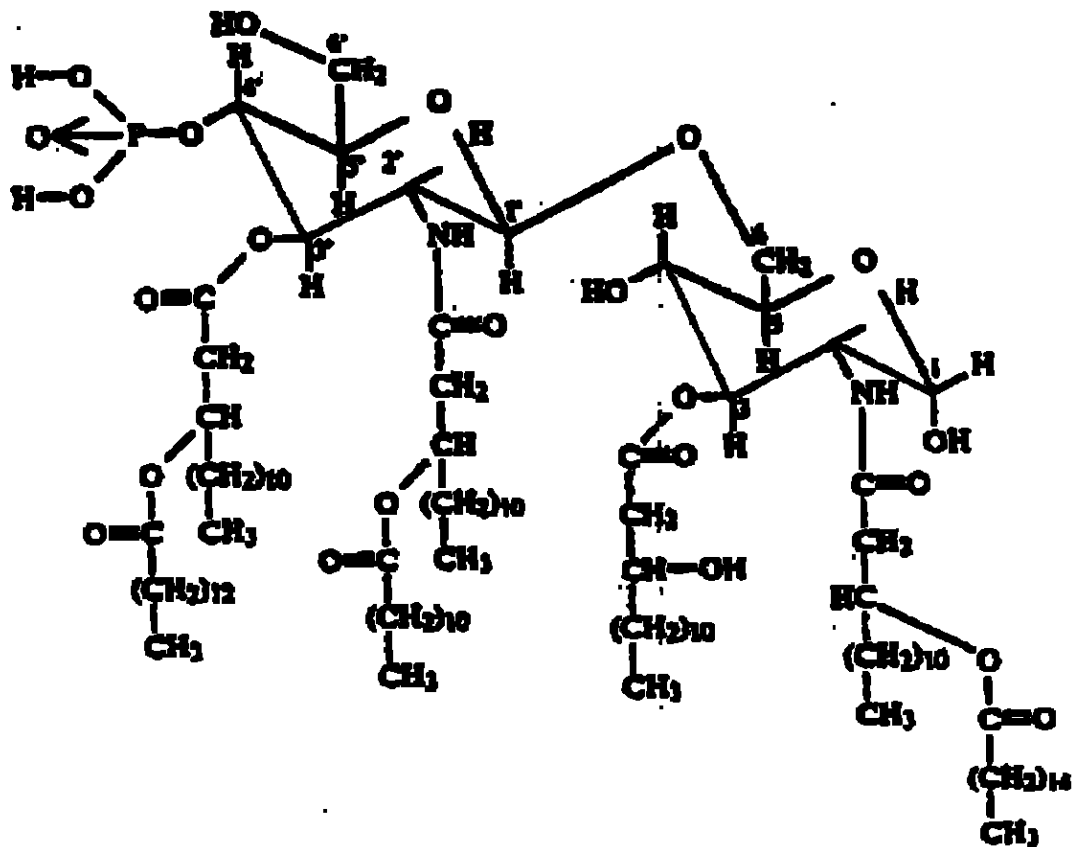
5 En un aspecto las PLV se preparan mediante técnicas de desacoplamientos y reagrupación, que pueden proporcionar las PLV de papilomavirus más estables y/o homogéneos. Por ejemplo, McCarthy y col., 1988 "Quantitative Disassembly and Reassembly of Human Papillomavirus Type 11 Viruslike Particles In Vitro" J. Virology 72 (1): 33 - 41, describe el
10 desacoplamiento y reagrupación de las PLV de VHP 11 L1 purificados a partir de células de insecto con el fin de obtener una preparación homogénea de las PLV. Los documentos WO 9913056 y US 6245568 también describen procedimientos de desacoplamiento y reagrupación para preparar las PLV de VHP.

15 En un aspecto las PLV de VHP de la invención se preparan como se describe en los documentos WO 9913056 y US 6245568.

 Las PLV de la invención se pueden combinar con un adyuvante o inmunoestimulante tal como, pero sin limitación, lípido A detoxificado a partir cualquier secuencia y derivados no tóxicos del lípido A, saponinas y otros
20 reactivos capaces de estimulación de una respuesta de tipo de TH1.

 Se ha conocido desde hace tiempo que el lipopolisacárido enterobacteriano (LPS) es un potente estimulador del sistema inmune, aunque su uso en adyuvantes se ha restringido por sus efectos tóxicos. Un derivado no tóxico de LPS, lípido A monofosforilo (MPL), producido por la eliminación de
25 grupo carbohidrato central y el fosfato de la glucosamina de extremo reductor, lo

han descrito Ribi y col (1986, Immunology and Immunopharmacology of bacterial endotoxins, Plenum Publ. Corp., NY, p. 407 - 419) y tiene la siguiente estructura:



5

Una versión adicional detoxificada de MPL se produce por la eliminación de la cadena acilo de la posición 3 de la estructura central de disacárido, y se llama lípido A monofosforilo 3-O-desacilado (3D-MPL). Se puede purificar y preparar mediante los procedimientos mostrados en GB 2122204B, cuya
10 referencia también describe la preparación de lípido A difosforilo, y las variantes 3-O-desaciladas del mismo.

En un aspecto el 3D-MPL está en la forma de una emulsión que tiene un

tamaño medio de partícula menor que 0,2 μm de diámetro, y su procedimiento de fabricación se describe en el documento 94/21292. Las formulaciones acuosas que comprenden el lípido A monofosforilo y un tensioactivo se han descrito en el documento WO 984367OA2.

5 El lipopolisacárido bacteriano derivado de adyuvantes a formularse en las composiciones de la presente invención se pueden purificar y procesar a partir de fuentes bacterianas, o como alternativa pueden ser sintéticas. Por ejemplo, el lípido A monofosforilo purificado se describe en Ribi y col., 1986 (anteriormente), y lípido A monofosforilo o difosforilo 3-O-desacilado derivado
10 de *Salmonella sp.* se describe en el documento GB 2220211 y US 4912094. Se han descrito otros lipopolisacáridos purificados y sintéticos (Hilgers y col., 1986, *Int. Arch. Allergy. Immunol.*, 79 (4): 392 - 6; Hilgers y col., 1987, *Immunology*, 60 (1): 141 - 6; y el documento EP 0 549 074 B1). En un aspecto el adyuvante de lipopolisacárido bacteriano es 3D - MPL.

15 De acuerdo a lo anterior, los derivados de LPS que se pueden usar en la presente invención son aquellos inmunoestimulantes que son similares en estructura a la de LPS o MPL o 3D - MPL. En otro aspecto de la presente invención los derivados de LPS pueden ser un monosacárido acilado, que es una sub - porción de la estructura anterior de MPL.

20 Las saponinas se describen en: Lacaille - Dubois, M y Wagner H. (1996. A review of the biological and pharmacological activities of saponinas. *Phytomedicine* vol. 2 pp. 363 - 386). Las saponinas son glicósidos esteroides o triterpenos ampliamente distribuidas en el reino vegetal y animal marino. Las saponinas se observa que forman soluciones coloidales en agua que forman
25 espuma tras agitación, y que precipitan colesterol. Cuando las saponinas están

cerca de las membranas celulares crean estructuras de tipo poro en la membrana que produce que la membrana revienta. La hemólisis de eritrocitos es un ejemplo de este fenómeno, que es una propiedad de ciertas, pero no todas, las saponinas.

5 Las saponinas se conocen como adyuvantes en vacunas para la administración sistémica. El adyuvante y actividad hemolítica de saponinas individuales se ha estudiado extensivamente en la técnica (Lacaille - Dubois y Wagner, anteriormente). Por ejemplo, Quil A (derivada de la corteza del árbol de Sur América Quillaza *Saponaria* Molina), y las fracciones de la misma, se describen en el documento US 5.057.540 y "Saponins as vaccine adjuvants", 10 Kensil, C. R., *Crit Rev Ther Drug Carrier Syst*, 1996, 12 (1 - 2): 1 - 55; y el documento EP 0 362 279 B1. Las estructuras particuladas, denominadas complejos estimulantes inmunes (ISCOMS), que comprenden fracciones de Quil A son hemolíticas y se han usado en la fabricación de vacunas (Morein, B., 15 documentos EP 0 109 942 B1; WO 96/11711; WO 96/33739). Las saponinas hemolíticas QWS21 y QS17 (fracciones purificadas por HPLC de Quil A) se han descrito como adyuvantes sistémicos potentes, y el procedimiento de su producción se describe en la patente de Estados Unidos Nº. 5.057.540 y el documento EP 0 362 279 B1. Otras saponinas que se han usado en estudios 20 de vacunación sistémica incluyen las derivadas de otras especies vegetales tales como *Gypsophila* y *Saponaria* (Bomford y col., *Vaccine*, 10 (9): 572 - 577, 1992).

Un sistema potenciado implica la combinación de un derivado de lípido A no tóxico y un derivado de saponina particularmente la combinación de QS21 y 25 3D -MPL como se describe en el documento WO 94/00153, o una composición

menos reactogénica en la que la QS21 se inactiva con colesterol como se describe en el documento WO 96/33739.

En un aspecto el adyuvante es una formulación de adyuvante particularmente potente que implica QS21 y 3D - MPL en una emulsión aceite
5 en agua y se describe en el documento WO 95/17210.

De acuerdo a una realización de la presente invención se proporciona una composición acompañada de adyuvante con lípido A detoxificado o un derivado no tóxico del lípido A, más preferiblemente acompañada de adyuvante con un lípido A monofosforilo o los derivados del mismo.

10 En un aspecto la composición adicionalmente comprende una saponina, más preferiblemente QS21.

En un aspecto la formulación de adyuvante adicionalmente comprende una emulsión aceite en agua. La presente invención también proporciona un procedimiento para producir una formulación de vacuna que comprende la
15 mezcla de una PLV de la presente invención junto con un excipiente farmacéuticamente aceptable, tal como 3D - MPL.

Los componentes adicionales que están preferiblemente presentes en una composición acompañada de adyuvante según la invención incluyen detergentes no tóxicos tales como los octoxinóles y polioxietilén ésteres como
20 se describe en esta memoria descriptiva, particularmente t-hidroxiapatita-fenoxi polietoxietanol (Triton X - 100) y monooleato de polioxietilén sorbitán (Tween 80); y sales biliares o derivados de ácido cólico como se describe en esta memoria descriptiva, en particular desoxicolato o taurodesoxicolato sódico. En un aspecto la formulación de adyuvante comprende 3D - MPL, Triton - 100,
25 Tween 80 y desoxicolato sódico, que se puede combinar con una PLV de VPH

proporcionando una vacuna adecuada.

En una realización de la presente invención, la composición comprende una formulación de adyuvante vesicular que comprende colesterol, una saponina y un derivado de LPS. A este respecto la formulación de adyuvante
5 puede comprender una vesícula unilamelar que comprende colesterol, que tiene una bicapa lipídica convenientemente comprendiendo dioleil fosfatidil colina, en la que la saponina y el derivado de LPS se asocian a, o están embebidas dentro, de la bicapa lipídica. Más preferiblemente, estas formulaciones de adyuvantes comprenden QS21 como la saponina, y 3D - MPL
10 y el derivado de LPS, en los que la relación de QS21: colesterol está entre 1:1 a 1:100 peso/peso, y lo más preferiblemente 1:5 peso/peso. Tales formulaciones de adyuvantes se describen en el documento EP 0 822 831 B, cuya descripción se incorpora en esta memoria descriptiva como referencia.

En un aspecto la composición de la invención se usan en combinación
15 con aluminio, y se absorben convenientemente o parcialmente absorbidos en adyuvante de aluminio. Convenientemente el adyuvante es una sal de aluminio, que en un aspecto está en combinación con 3D MPL, tal como fosfato de aluminio y 3D MPL.

En otro aspecto el adyuvante es hidróxido de aluminio, opcionalmente en
20 combinación con 3D MPL. En otros aspecto la composición es la combinación de las PLV con una sal de aluminio o con una sal de aluminio + 3D MPL. En un aspecto de la invención la sal de aluminio es hidróxido de aluminio.

La composición de la invención también puede comprender aluminio o un compuesto de aluminio como estabilizador.

25 La presente invención generalmente se refiere a combinaciones de PLV.

Sin embargo, se aprecia que el componente esencial de la PLV es una proteína L1. Las proteínas L1 se asocian para formar pentámeros (capsómeros) que después se teselan (agrupan) para formar las PLV. Como tal la presente invención se refiere a composiciones inmunogénicas como se ha descrito
5 anteriormente comprendiendo proteína L1, o capsómeros que comprenden proteína L1, en lugar de PLV como se describe en esta memoria descriptiva. Convenientemente las proteínas L1 son capaces de estimular una respuesta inmune protector. Convenientemente las proteínas L1 son conformacionalmente correctas.

10 Para evitar dudas la invención de este modo también se refiere al uso de derivados de L1 funcionales como se ha descrito anteriormente, tales como truncamientos de L1, mutantes por supresión, sustitución o inserción, y proteínas de fusión, convenientemente aquellos que son capaces de provocar una respuesta inmune capaz de reconocer un virus de VPH. Los capsómeros
15 que comprenden tales proteínas también se incluyen en la presente invención. Los capsómeros como agentes inmunogénicos se describen en, por ejemplo, el documento WO 0204007. El documento WO 9901557 también describe composiciones que contiene capsómeros de VPH. Las proteínas L1, derivados y capsómeros se pueden usar de al misma forma como se ha descrito para las
20 PLV anteriormente.

De este modo la invención también se puede ver que se refiere a una composición inmunogénica que comprende una proteína L1, o un derivado funcional de la misma, a partir de VPH 16 y 18 y al menos otro tipo canceroso de VPH, el otro tipo de cáncer seleccionándose entre la lista constituida por los
25 tipos de VPH 31, 45 y 52, en las que la dosis de proteína L1, o derivado de la

misma, de al menos otro tipo de cáncer se reduce con relación a la del VPH 16 ó 18.

De este modo la invención se puede observar que se refiere a una composición inmunogénica que comprende un capsómero de VPH de VPH 16 y 18 y al menos otro tipo de cáncer de VPH, el otro tipo de cáncer seleccionándose entre la lista constituida por los tipos de VPH 31, 45 y 52, en los que la dosis del capsómero de al menos otro tipo de cáncer se reduce con relación a la del VPH 16 ó 18.

En un aspecto la invención se refiere a una composición inmunogénica como se ha descrito anteriormente en combinación con un excipiente farmacéuticamente aceptable. Los excipientes adecuados se conocen bien en la técnica e incluyen por ejemplo tampones y agua.

Las composiciones y vacunas de la invención se pueden proporcionar y distribuir mediante cualquiera de una diversidad de vías tales como oral, tópica, subcutánea, mucosal (típicamente intravaginal), intravenosa, intramuscular, intranasal, sublingual, intradérmica y mediante supositorio.

En un aspecto de la invención la composición o vacuna se puede formular o co - administrar con un antígeno temprano de VPH, por ejemplo un antígeno seleccionado entre la lista constituida por VPH E1, E2, E3, E4, E5, E6, E7, y E8. En un aspecto alternativa vacuna puede carecer de un antígeno temprano de VPH, por ejemplo un antígeno seleccionado entre la lista constituida por VPH E1, E2, E3, E4, E5, E6, E7 y E8.

Opcionalmente la composición o vacuna también se puede formular o co - administrar con antígenos no - VPH. Convenientemente estos antígenos no VPH pueden proporcionar protección contra otras enfermedades, lo más

preferiblemente enfermedades transmitidas sexualmente tales como virus herpes simplex, VHB, clamidia y VIH. Los inventores particularmente prefieren que la composición o vacuna comprenda gD o un truncamiento de la misma de VSH, convenientemente un truncamiento C terminal de de VSH - 2
5 conocido como gD2t. De esta forma la composición o vacuna proporciona protección contra tanto VPH como VSH.

La dosificación de la composición o componentes de vacuna variarán con la condición, sexo, edad y peso del individuo, la vía de administración y VPH de la vacuna. La cantidad también puede variar con el número de tipos de
10 PLV. Convenientemente la distribución es de una cantidad de vacuna adecuada para generar una respuesta inmunológicamente protectora. Convenientemente cada dosis de vacuna comprende 1 - 100 μ g de cada PLV, en un aspecto 5 - 80 μ g, en un aspecto adicional 5 - 30 μ g de cada PLV, en un aspecto adicional 5 - 20 μ g de cada μ g de cada PLV con todavía aspectos
15 adicionales siendo específicamente 5 μ g, 6 μ g, 10 μ g, 15 μ g o 20 μ g.

Las dosis adecuadas para uso en seres humanos típicamente incluyen 20 - 40 μ g de PLV de VPH 16 y VPH 18, con dosis reducidas de los otros tipos de cáncer de VPH (31, 45, 52) como se ha descrito en esta memoria descriptiva, convenientemente a un nivel menor de 20 μ g por PLV,
20 convenientemente a un nivel que es capaz de provocar una respuesta protectora inmune en al menos algunos individuos vacunados.

Otras dosis adecuadas para uso en seres humanos pueden comprender cantidades inferiores de VPH 16 y/o 18, con tal que tales dosis sean protectoras en seres humanos como se puede determinar usando ensayos
25 indicados en esta memoria descriptiva. Por ejemplo tales dosis pueden ser

apropiadas cuando las PLV de la invención se combinan con adyuvantes fuertes.

En un aspecto la composición de la invención comprende 20 μg de VPH 16, 20 μg de VPH 18 y entre 5 - 18 μg de cada PLV del otro tipo de cáncer (31, 45 ó 52), por ejemplo 5 - 15 μg , y en un aspecto adicional específicamente 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 ó 15 μg de PLV de cada tipo de cáncer de no VPH 16/18.

En un aspecto la composición de la invención comprende 10 - 15 μg de VPH 16, 10 - 15 μg de VPH 18 y entre 5 - 9 μg de cada PLV del otro tipo de cáncer (31, 45 ó 52), y en un aspecto específicamente 5, 6, 7, 8 ó 9 μg de PLV de cada tipo de cáncer de VPH 16/18.

En un aspecto la relación en peso de PLV de VPH 16 a otro PLV de tipo canceroso (31, 45 ó 52) está en el intervalo de 1: 0,9 - 1:0,1 (VPH 16: otro tipo), convenientemente en el intervalo de 1: 0,9 - 1:0,3, convenientemente 1:0,8 - 1:0,4.

En un aspecto la relación en peso de PLV de VPH 18 a otro PLV de tipo canceroso (31, 45 ó 52) está entre 1:0,9 - 1:0,1, (VPH 18: otro tipo), convenientemente en el intervalo de 1:0,9 - 1:0,32, convenientemente 1:0,8 - 1:0,4.

En otras palabras, una dosis reducida es convenientemente 10 - 90% de la dosis de las PLV de VPH 16 o VPH 18, y en un aspecto es 20 - 80% de la dosis de las PLV de VPH 16 o VPH 18, en un aspecto adicional 30 - 70%, todavía en un aspecto adicional 30 - 60%, y específicamente 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% o 90% de la dosis de VPH 16 o VPH18.

En un aspecto la composición se usa convenientemente para prevenir

uno o más de: infección por VPH - 16 y/o VPH - 18, infección por VPH - 16 y/o VPH - 18 persistente y neoplasia de cuello de útero asociada a VPH - 16 y/o VPH - 18.

Convenientemente el uso de la composición inmunogénica de la invención se usa para prevenir neoplasia de cuello de útero y/o infección incidente y/o infección persistente asociada a infección por otros tipos oncogénicos (no VPH 16, 18).

Convenientemente la composición inmunogénica de la invención se usa en la inmunización activa de adultos y mujeres adolescentes desde la edad de 10 años en adelante. Para todas las composiciones y vacunas de la invención la composición o vacuna se usa convenientemente para la vacunación de chicas adolescentes de edad entre 10 y 15, preferiblemente 10 - 13 años. La composición o vacuna también se puede administrar a mujeres adultas de más edad después de un frotis de PAP anómalo o después de cirugía que sigue a la retirada de una lesión producida por VPH, o que son seronegativos y ADN negativo para tipos de cáncer de VPH. Las mujeres de 10 - 55 años son otro grupo diana adecuado. En otro aspecto la vacuna se puede usar en chicas y mujeres de todas las edades, desde niñas en adelante, en un aspecto adicional se puede proporcionar a chicos u hombres. En un aspecto adicional la vacuna se puede usar terapéuticamente en mujeres que son seropositivos para el virus de VPH.

En un aspecto la composición de la invención se usa para prevenir o tratar cáncer de cuello de útero, o estados patológicos CIN I, CIN II o CIN III producidos por infección por VPH.

En un aspecto la invención la vacuna se distribuye en un régimen de 2 ó

3 dosis, por ejemplo un régimen de 0, 1 mes o un régimen de 0, 2 meses, o un régimen de 0, 2, 6 meses o un régimen 0, 1 y 6 meses respectivamente. Convenientemente el régimen de vacunación incorpora una inyección de recuerdo después de 3 a 10 años, o 5 - 10 años, tal como preferiblemente 3, 4, 5 5, 6, 7, 8, 9 ó 10 años.

En un aspecto la composición o vacuna de la invención es una formulación de vacuna líquida, aunque la vacuna se puede liofilizar y reconstituir antes de administración. También se pueden usar las formulaciones tópicas tales como, por ejemplo, cremas intravaginales.

10 La descripción de todas las referencias en la presente solicitud, que incluye solicitudes de patente y patentes concedidas, se incorporan en esta memoria descriptiva en su totalidad como referencia.

Las composiciones y vacunas de la invención comprenden ciertos componentes de VPH como se ha establecido anteriormente. En un aspecto 15 adicional de la invención la vacuna consta esencialmente de, o está constituida por dichos componentes.

La presente invención se ilustra en esta memoria descriptiva como referencia a los siguientes ejemplos no limitantes con relación a la protección cruzada por las PLV de VPH 16 y VPH 18, y que muestra la producción de las 20 PLV de VPH.

Ejemplo 1

Mujeres sanas entre las edades de 15 y 25 años se inmunizaron con una mezcla de PLV de L1 de VPH 16 y VPH 18. Las mujeres en el alistamiento 25 eran: 1) seronegativas para VPH - 16 y VPH - 18; 2) negativas para infección

por VPH de alto riesgo del cuello uterino (detectado por PCR de VPH); 3) tenían 6 o menos parejas sexuales vitalicias y 4) tenían frotis de PAP.

La mezcla comprendía, por dosis de 0,5 ml, 20 μ g de PLV de L1 de VPH - 16, 20 μ g de PLV de L1 de VPH - 18 y se acompañó de adyuvante con 500 μ g de hidróxido de aluminio y 50 μ g de 3D MPL. El grupo de placebo se inyectó con 500 μ g de hidróxido de aluminio solo.

Se evaluó la eficacia de la vacuna (V. E.) contra tipos de VPH de cáncer de alto riesgo, en la que la V. E. es la % de mejora en la protección contra la infección por la vacuna comparada con un grupo placebo.

La protección cruzada se determinó detectando la presencia de ácido nucleico específico para diversos tipos oncogénicos en las vacunas y grupo de control. La detección se llevó a cabo usando técnicas como se describe en el documento WO 03014402, y las referencias en esta memoria descriptiva, particularmente para ampliación no específica de ADN de VPH y posterior detección de tipos de ADN usando un sistema de LIPA como se describe en el documento WO 99/14377, y en Kleter y col., [Journal of Clinical Microbiology (1999), 37 (8): 2508 - 2517], cuyos contenidos totales se incorporan en esta memoria descriptiva como referencia.

Sin embargo, cualquier procedimiento adecuado se puede usar para la detección de ADN de VPH en una muestra, tal como PCR específica de tipo que usa cebadores específicos para cada tipo de VPH de interés. Los cebadores adecuados los conocen los expertos en la técnica, o se pueden construir fácilmente dado que se conocen las secuencias de los tipos de VPH oncogénicos.

La eficacia de las vacunas se determinó contra las infecciones para los

12 tipos de cáncer de alto riesgo, tipos relacionados filogenéticamente con VPH - 16 (los grupos de, 31, 35, y 58; 31, 33, 35, 52 y 58) y los tipos relacionados filogenéticamente 30 VPH - 18 (45 y 59).

Un análisis inicial se llevó a cabo sobre una "ITT" (intención para tratar
5 cohorte, que representa todos los individuos que recibieron al menos una dosis de vacuna). Estos datos se muestran en la tabla 1.

Los resultados presentados en las tablas 2 y 3 se refieren al grupo "ATP" (según el protocolo) para aquellos pacientes que cumplen con todos los criterios del ensayo. La tabla 2 es un análisis de punto medio con los datos
10 tomados de todos los pacientes en el instante en que al menos el 50% de la cohorte estaba 18 meses después de su primera vacunación. La tabla 3 proporciona los resultados finales, todos los datos estando entre los sujetos a 18 meses después de la primera vacunación (mes 0). En el grupo PTA todos los pacientes recibieron 3 dosis de vacuna a los meses 0, 1 y 6 eran
15 seronegativas a los 6 meses.

Como se demuestra por los datos presentados en la tabla 1, la inmunización con una mezcla de PLV de VPH 16 y VPH 18 proporcionó protección cruzada evidente contra otros tipos de VPH. En este punto los tamaños de muestra son demasiado pequeños para proporcionar un análisis
20 estadístico riguroso, sin embargo los datos demuestran una tendencia positiva y sugieren que la inmunización con las PLV de VPH 16 y VPH 18 será eficaz contra infección con otros tipos de VPH.

Esto se confirmó a medida que el estudio progresaba.

Los detalles del protocolo se describen adicionalmente en el ejemplo 3.

25 La tabla 2 demuestra que el VPH 16 y VPH 18 proporcionan protección

cruzada estadísticamente significativa contra el grupo de tipos de cáncer de alto riesgo 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 y 68.

La tabla 3 demuestra que, excepto para los tipos relacionados con VPH - 18 (que muestran una tendencia muy baja), existe protección cruzada estadísticamente significativa contra los grupos de: VPH 31, 35, 58; VPH 31, 33, 35, 52, 58; y los 12 tipos de alto riesgo (tipos de no VPH - 16/18) evaluados.

El análisis posterior de los datos de ensayo han indicado que la vacuna de VPH 16 y 18 usada en los ejemplos 1 proporciona protección cruzada estadísticamente significativa contra la infección estadística incidente por VPH 31 (eficacia de vacuna 75,1%, $p = 0,007$). Mientras el tamaño de muestra no permita todavía conclusiones estadísticamente significativas a dibujarse sobre otros tipos, los datos sobre otros tipos tales como 39, 45, 51 y 52 demuestran una tendencia positiva y sugieren que la inmunización con las PLV de VPH 16 y VPH 18 serán eficaces contra la infección con otros tipos de VPH.

Los datos presentados en el ejemplo 3 proporcionan datos adicionales obtenidos en la muestra de estudio, y se centra en la protección cruzada proporcionada contra ciertos tipos específicos.

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Tabla 1

Tipo de VPH analizado	Número de mujeres infectadas (grupo de vacuna)	% de mujeres infectadas (grupo de vacuna) = A	Número de mujeres infectadas (grupo de placebo)	% de mujeres infectadas (grupo de placebo) = B	% de eficacia de vacuna (1 - (A/B) x 100, ajustado para tamaño relativo de vacuna y grupo de placebo)	límite de confianza de 95% - límite inferior	límite de confianza de 95% - límite superior	P
VPH 31, 35, 58	5	1,1	11	2,4	55,1	-29,1	84,4	0,127
VPH 31, 33, 35, 52, 58	17	3,8	24	5,4	30,3	-29,7	62,6	0,252
VPH 45, 59	3	0,7	6	1,3	50,6	-99,7	87,6	0,309

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VPH 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68.	27	6,3	40	9,4	34,6	-6,5	59,9	0,086
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Se tomaron muestras de pacientes a los 9, 12, 15 y 18 meses y se ensayaron para infección por VPH por los otros tipos especificados anteriormente.

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Tabla 2 - Eficacia de vacuna después d tres dosis en la prevención d infecciones heterólogas incidentes

Tabla 2: Eficacia de vacuna contra la infección con tipos relacionados filogenéticamente con VPH - 16, tipos relacionados filogenéticamente con VPH - 18, tipos relacionados filogenéticamente con VPH - 16 y/o VPH - 18 y todos los tipos exclusivos de alto riesgo exclusivos de la cohorte de VPH - 16 y VPH - 18 (mes 6 - 18)

Tipo de infección	Tasa de ataque						Eficacia de vacuna		
	Vacuna			Placebo					
	N	n	AR	N	n	AR	%	CI al 95%	valor d p
Relacionado con VPH - 16	443	12	2,8	438	24	5,5	49,4	0,2 74,4	0,080
* Relacionado con VPH - 16	423	29	6,9	423	46	10,9	37,0	1,6 59,6	0,052
Relacionado con VPH - 18	442	9	2,0	449	16	3,6	42,9	-27,9 74,5	0,223
Relacionado con VPH - 16/18	433	21	4,9	438	41	9,4	48,2	13,8 68,9	0,012
* Relacionado con VPH - 16	423	34	8,0	453	56	13,2	39,3	9,0 59,5	0,019
** Alto riesgo	385	53	13,8	386	88	22,8	39,8	17,7 55,7	0,001

N = número d sujetos un la cohorte³¹ especificada

n = número de sujetos con infección por VPH incidente

AR = tasa de ataque = n/N

CI al 95% = intervalo de confianza al 95%

5 Límite inferior = $1 - \exp (\log (\text{arvg} / \text{arp}) + 1,96 * \text{raíz cuadrada de } (1/nv - 1/Nv + 1/np - 1/Np))$

Límite superior = $1 - \exp (\log (\text{arv} / \text{arp}) - 1,96 * \text{raíz cuadrada de } (1/nv - 1/Nv + 1/np - 1/Np))$

cuando el número de casos en vacuna = 0

Límite inferior * = $1 - \exp (\log (\text{arvg} * / \text{arp} *) + 1,96 * \text{raíz cuadrada de } (1/(nv + 0,5) - 1/(Nv + 0,5) + 1/(np + 0,5) - 1/(Np + 0,5)))$

10 Límite superior * = $1 - \exp (\log (\text{arv} * / \text{arp} *) - 1,96 * \text{raíz cuadrada de } (1/(nv + 0,5) - 1/(Nv + 0,5) + 1/(np + 0,5) - 1/(Np + 0,5)))$

con: arv = tasa de ataque en receptores de vacuna

arp = tasa de ataque en receptores de placebo

nv = número de casos en receptores de vacuna

15 Nv = número de casos y no casos en receptores de vacuna

np = número de casos en receptores de placebo

Np = número de vasos y no casos en receptores de placebo

relacionados con VPH - 16: tipos 35, 31, 58 relacionados filogenéticamente con VPH - 16 sin considerar otros tipos de VPH

relacionados con VPH - 16 *: tipos 35, 31, 58, 33, 52 relacionados filogenéticamente con VPH - 16 sin
5 considerar otros tipos de VPH

relacionados con VPH - 18: tipos 45, 59 relacionados filogenéticamente con VPH - 16 sin considerar otros tipos de VPH

relacionados con VPH - 16 y/o VPH - 18: tipos 35, 31, 58, 45, 59 relacionados filogenéticamente con VPH - 16 y/o VPH - 18 sin considerar otros tipos de VPH

10 relacionados con VPH - 16 y/o VPH - 18 *: tipos 35, 31, 58, 33, 52, 45, 59 relacionados filogenéticamente con VPH - 16 y/o VPH - 18 sin considerar otros tipos de VPH

* * = tipos de alto riesgo exclusivos de VPH - 16 y VPH - 18

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Tabla 3

Tipos de VPH analizados	Número total de número de sujetos con información disponible por grupo	Número de mujeres infectadas (grupo de vacuna)	% de mujeres infectadas (grupo de vacuna) = A	Número de mujeres infectadas (grupo de placebo)	% de mujeres infectadas (grupo de placebo) = B	% de eficacia de vacuna (1 - (A/B) x 100, ajustado para tamaño relativo de vacuna y grupo de placebo	límites de confianza al 95% - límite inferior	límites de confianza al 95% - límite superior	P
VPH 31, 35, 58	412	11	2,7	26	6,3	57,9	15,9	78,9	0,012
VPH 31, 33, 35,	403	28	6,9	48	12,2	43,0	11,0	63,5	0,015

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52, 58									
VPH 45, 59	421	10	2,4	15	3,6	33,5	-46,3	69,8	0,319
VPH 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68.	368	58	15,8	90	25,3	37,7	16,2	53,6	0,002

Se tomaron muestras de pacientes a los 18 meses y se ensayaron para infección por VPH por los otros tipos especificados anteriormente.

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Ejemplo 2

Las PLV de VPH 16 y VPH 18 se produjeron de la siguiente manera:

Ejemplo 1:

La combinación de las PLV de VPH 16 y VPH 18 se detalla en esta memoria descriptiva. La proteína L1 de otros genotipos de VPH se pueden producir fácilmente mediante procedimientos similares, ya conocidos en la técnica.

A Preparación de PLV de VPH 16/18

Producción de PLV de VPH 16 y VPH 18 se llevó a cabo usando protocolos convencionales - por ejemplo, véase el documento WO 9913056, las proteínas de VPH 16/18 se expresan en células de *Trichoplusia ni* (High Five™) (a una densidad de aproximadamente 350000 células/ml) infectadas con baculovirus recombinante (MOI de 0,3) que codifica el gen de L1 de VPH 16 ó 18 de interés. Las células se recogieron aproximadamente 72 horas después de la infección.

4.1 B Recogida de células / extracción de antígeno

El antígeno (L1 - 16/18) se extrajo de células HI5 en un procedimiento de tres etapas de concentración, extracción, clarificación. La etapa de concentración retira hasta el 90% del medio de cultivo, y se realizó mediante filtración de flujo tangencial. La etapa de extracción se realizó con un tampón hipotónico (Tris 20 mM, pH 8,5). Un volumen igual al volumen del cultivo se usó para realizar la extracción. Se usó un tiempo de contacto de mínimo una hora y media en agitación suave. La clarificación se realizó mediante filtración de flujo tangencial.

C Purificación

El procedimiento de purificación se llevó a cabo a temperatura ambiente. Se añadió β - mercaptoetanol (4% p/p) al extracto con el fin de disociar las PLV en cápsomeros, para ambos antígenos, L1 - 16/18. Se añadió glicerol hasta una concentración de 10% en peso justo antes de la adición de β - mercaptoetanol.

Todos los tampones usados se filtraron sobre filtros de 0,22 μ m antes de almacenar a 2°C - 8°C. Antes de cada purificación, las matrices de gel se sanitizan y se equilibran con tampón apropiado antes de cargar la muestra.

Los regímenes de purificación se proporcionan para la purificación separada de L1 a partir de tanto VPH 16 como VPH 18. Estos esquemas son en sentido amplio similares, e implican las etapas de:

- Cromatografía de intercambio aniónico (Di metil amino etilo - DMAE),
- Cromatografía de intercambio aniónico (Tri metil amino etilo - TMAE),
- Cromatografía en hidroxapatita,
- Filtración nanométrica (Planova),
- Ultrafiltración,
- Cromatografía de interacción hidrófoba (usando octil sefarosa) para VPH 18 o cromatografía de intercambio aniónico (DEAE) para VPH 16; y filtración estéril.

4.1.1 Específicamente:

4.1.2 C1 Purificación de antígeno L1 - 18

4.1.2.1 Cromatografía de intercambio aniónico DMAE

El extracto clarificado (proteína a una concentración de

aproximadamente 1g/ml, con la proteína L1 a aproximadamente 150 mg/ml) se aplica a una columna de intercambio aniónico (Di metil amino etilo). La elución se realiza con tampón (Tris 20 mM / NaCl 200 mM / β - mercaptoetanol BME al 4%) pH $7,9 \pm 0,2$. El antígeno se eluye con aproximadamente 5 volúmenes de columna y el perfil de elución se controla a 280 nm.

4.1.2.2 Cromatografía de intercambio aniónico TMAE

El eluido de la primera etapa se diluye con 1 volumen de H₂O/BME al 4%. Después el eluido diluido se aplica a una segunda columna de intercambio aniónico (Trimetil amino etilo). La elución se realiza con tampón (Tris 20 mM / NaCl 200 mM / BME al 4%) pH $7,9 \pm 0,2$. El antígeno se eluye con aproximadamente 4 volúmenes de columna y el perfil de elución se controla a 280 nm.

4.1.2.3 Cromatografía de hidroxapatita

El eluido de la etapa de TMAE se aplica a una columna de hidroxapatita (HA). Después de la aplicación de la muestra, el gel se eluye con aproximadamente 2,5 volúmenes de columna de tampón (NaH₂PO₄ 100 mM / NaCl 30 mM / BME al 4%), pH $6,0 \pm 0,2$.

20

4.1.2.4 Filtración nanométrica (Planova)

El eluido de HA se diluye con el fin de alcanzar las siguientes condiciones: tampón (NaH₂PO₄ 25 mM / NaCl 10 mM / BME al 4%), pH $7,5 \pm 0,2$.

Después se filtra sucesivamente sobre un prefiltro de 0,2 μ m y sobre un

filtro 15 N de 0,12 m². La filtración se realiza a una presión constante de 200 mbar (20 kPa) $\pm$ 20 (2 kPa) mbar.

4.1.2.5 Ultrafiltración

- 5 La ultrafiltración se realiza a con un sistema de ultrafiltración de flujo tangencial equipado con membranas de poliétersulfona (módulo de Centramate 0,1 m², 100 kD).

El eluido de Planova se trata para alcanzar las siguientes condiciones: (NaH₂PO₄ 100 mM / NaCl 30 mM / BME al 4%), pH 6,0 $\pm$ 0,2; después se carga
10 en el sistema, se concentra cinco veces y se dia - filtra con inyección continua de aproximadamente 10 volúmenes de partida de tampón (NaH₂PO₄ 20 mM/ NaCl 500 mM), pH 6,0 $\pm$ 0,2.

4.1.2.6 Cromatografía de interacción hidrófoba (Octil Sefarosa)

- El permeado de ultrafiltración se aplica a una columna de octil Sefarosa.
15 Esta etapa de cromatografía se lleva a cabo en el modo negativo con aproximadamente 5 volúmenes de columna de tampón (Na₃PO₄ 20 mM/ NaCl 500 mM), pH 6,0 $\pm$ 0,2.

4.1.2.7 Filtración estéril

- 20 La solución de antígeno L1 - 18 purificada se esteriliza mediante filtración sobre una membrana de 0,22 μ m.

4.1.3

- 25 4.1.4 C2 Purificación de antígeno L1 - 16

4.1.4.1 Cromatografía de intercambio iónico DMAE

El extracto clarificado se aplica a una columna de intercambio aniónico (Di metil amino etilo). La elución se realiza con tampón (Tris 20 mM / NaCl 180 mM / BME al 4%) pH 7,9 $\pm$ 0,2. El antígeno se eluye con aproximadamente 4 volúmenes de columna y el perfil de elución se controla a 280 nm.

4.1.4.2 Cromatografía de intercambio aniónico TMAE

El eluido de la primera etapa se diluye con 1 volumen de H₂O/BME al 4%. Después el eluido diluido se aplica a una segunda columna de intercambio aniónico (Trimetil amino etilo). La elución se realiza con tampón (Tris 20 mM / NaCl 180 mM / BME al 4%) pH 7,9 $\pm$ 0,2. El antígeno se eluye con aproximadamente 5 volúmenes de columna y el perfil de elución se controla a 280 nm.

15

4.1.4.3 Cromatografía de hidroxilapatita (HA)

El eluido de la etapa de TMAE se aplica a una columna de HA. Después de la aplicación de la muestra, el gel se eluye con aproximadamente 3 volúmenes de columna de tampón (NaH₂PO₄ 100 mM/ NaCl 30 mM/ BME al 4%), pH 6,0 $\pm$ 0,2.

20

4.1.4.4 Filtración nanométrica (Planova)

El eluido de HA se diluye con el fin de alcanzar las siguientes condiciones: tampón (NaH₂PO₄ 25 mM / NaCl 10 mM / BME al 4%), pH 7,5 $\pm$ 0,2.

25

Después se filtra sucesivamente sobre un prefiltro de $0,2 \mu\text{m}$ y sobre un filtro 15 N de $0,12 \text{ m}^2$. La filtración se realiza a una presión constante de 200 mbar (20 kPa) ± 20 (2 kPa) mbar.

5 4.1.4.5 Ultrafiltración

La ultrafiltración se realiza a con un sistema de ultrafiltración de flujo tangencial equipado con membranas de poliótersulfona (módulo de Centramate $0,1 \text{ m}^2$, 100 kD).

El eluido de Planova se trata para alcanzar las siguientes condiciones:
10 (NaH_2PO_4 100 mM / NaCl 30 mM / BME al 4%), pH $6,0 \pm 0,2$; después se carga en el sistema, se concentra cinco veces y se dia - filtra con inyección continua de aproximadamente 10 volúmenes de partida de tampón (NaH_2PO_4 20 mM / NaCl 500 mM), pH $6,0 \pm 0,2$.

15 4.1.4.6 Cromatografía de intercambio aniónico DEAE

El eluido de ultrafiltración se ajusta a la conductividad del tampón de equilibrio, (Na_3PO_4 20 mM / NaCl 250 mM), pH $6,0 \pm 0,2$ y se aplica a una columna de intercambio aniónico /Di etil amino etilo).

La elución se realiza con tampón (NaH_2PO_4 20 mM / NaCl 500 mM), pH
20 $6,0 \pm 0,2$. El antígeno se eluye con aproximadamente 3 volúmenes de columna y el perfil de elución se controla a 280 nm .

4.1.4.7 Filtración estéril

La solución de antígeno L1 - 16 purificada se esteriliza mediante
25 filtración sobre una membrana de $0,22 \mu\text{m}$.

C3

Cada tipo de PLV se adsorbe independientemente para producir un monovalente adsorbido concentrado.

Preparación de monovalente adsorbido concentrado de PLV16.

- 5 60 μg de PLV purificados de VPH16 se adsorben en 150 μg de Al^{+3} de $\text{Al}(\text{OH})_3$, a un pH de $6,0 \pm 0,2$, durante una hora a temperatura ambiente con agitación suave. Este monovalente adsorbido concentrado se almacena a $+4^\circ\text{C}$. La adsorción se comprueba mediante centrifugación de la preparación y cuantificación de las PLV en el sobrenadante.

- 10 Preparación de monovalente adsorbido concentrado PLV18:

- 60 μg de PLV purificados de VPH18 se adsorben en 150 μg de Al^{+3} de $\text{Al}(\text{OH})_3$, a un pH de $6,0 \pm 0,2$, durante una hora a temperatura ambiente con agitación suave. Este monovalente adsorbido concentrado se almacena a $+4^\circ\text{C}$. La adsorción se comprueba mediante centrifugación de la preparación y
15 cuantificación de las PLV en el sobrenadante.

D. Preparación de vacuna final

- Los monovalentes adsorbidos concentrados preparados mediante el procedimiento anterior se pueden combinar para formar una suspensión que
20 contiene 20 μg de cada uno de las PLV por dosis. La vacuna final se almacena a $+4^\circ\text{C}$.

- La adición de las PLV de otros tipos de cáncer se puede añadir según sea apropiado, a concentración adecuada de acuerdo con la invención. Las
25 secuencias de tales tipos se conocen en la técnica y los expertos en la técnica

pueden fácilmente expresar las PLV que comprenden tales proteínas.

Las masas adsorbidas combinadas, o masas adsorbidas individuales, se pueden además mezclar con adyuvantes tales como 3D - MPL.

5 Ejemplo 3

Los detalles precisos del experimento llevado a cabo se proporcionan en Harper y col., the Lancet 2004 Nov 13; 364 (9447): 1757 - 65.

En resumen, mujeres sanas entre las edades de 15 y 25 años se inmunizaron con una mezcla de PLV L1 de VPH 16 y VPH 18. Las mujeres en el
10 alistamiento eran: 1) seronegativas para VPH - 16 y VPH - 18; 2) negativas para infección por VPH de alto riesgo del cuello uterino (detectado por PCR de VPH); 3) tenían 6 o menos parejas sexuales vitalicias y 4) tenían frotis de PAP.

La mezcla comprendía, por dosis de 0,5 ml, 20 µg de PLV de L1 de VPH - 16, 20 µg de PLV de L1 de VPH - 18 y se acompañó de adyuvante con 500 g
15 de hidróxido de aluminio y 50 µg de 3D MPL. El grupo de placebo se inyectó con 500 µg de hidróxido de aluminio solo.

Las PLV de VPH 16 estaban constituidos por una proteína L1 de VPH de 471 aminoácidos, truncada terminalmente en C, con una supresión de 34 aminoácidos. Las PLV de VPH 18 están constituidas por una proteína L 1 de
20 VPH truncada terminalmente en C de 472 aminoácidos, con una supresión de 35 aminoácidos.

Se determinó la eficacia de vacuna (V. E.) contra ciertos tipos de VPH cancerosos, en los que la V. E. es el % de mejora en protección contra infección por la vacuna comparado con un grupo de placebo.

25 La protección cruzada se determinó detectando la presencia de ácido

nucleico específico para diversos tipos oncogénicos en las vacunas y grupo control. La detección se llevó a cabo usando técnicas como se describe en el documento WO 03014402 y las referencias en él, particularmente para la amplificación no específica de ADN de VPH y posterior detección de tipos de ADN usando un sistema de LiPA como se describe en el documento WO 99/14377, y en Kleter y col., [Journal of Clinical Microbiology (1999), 37 (8): 2508 - 2517], cuyo contenido total se incorporan en esta memoria descriptiva como referencia específicamente.

Sin embargo, cualquier procedimiento adecuado, se puede usar para la detección de ADN de VPH en una muestra, tal como PCR específico de tipo usando cebadores específicos para cada tipo de VPH en una muestra. Los cebadores adecuados los conocen los expertos en la técnica, o se pueden construir fácilmente con tal que las secuencias de los tipos de VPH oncogénicos se conozcan. En detalle, la sección de procedimientos de la publicación de Lancet se reproduce en esta memoria descriptiva como referencia, para la verla íntegramente:

Harper y col., the Lancet, 13 de noviembre de 2004; 364 (947); 1757 - 85 - detalles experimentales.

El objetivo principal de este estudio era para determinar la eficacia de vacuna en la prevención de infección con VPH - 16, VPH - 18, o ambos (VPH - 16/18), entre los meses 6 y 18 en participantes que se mostró inicialmente que eran seronegativos para VPH - 16/18 mediante ELISA y negativos para ADN de VPH - 16/18 mediante PCR. Los objetivos secundarios incluían: evaluación de eficacia de vacuna en la prevención de infección persistente con VPH - 16/18, y la evaluación de la eficacia de la vacuna en la prevención de lesiones

intraepiteliales escamosas de grado bajo confirmadas citológicamente (LSIL), lesiones intraepiteliales escamosas de grado alto (HSIL), y cáncer de células escamosas LSIL (CIN 1), HSIL (CIN 2 ó 2) confirmado histológicamente, adenocarcinoma asociado a infección por VPH - 16/18 entre los meses 6 y 8, y
5 meses 6 y 27. La prevención de células escamosas atípicas de citología significación no determinada (ASCUS) asociada a la infección por VPH - 16/18 se añadió post - hoc a los análisis de los resultados.

Los inventores no hicieron un análisis exploratorios de los puntos finales histopatológicos CIN 1 y 2 asociados al ADN de VPH - 16/18 detectado
10 mediante PCR en tejido de lesionado. Otros objetivos incluían la determinación de inmunogenicidad de vacuna, seguridad, y tolerancia.

Los investigadores en Norte América (Canadá y Estados Unidos) y Brasil reclutaron mujeres para este estudio de eficacia mediante anuncios o participación previa en el estudio de epidemiología seccional cruzado de VPH
15 que tuvo lugar entre julio y diciembre de 2000.

Para cada uno de los 32 sitios de estudio, un panel de revisión institucional aprobó el protocolo, formas de consenso, y enmiendas. Las mujeres firmaron consentimientos escritos separados para la participación y colposcopia. Para las que eran menores de 18 años, eran obligatorios
20 consentimiento de los padres y asentimiento del participante

Existían dos fases de estudio: una fase inicial para la vacunación y el seguimiento que concluía en el mes 18; y una fase de extensión de seguimiento a ciegas que concluyó el mes 27.

Las mujeres que se pueden elegir para la fase inicial (meses 0 - 18)
25 incluían mujeres sanas de 15 - 25 años de edad, que no habían tenido más de

6 parejas sexuales, sin historial de un ensayo Pap anómalo o tratamiento por ablación o escisión del cuello uterino, y ningún tratamiento en curso para condilomata externo; y que eran citológicamente negativas, seronegativas para anticuerpos de VPH - 16 y VPH - 18 mediante ELISA, y negativas para ADN de VPH mediante PCR para 14 tipos de VPH de alto riesgo 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, y 68) no más de 90 días antes del comienzo del estudio.

Las mujeres que completaron la fase inicial del mas temprano de los estudios, y que no tenían terapia por ablación o escisión del cuello uterino, o histerectomía después del alistamiento, se pudieron elegir para participar en la fase de extensión del estudio (meses 18 - 27).

Procedimientos

Cada dosis de las vacunas de partículas que parecen virus de VPH - 16/18 (GlaxoSmithKline Biologicals, Rixensart, Bélgica) contenía 20 μ g de partícula que parece virus de L1 de VPH - 16 y 20 μ g de partícula que parece virus de L1 de μ g - 18. Cada tipo de partícula que parece virus se produjo sobre sustrato de células de *Spodoptera frugiperda* Sf - 9 y *Trichoplusia ni* Hi - 5 con adyuvante AS04 que contenía 500 μ g de hidróxido de aluminio y 50 μ g de 3-lípido monofosforilo 3-desacilado (MPL, Corixa, Montana, Estados Unidos) proporcionado en un vial monodosis. El placebo contenía 500 μ g de hidróxido de aluminio por dosis, y era idéntico a en apariencia a la vacuna de μ g - 16/18. Cada participante en el estudio recibió una dosis de 0 - 5 ml de vacuna o placebo a los 0 meses, 1 mes, y 6 meses.

Los Proveedores de asistencia sanitaria obtuvieron muestras de cuello

uterino con un cepillo y espátula de cuello uterino (lavadas en PreservCyt, Cytyc Corporation, Boxborough, MA, Estados Unidos) para ensayo de citología y ADN de VPH en la selección y los meses 6, 12, y 18. En los meses 0 y 6, y posteriormente cada 3 meses, muestras cervicovaginales obtenidas de las
5 propias mujeres con dos toma de muestras con escobillones sucesivas (colocados en PreservCyt) para ensayo de ADN de VPH. [DM Harper, WW Noll, DR Belloni y BF, Cole, Randomized clinical trial of PCR - determined human papillomavirus detection methods: self - sampling versus clinician - directed - biologic concordance and women's preferences. *Am. J Obstet*
10 *Gynecol* 186 (2002), pp. 365 - 373]. Un laboratorio central (Quest Diagnostics, Teterboro, NJ, Estados Unidos) reseño resultados citológicos (ThinPrep, Cytyc Corporation) mediante el uso del sistema de clasificación de Bethesda de 1991.

Las directrices de protocolo recomendaban colposcopia después de dos informes de ASCUS, o un informe de células glandulares atípicas de
15 significación no determinada, LSIL o HSIL, carcinoma de células escamosas, adenocarcinoma *in situ*, o adenocarcinoma. Estas directrices también recomendaron biopsia para cualquier lesión sospechosa.

El laboratorio de histología central realizó una diagnosis inicial de las muestras de tejidos fijados por formalina para tratamiento clínico. Un panel de
20 tres patólogos realizaron una diagnosis de consenso posterior para lesiones asociadas a VPH - 16 y VPH - 18 con el sistema CIN. Esta diagnosis de consenso también incluía una revisión de las secciones tomadas en el momento de la microsección para la detección por PCR de ADN de VPH de lesión.

25 El ADN de VPH aislado de la muestra citológica (MagNaPure Total

Nucleic Acid system, Roche Diagnostics, Almere, Holanda) y de muestras de biopsia de cuello uterino (extracción de proteinasa K) se amplificó a partir de una alícuota de ADN total purificada con los cebadores de amplio espectro SPF10 que amplifican una región de 65 pares de bases del gen L1 [B. Kleter, LJ van Doorn, J ter Schegget y col., Novel short - fragment PCR assay for highly sensitive broad - spectrum detection of anogenital human papillomaviruses. *Am J Pathol* 153 (1998), pp. 1731 - 1739; LJ van Doorn, W Quint, B Kleter y col., Genotyping of human papillomavirus in liquid cytology cervical specimens by the PGMY line blot assay and the SPF (10) line probe assay. *J Clin Microbiol* 40 (2002), pp. 979 - 983 y WG Quint, G Scholte, LJ van Doorn, B Kletter, PH Smits y J. Lindeman, Comparative analysis of human papilloma virus infections in cervical scrapes and biopsy specimens by general SPF (10) PCR and HPV genotyping. *J. Pathol* 194 (2001), pp. 51 - 58]. Los productos de amplificación se detectaron mediante un inmunoensayo de enzimas de ADN. Un ensayo de sonda lineal kit LiPA, ensayo de identificación de genotipo de VPH INNO LiPA VPH, sistema SPF - 10, versión 1, Inogenetics, Gent, Bélgica, fabricado por Labo Bio - medical Products Rijswijk, Holanda) detectó 25 genotipos de VPH (6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, y 74). [B. Kleter, LJ van Doorn, L Schauwen y col., Development and clinical evaluation of a highly sensitive PCR - reverse hybridization line probe assay for detection and identification of anogenital human papillomavirus *J. Clin Microbiol* 37 (1999), pp. 2508 - 2517]. Cualquier muestra que era positiva mediante inmunoensayo de enzimas de ADN se ensayó mediante PCR de VPH - 16 y VPH - 18 específico de tipo. Los cebadores de PCR específico del tipo VPH - 16 amplificaron un segmento de

92 pares de bases del gen E6/E7 y cebadores de PCR específico del tipo VPH - 18 amplificaron un segmento de 126 pares de bases del gen L1 [MF Baay, WG Quint, J, Koudstaal y col, Comprehensive study of several general and type - specific primer pairs for detection of human papillomavirus DNA by PCR in paraffin - embedded cervical carcinomas. *J. Clin Microbiol* 34 (1996), pp. 745 - 747]

Los inventores definieron invención del cuello uterino incidente con VPH - 16/18 con al menos un resultado de PCR positivo para VPH - 16 o VPH - 18 durante el ensayo, e infección persistente con VPH - 16/18 como al menos dos ensayos de VPH - ADN positivos para el mismo genotipo viral separado en al menos 6 meses. [H Richardson, Kelsall, P Tellier y col., The natural history of type - specific human papillomavirus infections in female university students. *Cancer Epidemiol Biomarkers Prev* 12 (2003), pp. 485 - 490 y AB Moscicki, JH Ellenberg, S Farhat y J. Xu, Persistence of human papillomavirus infection in HIV - infected and uninfected adolescent girls: risk factors and differences, by phylogenetic type. *J. Infect Dis* 190 (2004), pp. 37 - 45] Los resultados de ensayos de HPV - DNA se escondieron a los investigadores durante el estudio y las diagnósis citológicas e histológicas se revelaron solamente para propósitos de tratamiento clínico. Los análisis incluían resultados de ADN de VPH - 16/18 para muestras de cuello uterino y muestras de cuello uterino y cervicovaginales combinadas.

Los inventores recogieron suero de los participantes de estudio en los meses 0, 1, 6, 7, 12, y 18 para las determinaciones de inmunogenicidad. El ensayo sexológico para anticuerpos a las partículas que parecen virus de VPH - 16 y VPH - 18 fue mediante ELISA. Se usaron partículas que parecen virus de

VPH - 16 y VPH - 18 como antígenos de recubrimiento para la detección de anticuerpos (véase el apéndice de la página web [http://image.thelancet.com/extras/04art10103webappendix .pdf](http://image.thelancet.com/extras/04art10103webappendix.pdf)). La seropositividad se definió como un título mayor que o igual al título de corte del ensayo establecido en 8 unidades/ml de ELISA para VPH - 16 y 7 unidades/ml de ELISA para VPH - 18. Los títulos naturales típicos se determinaron mediante el uso de muestras de sangre obtenidas de mujeres en el estudio de epidemiología anterior que se encontraron que eran seropositivas para VPH - 16 o VPH - 18 mediante ELISA.

Las mujeres registraron síntomas experimentados durante los 7 primeros días después de la vacunación en tarjetas diarias con una escala de tres grados de intensidad de síntomas. Adicionalmente, reseñaron al personal de estudio mediante entrevista de todos los episodios adversos en los 30 primeros días después de la vacunación. La información sobre los episodios adversos graves y embarazos se recogió a lo largo del estudio.

Procedimientos estadísticos

Asumiendo una tasa acumulada de incidencia del 60% de infecciones tanto del tipo VPH - 16 como VPH - 18 durante 12 meses, los inventores estimaron que 500 mujeres por grupo de tratamiento proporcionarían un poder de 80% para evaluar un límite inferior del 95% de CI de la eficacia de vacuna por encima de cero. Los inventores asumían un 80% de tasa de retención durante 18 meses. Los análisis provisionales para eficacia, seguridad, e inmunogenicidad se hicieron para propósitos de planificación del estudio futuro solamente; el procedimiento de O'Brien y Fleming se usó para ajustar el valor

para el análisis final después de que se produjeron los análisis provisionales (p global = 0,05; ensayo de dos vías). [PC O'Brien y TR Fleming, A multiple testing procedure for clinical trials. *Biometrics* 35 (1979), pp. 549 - 556]

La distribución aleatoria estratificada, de bloque según algoritmos validados se centralizó con un sistema de distribución aleatoria de internet. La estratificación era según la edad (15 - 17, 18 - 21, y 22 - 25 años) y región (Norte América y Brasil). Cada dosis de vacuna se atribuyó a un número elegido al azar basándose en la información del participante específica metida en el sistema de distribución aleatoria computarizado por el personal del estudio. La localización del tratamiento permanece oculta para los investigadores y las mujeres participantes en un estudio de seguimiento a largo plazo.

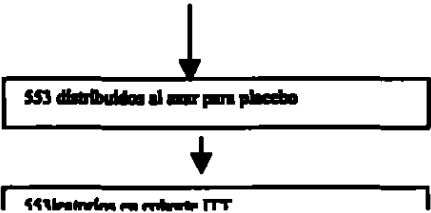
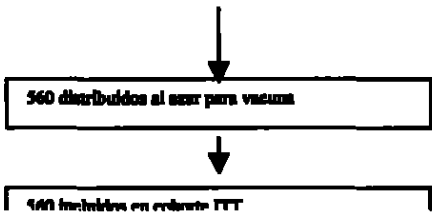
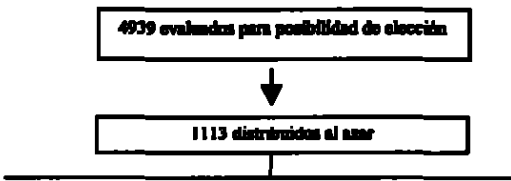
Las cohortes de intención - de - tratar y según - el - protocolo se muestran en la figura, en las que las razones para la exclusión de los análisis se enumeran por orden de categoría mujeres que reunían más de un criterio de exclusión se contaron solamente según el criterio de categoría más alta. Los inventores se refieren a conjuntos de participantes introducidos en los análisis de intención - de - tratar y según - el - protocolo como cohortes, aunque la información usada para restringir la inclusión de sujetos en el según - el - protocolo solamente se conocía después del seguimiento.

Los inventores realizaron tanto según - el - protocolo como intención - de - tratar para eficacia. El cálculo de la eficacia de vacuna en el análisis según - el - protocolo se basó en la proporción de participantes con infección por VPH - 16/18 en los grupos vacunados frente placebo. La eficacia de vacuna se definió como 1 menos la relación entre estas dos proporciones; 95% de CI medía la

precisión de las estimaciones de eficacia, los valores de p se calcularon con el ensayo exacto de Fisher de dos vías. Las tasas correspondientes se expresaron como el número de casos con el resultado dividido por el número de participantes en riesgo. La cohorte según - el - protocolo de 18 meses
5 incluía las mujeres alistadas que recibieron tres dosis programadas de vacuna y cumplían con el protocolo como se describe en la figura.

El cálculo de la eficacia de vacuna en los análisis intención - de - tratar y según - el - protocolo de 27 meses se basaron en el modelo proporcional de Cox usando el tiempo de ocurrencia de los casos con infección por VPH 16/18
10 en los grupos vacunados frente placebo. Esto permitió el control para los datos acumulados de persona - tiempo. La eficacia de vacuna se calculó usando 1 menos la relación de azar y los valores de p calculados usando el ensayo de log de categoría. Las tasas correspondientes se expresaron como el número de casos divididos por el persona - tiempo total. Todas las mujeres alistadas que
15 recibieron al menos una dosis de vacuna o placebo, eran negativos para VPH - ADN de alto riesgo el mes 0, y tenía cualquier dato disponible para la medición de resultados se incluyeron en la cohorte intención - de - tratar. La cohorte según - el - protocolo de 27 meses incluía los resultados de la cohorte según - el - protocolo de 18 meses y los resultados que se produjeron durante la fase
20 de extensión (de los 18 meses a los 27 meses).

El cálculo de los valores de p para los análisis de seguridad se realizó usando las comparaciones del ensayo exacto de Fisher. La cohorte para los análisis de seguridad incluyeron todas las mujeres alistadas que recibieron al menos una dosis de vacuna o placebo y cumplían con los requerimientos de
25 protocolo mínimos, especificados (véase la figura a continuación).



La inmunogenicidad se evaluó en un subconjunto de la cohorte de seguridad según el protocolo que incluía mujeres con resultados de serología en los meses 0, 7, y 18, que recibieron las tres dosis de vacuna o placebo del estudio según el programa, cumplieran con el programa de muestreo de sangre, y no llegaron a ser positivas para ADN de VPH - 16/18 durante el ensayo. Las tasas de seropositividad entre los grupos de vacuna y placebo se compararon con el ensayo exacto de Fisher ($p < 0,001$ juzgado significativamente). La media geométrica de los títulos se comparó con ANOVA y ensayo de Kruskal - Wallis.

Los análisis de distribución a azar en bloque y estadísticos se realizaron con SAS versión 8,2 (SAS Institute, Cary, North Carolina).

Resultados

Los resultados del análisis inicial sobre protección cruzada se presentan en la solicitud de patente WO 2004/056389, cuyo contenido total se incorpora en esta memoria descriptiva como referencia.

Análisis adicional

Se llevó a cabo un análisis sobre un grupo "ATP" (según el protocolo) para aquellos pacientes que cumplieran con todos los criterios del ensayo. En el grupo de ATP todos los pacientes recibieron 3 dosis de vacuna en los meses 0, 1 y 6 y eran seronegativos a los 6 meses.

Como se demuestra por los datos presentados en la tabla 4, la

inmunización con una mezcla de las PLV de VPH 16 y VPH 18 proporcionó protección cruzada estadísticamente significativa contra infección incidente por los tipos de VPH 31, 52 y 45 comparados con el control.

La protección cruzada estadísticamente significativa infección incidente también se observó contra el grupo de todos los tipos relacionados con VPH 16 (VPH - 31, 33, 35, 52 y 58) y el grupo de todos los tipos de alto riesgo, excluyendo 16 y 18 (VPH 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, y 68),

La protección cruzada estadísticamente significativa contra la infección persistente también se observó contra los tipos 31 y 52 (véase tabla 5), y también se observó contra el grupo de todos los tipos relacionados con VPH 16 (véase la tabla 5).

También se observó protección cruzada estadísticamente significativa

Tipo VPH	Vacuna		Control		Eficacia de vacuna	
	N	AR	N	AR	%	Valor de P
VPH - 31	1	0,2	10	2,4	99,0	0,006
VPH - 33	6	1,4	6	1,4	-0,2	1,000
VPH - 35	1	0,2	3	0,7	66,5	0,624
VPH - 52	6	1,4	16	3,9	63,0	0,031
VPH - 58	5	1,2	5	1,2	0,0	1,000
VPH - 45	0	0,0	5	1,2	100,0	0,031
VPH - 59	4	0,9	2	0,5	-100,5	0,448

Todos relacionados con 16	16	4,0	32	8,1	51,1	0,017
Todos relacionados con 18	4	1,0	7	1,7	43,0	0,384
Todos HR	32	9,0	53	15,6	42,3	0,011

**

* Muestras de cuellos de útero: cohorte de ATP

Tabla 5

5 Eficacia contra infecciones persistentes con los tipos relacionados con 16/18 *

	Tipo VPH	Vacuna		Control		Eficacia de vacuna	
		N	AR	N	AR	%	Valor de P
**	VPH - 31	2	0,48	9	2,15	78,5	0,030
	VPH - 33	3	0,71	5	1,18	40,2	0,476
	VPH - 35	1	0,24	1	0,24	0,4	0,998
	VPH - 52	5	1,2	21	5,10	77,1	0,001
	VPH - 58	4	0,95	6	1,42	34,1	0,515
***	VPH - 45	1	0,24	4	0,94	75,4	0,174
	VPH - 59	3	0,71	0	0,00	-	0,083
Todos relacionados con 16		11	2,7	30	7,8	65,1	0,002
Todos relacionados con 18		4	1,0	4	1,0	1,0	0,989
Todos HR (excepto 16/18)		36	10,1	46	13,5	27,1	0,155

* Todas las muestras; cohorte de ATP

5

Tabla 6

Eficacia contra anomalías citológicas. Con los tipos relacionados con 16/18 *

10

	Tipo VPH	Vacuna		Control		Eficacia de vacuna	
		N	AR	N	AR	%	Valor de P
** {	VPH - 31	1	0,24	5	1,20	80,1	0,123
	VPH - 33	2	0,47	4	0,94	49,9	0,686
	VPH - 35	0	0,00	2	0,47	100	0,499
	VPH - 52	1	0,24	11	2,67	91	0,003
	VPH - 58	2	0,47	2	0,47	0,2	1,000
	VPH - 45	0	0,00	2	0,47	100	0,249
	VPH - 59	4	0,94	2	0,47	-101	0,451
Todos relacionados con 16		5	1,2	18	4,6	72,8	0,005
Todos relacionados con 18		4	1,0	4	1,0	0,2	1,000
Todos HR (excepto 16/18)		10	2,8	30	8,8	68,2	< 0,001

16A

*** Cohorte de ATP**

En las tablas 4, 5 y 6

5 **N = número de sujetos en la cohorte específica**

AR = tasa de ataque = n (número de sujetos con VPH o bien infección incidente, infección persistente o anormalidad citológica, según sea apropiado para la tabla) / N

% de eficacia de vacuna es $1 - (A/B) \times 100$, ajustada para el tamaño relativo de grupo de vacuna y placebo

*** **A = % de mujeres en el grupo de vacuna con infección incidente, infección persistente o anormalidad citológica, según sea apropiado para la tabla**

15 **B = % de mujeres en el grupo de placebo con infección incidente, infección persistente o anormalidad citológica, según sea apropiado para la tabla**

**** relacionados con 16**

***** relacionados con 18**

20

25

Habiendo descrito la invención como antecede, se declara como
5 propiedad lo contenido en las siguientes

REIVINDICACIONES

- 10 1. Una composición inmunogénica que comprende las PLV o cápsomeros de VPH 16 y VPH 18 y al menos otro tipo de cáncer de VPH, el otro tipo de cáncer seleccionándose entre la lista constituida por los tipos de VPH 31, 45 y 52, en la que la dosis de la PLV o capsómero de al menos otro tipo de cáncer se reduce con relación al del VPH 16 ó 18.
- 15 2. Una composición según la reivindicación 1 en la que el otro tipo de cáncer es VPH 31.
3. Una composición según la reivindicación 1 en la que el otro tipo de cáncer es VPH 45.
- 20 4. Una composición según la reivindicación 1 en la que el otro tipo de cáncer es VPH 52.
- 25 5. Una composición según la reivindicación 1 en la que los otros tipos de cánceres son VPH 31 y VPH 45.

6. Una composición según la reivindicación 1 en la que los otros tipos de cánceres son VPH 31 y VPH 52.
- 5 7. Una composición según la reivindicación 1 en la que los otros tipos de cánceres son VPH 52 y VPH 45.
8. Una composición según la reivindicación 1 en la que los otros tipos de cánceres son VPH 31, VPH 45 y VPH 45.
- 10 9. Una composición inmunogénica según una cualquiera de las reivindicaciones preferentes en la que la composición comprende 10 μg , o más, de las PLV de VPH 16 y/o VPH 18 o capsómero y entre 2 - 9 μg de PLV o capsómero del otro tipo de cáncer.
- 15 10. Una composición inmunogénica según una cualquiera de las reivindicaciones 1 - 8 en la que la composición comprende 20 μg , o más, de las PLV de VPH 16 y/o VPH 18 o capsómeros y entre 5 - 15 μg de PLV o capsómero del otro tipo de cáncer.
- 20 11. Una composición inmunogénica según la reivindicación 10 en la que la composición comprende 10 μg de PLV o capsómero del otro tipo de cáncer.
- 25 12. Una composición inmunogénica según una cualquiera de las

reivindicaciones 1 - 11 en combinación con un adyuvante.

13. Una composición según la reivindicación 12 en la que el adyuvante es una sal de aluminio.

5

14. Una composición según la reivindicación 13 en la que el adyuvante es hidróxido de aluminio.

10

15. Una composición según la reivindicación 12 en la que el adyuvante es un derivado del lípido A.

16. Una composición según la reivindicación 15 en la que el adyuvante es 3D MPL.

15

17. Una composición según la reivindicación 16 en la que el adyuvante es 3D MPL e hidróxido de aluminio.

20

18. Una vacuna que comprende una composición inmunogénica según cualquier reivindicación precedente con un excipiente farmacéuticamente aceptable.

25

19. Un procedimiento de prevención de infección de VPH y/o enfermedad que comprende la administración a un individuo en necesidad del mismo de una composición o vacuna como se ha definido anteriormente.

20. Un procedimiento de preparación de una composición inmunogénica como se ha definido anteriormente que comprende la mezcla de las PLV o cápsomeros de VPH 16 y 18 con al menos otro tipo de cáncer de VPH, el otro tipo de cáncer seleccionándose entre la lista constituida por los tipos 31, 45, y 52 de VPH, en los que la dosis de la PLV o cápsomero de al menos otros tipos de cáncer se reduce con relación al de VPH 16 ó 18.

21. Un procedimiento de prevención o tratamiento de infección y/o enfermedad por VPH que comprende la distribución a un individuo de una composición inmunogénica que comprende las PLV o cápsomeros de VPH 16 y 18 y al menos otro tipo de cáncer de VPH, el otro tipo de cáncer seleccionándose entre la lista constituida por los tipos 31, 45 y 52 de VPH, en los que la dosis de la PLV o cápsomero de al menos otros tipos de cáncer se reduce con relación a la de VPH 16 ó 18.

**REQUISITOS MINIMOS PARA ADMISION A TRAMITE
PCT**

PATENTE DE INVENCION [X]

MODELO DE UTILIDAD []

- ♦ Indicación de que se solicita la concesión de una patente [X] 1
- ♦ Datos de identificación del solicitante o de la persona que presenta la solicitud [X] 1
- ♦ Descripción de la invención [X]
- ♦ Dibujos de ser estos pertinentes []
- ♦ Comprobante de pago de las tasas establecidas [X] 2

COMPLETA [X]

INCOMPLETA []

DISEÑO INDUSTRIAL []

- ♦ Indicación de que se solicita el registro de Diseño Industrial []
- ♦ Datos de identificación del solicitante o de la persona que presenta la solicitud []
- ♦ Representación gráfica y fotográfica del Diseño Industrial o muestra del material que incorpora el diseño []
- ♦ Comprobante de pago de las tasas establecidas []

COMPLETA []

INCOMPLETA []

ESQUEMA DE TRAZADO []

- ♦ Indicación de que se solicita el registro de un Esquema de Trazado []
- ♦ Datos de identificación del solicitante o de la persona que presenta la solicitud []
- ♦ Representación gráfica del esquema trazado []
- ♦ Comprobante de pago de las tasas establecidas []

COMPLETA []

INCOMPLETA []

Fdo B
Firma Responsable

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REPUBLICA DE COLOMBIA
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

Bogotá,
2020

Doctor(a)
LLOREDA RICAURTE ALICIA
Apoderado(a) y/o Representante de
GLAXOSMITHKLINE BIOLOGICALS S.A.

REFERENCIA	Radicación No.	6 120701
	Solicitud internacional	PCT/EP2005/006461
	Fecha inicio fase nacional	16/01/2007
	Trámite	11
	Evento	379
	Actuación	430
	Oficio No.	1877

Como resultado del examen de forma, realizado con fundamento en el artículo 38 de la Decisión 486 de la Comisión de la Comunidad Andina, artículo 27 del Tratado de Cooperación en Materia de Patentes (PCT), regla 51 bis del Reglamento y Circular Única de la Superintendencia de Industria y Comercio, se le comunica que:

1. La solicitud no contiene la copia del documento en que consta la cesión del derecho a la patente del inventor al solicitante o a su causante. (Art. 26-k) Decisión 486.

En cumplimiento de lo dispuesto por el artículo 39 de la Decisión 486, se le requiere para que dentro del término de dos meses siguientes a la fecha de notificación del presente oficio, complete los requisitos anteriormente enunciados. En caso de no dar cumplimiento al presente requerimiento, la solicitud se considerará abandonada y perderá su prelación.

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.

ALIX CARMENZA CESPEDES DE VERGEL
Jefe de la división de Nuevas Creaciones

El anterior requerimiento fue notificado por fijación en lista de
fecha 09 FEB. 2007
lerazo