

JOSE LLOREDA CAMACHO & CO.

ABOGADOS

Calle 72 No. 5-83 Piso 5, Bogotá, D.C. Tel: 6069700 Fax 6069701

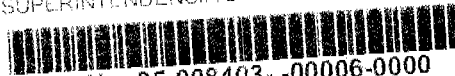
Señor

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO
DIVISIÓN DE NUEVAS CREACIONES

E.

S.

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 05-008403- -00006-0000

Fecha: 2010-04-30 17:04:07 Dep. 2020 DIR NUEVAS CR
Tra 11 PATENTE Y Lvs 379 FASE NACIONAL II
Act 444 RESPUESTA RIQUEL Folios: 24

Re: P1.-GLAXO GROUP LIMITED.-Solicitud de Patente de Invención en Fase Nacional PCT para "ANTICUERPOS DE GLICOPROTEÍNA ASOCIADA CON ANTI-MIELINA (MAG)".-Clase A61K 39/395.-Expediente número 05-008.403.-

1. Me refiero al auto dictado por ese Despacho notificado por fijación en lista de 5 de Febrero de 2010.
2. Con el fin de responder las objeciones presentadas por el Señor Examinador en su Concepto Técnico No. **484** bajo los acápites de Claridad del Capítulo Reivindicatorio y Nivel inventivo, me permito dirigir su atención a las siguientes modificaciones y argumentos técnicos, por medio de los cuales se superan completamente dichas objeciones.
3. **CLARIDAD DEL CAPÍTULO REIVINDICATORIO.**

En primera instancia, el Señor Examinador objeta la claridad del capítulo reivindicatorio actualmente en estudio por considerar que la reivindicación 7 define un mismo polinucleótido con secuencias diferentes entre sí, las cuales además redundarían con lo expuesto en las reivindicaciones 9 a 14.

Tomando en consideración esta objeción, me permito manifestar que es deseo del solicitante eliminar la mencionada reivindicación **7** del capítulo reivindicatorio.

Como consecuencia de lo anterior, el capítulo reivindicatorio modificado queda constituido por **14** reivindicaciones que corresponden a las reivindicaciones 1 a 6 y 8 a 15 anteriormente presentadas, las cuales han sido reenumeradas como reivindicaciones 1 a 14.

De esta forma, en virtud de la mencionada eliminación de la reivindicación **7** el capítulo reivindicatorio modificado es totalmente claro y conciso al reclamar el anticuerpo humanizado objeto de la presente invención, y por lo tanto dicho capítulo

cumple a cabalidad con lo preceptuado en el Artículo 30 de la Decisión Andina 486 de 2000.

4. **NIVEL INVENTIVO.**

El Señor Examinador objeta la altura inventiva de la solicitud en estudio a la luz de las enseñanzas de los documentos **WO9522344 (D1)**, y **Fujita sato et al. Biochem. Biophys. Res. Commun. 165(3), 1162-1169, 1989 (D2)**.

Con respecto a estos documentos el Señor Examinador declara *"siendo el problema técnico persistente, sin evidencia concluyente de un efecto técnico extraordinario, y teniendo en cuenta que los productos y procedimientos relacionados en la solicitud eran del conocimiento de un técnico medio al momento de la invención, el desarrollo reclamado se deriva evidentemente del estado de la técnica y por lo tanto carece de actividad inventiva"*.

Sobre este particular me permito manifestar que la presente solicitud si resuelve el problema técnico planteado, y que no existen dentro de las enseñanzas de ninguno de los documentos citados, D1 y D2, información suficiente que llevara a la persona versada en la materia a obtener específicamente los anticuerpos humanizados materia de la presente solicitud, tal como se mostrará a continuación.

4.1. **El documento D1 es el estado del arte más próximo pero no conduce de forma evidente a la presente invención.**

Como se estableció en mi memorial de Mayo 28 de 2008, el citado documento D1 hace referencia al uso de la glicoproteína asociada a mielina (MAG) en la regulación del crecimiento de neuronas tanto en el sistema nervioso periférico (PNS) como en el sistema nervioso central (CNS), y está dirigido principalmente a identificar que tipo de proteínas asociadas a mielina se encuentran relacionadas con la inhibición de crecimiento de células nerviosas:

"A la fecha ninguna de las proteínas inhibitorias de mielina ha sido identificada a nivel molecular. Sería altamente deseable proveer este tipo de proteínas inhibidoras derivadas de mielina para poder encontrar agentes que superen la inhibición del crecimiento y que puedan permitir el crecimiento regenerativo de axones dañados en el CNS de mamíferos". (Página 2, líneas 9 a15, D1).

En este sentido, el documento D1 revela un método para identificar antagonistas de MAG (página 12, línea 3), resaltando dentro de dichos antagonistas útiles los anticuerpos anti-MAG o fragmentos de los mismos. Sin embargo, la alusión a este respecto es muy general, y D1 no proporciona la constitución específica de dichos anticuerpos:

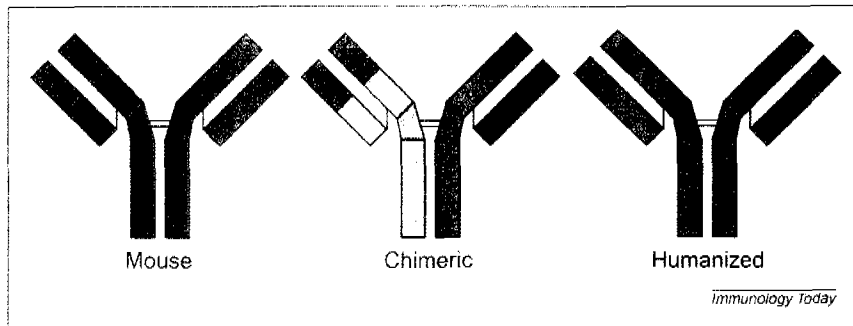
"Antagonistas de MAG útiles incluyen anticuerpos hacia MAG y los fragmentos de unión de dichos anticuerpos. Los anticuerpos, que son tanto monoclonales como policlonales, y que reconocen MAG y sus diversos epítopes, pueden ser producidos por procedimientos de rutina." (Página 14, líneas 19 a 23, D1).

*"Los anticuerpos monoclonales para uso terapéutico pueden **ser anticuerpos monoclonales humanos, o anticuerpos quiméricos humano-ratón** (o otras especies)"* (página 15, líneas 16 a 18, D1, resaltado fuera de texto).

En vista de estas revelaciones, es claro que la persona versada en la materia al partir de las enseñanzas de D1 no tendría herramientas suficientes para seleccionar específicamente **anticuerpos humanizados**, con unos CDRs particulares tanto en V_L como en V_H en busca de antagonistas de MAG, máxime cuando el documento D1 no indica la utilidad de emplear este tipo de anticuerpos (D1 se refiere sólo a anticuerpos humanos o quiméricos), ni revela de ninguna manera las secuencias de las regiones variables de los anticuerpos allí empleados.

En este punto vale la pena resaltar que la persona versada en la materia estaría totalmente conciente del hecho que existen diferencias significativas entre un anticuerpo humano, uno quimérico y uno humanizado. Los anticuerpos humanos son los generados por un organismo humano en reacción a un antígeno específico, los quiméricos son aquellos en donde las regiones variables tanto de las cadenas pesadas como livianas (región V) de un anticuerpo no-humano (generalmente murino) se unen sobre regiones constantes de cadena pesada y liviana humanas (región C), mientras que en los anticuerpos humanizados, las secuencias de las regiones determinantes de complementariedad (CDRs) de un anticuerpo no-humano son injertadas sobre un marco de regiones V humanas y expresadas con regiones C humanas (las líneas rojas representan las cadenas humanas y las azules las de ratón)¹:

¹ Clark, Mike. *Immunology Today* 21, 8, 397-402, 2000.



Consecuentemente, partiendo de las enseñanzas de D1 la persona versada en la materia no tendría ninguna motivación para emplear específicamente anticuerpos humanizados, y en particular que incluyan ciertas secuencias de CDRs y no otras, buscando un antagonista potente de MAG, toda vez que dicho documento no sugiere que tal tipo de anticuerpos puedan ser efectivos para este fin.

Sumado a lo anterior, me permito señalar que aún cuando el proceso de humanización pudiera ser conocido en el momento del desarrollo de la invención, la persona versada en la materia estaría conciente del hecho de que existe un alto grado de dificultad en dicho proceso, toda vez que no existe ninguna garantía con respecto a que un anticuerpo de ratón dado pueda ser humanizado y mantenga una suficiente afinidad de unión con respecto al anticuerpo parenteral.

En este sentido, dentro del campo técnico de la invención es un hecho ampliamente conocido que aún cuando la humanización de las regiones V de un anticuerpo puede llegar a reducir los problemas de inmunogenicidad, este proceso conlleva frecuentemente una reducción en la afinidad del anticuerpo².

Consecuentemente, la persona versada en la materia no tendría ninguna certeza con respecto a que al humanizar un anticuerpo murino con una buena afinidad por un cierto antígeno, la actividad biológica de dicho anticuerpo se mantendría intacta, o por lo menos sería suficiente para proveer una buena actividad del anticuerpo resultante.

De esta forma, **se constituye un hecho sorprendente en sí mismo que los anticuerpos humanizados objeto de la solicitud en estudio reconozcan el mismo epítipo y posean afinidades muy similares a MAG que el anticuerpo parenteral murino (página 82 de la descripción de la solicitud), toda vez que el estado del arte no permite de ninguna manera predecir este tipo de**

² Ibíd. Página 3.

comportamiento, y menos con anticuerpos que incluyan específicamente las secuencias de CDRs contenidas en los anticuerpos humanizados de la presente invención.

De esta forma, los anticuerpos reclamados por la solicitud en estudio poseen una menor inmunogenicidad con respecto a los anticuerpos murinos, pero mantienen una buena afinidad por MAG, por lo que son útiles para su uso terapéutico, siendo este el aporte que la invención le hace al campo técnico correspondiente, el cual resuelve completamente el problema planteado por dicha solicitud:

*"Por consiguiente los anticuerpos anti-MAG proporcionan agentes terapéuticos potenciales tanto para la neuroprotección aguda así como para la promoción de recuperación funcional después del derrame cerebral. Este anticuerpo es un anticuerpo de murino. Aunque los anticuerpos de murino por lo general se utilizan como agentes de diagnóstico, su utilidad como un agente terapéutico ha sido probada solamente en algunos casos. **Su aplicación limitada se debe en parte a la administración repetida de monoclonales de murino en humanos, que usualmente provocan respuestas inmunes humanas contra estas moléculas.**"*

(Página 6, líneas 16 a 25, descripción de la solicitud en estudio, resaltado fuera de texto).

En vista de todo lo anterior, es evidente que la persona versada en la materia no podría obtener de ninguna manera los anticuerpos humanizados objeto de la presente solicitud a partir de las enseñanzas de D1, por lo que dicho documento no puede afectar el Nivel Inventivo de la solicitud en estudio.

4.2. **El documento D2 no subsana las deficiencias del documento D1 para obtener la materia reclamada por la solicitud en estudio.**

De otra parte, el documento D2 revela las estructuras de 3 formas de mRNAs de glicoproteína asociada a mielina de ratón, a partir de clones de longitud completa cDNA, encontrando que una de dichas formas sería nueva toda vez que carece de los exones 2 y 12, por lo que tendría un patrón alternativo de empalme.

En relación con este documento, muy respetuosamente me permito señalar que este no brinda ninguna información que sea relevante para obtener la presente invención. En primer lugar, **contrario a lo afirmado por el Señor Examinador, dicho documento D2 se centra en los RNA mensajeros que codifican la glicoproteína asociada a mielina de ratón, pero no muestra ni enseña una secuencia de aminoácidos correspondiente a dicha proteína.**

Sumado a lo anterior, **aún si dicho documento señalara una secuencia de aminoácidos de la proteína asociada a mielina, esta información no sería útil para la persona versada en la materia para obtener la presente invención, toda vez que el objeto reclamado por la solicitud en estudio se relaciona con anticuerpos anti-MAG, y no con la proteína asociada a mielina *per se*.**

De esta forma aún cuando dicho documento D2 revelara la secuencia específica de aminoácidos de MAG murino y la correspondiente secuencia de nucleótidos que la codifica, esto no sería una herramienta que pudiera ser empleada por la persona versada en la materia para desarrollar un anticuerpo humanizado anti-MAG toda vez que los anticuerpos son generados por las células B de un organismo ya sea por el contacto directo con el antígeno (anticuerpos policlonales) o por la estimulación con dicho antígeno de hibridomas específicos (anticuerpos monoclonales). Es decir, dentro del campo técnico de la invención es claro que el conocimiento de la secuencia de un antígeno no ayuda de ninguna manera en el diseño específico del anticuerpo que reconoce dicho antígeno, toda vez que la secuencia de dicho anticuerpo será dada por la respuesta inmune específica que se elicit e en las células B.

Como consecuencia de lo anterior, **ninguna información proporcionada por D2 podría conducir a la persona versada en la materia a anticuerpos humanizados anti-MAG que incluyeran específicamente ciertas secuencias de CDRs, como los reclamados por la presente invención.**

Ahora bien, dentro del referenciado Concepto Técnico No. **484** el Señor Examinador indica también que *"en el estado del arte se conocían los anticuerpos anti-MAG de ratón, tal como lo divulga la misma solicitud (ver ejemplos 1,2); puesto que esta utiliza formas comerciales de estos anticuerpos para realizar las comparaciones de actividad de la forma quimérica y humanizada del mismo"*.

Sin embargo, aún cuando existiera el anticuerpo murino en el momento en el que fue desarrollada la invención, la información sobre su estructura no permitía que la persona versada en la materia supiera cómo realizar la humanización particular de este anticuerpo sin producir una reducción significativa en la afinidad del mismo, tal como lo hace la presente invención, y mucho menos indicaría las secuencias particulares de CDRs que podrían ser empleadas para este fin.

Como se discutió anteriormente, el proceso de humanización muchas veces conlleva la disminución de la afinidad de unión del anticuerpo, por lo que no es posible predecir si después del proceso de humanización de un cierto anticuerpo la molécula resultante se uniría o no a los mismos epítopes y mantendrá o no una afinidad similar al anticuerpo parenteral.

Dentro de este marco de ideas me permito referirme al señalamiento que hace el Tribunal de Justicia de la Comunidad Andina con respecto a las definiciones de "obvio" y "evidente" en la evaluación del Nivel Inventivo de una solicitud de patente³:

"Resulta pertinente, a los efectos del artículo 4 de la Decisión 344, definir qué se entiende por "obvio" y por "evidente" a fin de determinar qué se debe entender por nivel inventivo.

Lo obvio es aquello que salta a la vista de manera clara y directa; lo "que se encuentra o pone delante de los ojos, muy claro o que no tiene dificultad". Mientras que lo evidente consiste en la "certeza clara y manifiesta de la que no se puede dudar; cierto, claro, patente y sin la menor duda". De forma que algo que resulte obvio no es necesariamente evidente; empero lo que es evidente, es también obvio.

En este orden de ideas, se puede concluir que una invención goza de nivel inventivo cuando a los ojos de un experto medio en el asunto de que se trate, se necesita algo más que la simple aplicación de los conocimientos técnicos en la materia para llegar a ella, es decir, que de conformidad con el estado de la técnica el invento no sea consecuencia clara y directa de dicho estado."

En vista de lo anterior, dado que no "salta a la vista de manera clara y directa" la forma de realizar la humanización de los anticuerpos anti-MAG murinos, empleando específicamente las secuencias de CDRs que están incluidas dentro de la presente invención, ni se tiene "certeza clara y manifiesta" que dicha humanización no afectaría significativamente la afinidad del anticuerpo resultante, es claro que la solicitud en estudio conlleva Nivel Inventivo frente al estado del arte más próximo.

Adicionalmente me permito señalar que el solicitante ha comprobado la seguridad y tolerancia de la administración del anticuerpo humanizado de la invención, tal como se muestra en los ensayos clínicos Fase I registrados en los Institutos Nacionales de Salud de los Estados Unidos⁴, los cuales fueron llevados a cabo desde Febrero de 2008 y han terminado con éxito, permitiendo que en este momento se esté empezando la realización de los ensayos Fase II⁵ con pacientes que han sufrido derrame cerebral.

³ Tribunal de Justicia de la Comunidad Andina, PROCESO 192-IP-2005

⁴ <http://clinicaltrials.gov/ct2/show/NCT00622609>

⁵ <http://clinicaltrials.gov/ct2/show/NCT00833989>

Estos hechos demuestran que la solicitud en estudio claramente resuelve el problema técnico planteado de proveer nuevos anticuerpos no-murinos con alta actividad anti-MAG, útiles y seguros para inhibir la neurodegeneración y/o promover la recuperación funcional de un paciente que sufre o está en riesgo de desarrollar un derrame cerebral u otra enfermedad neurológica.

5. **CONCLUSIÓN.**

Para finalizar, me permito señalar que en virtud de la cancelación de la reivindicación 7 el capítulo reivindicatorio modificado es totalmente claro y conciso al reclamar el anticuerpo humanizado objeto de la presente solicitud.

Por su parte, los argumentos discutidos con respecto los citados documentos D1 y D2 establecieron de forma incontrovertible que la persona versada en la materia no podría obtener de manera evidente la presente invención a partir de sus enseñanzas, toda vez que ninguno de dichos documentos señala la manera más adecuada de realizar la humanización de un anticuerpo anti-MAG murino sin afectar significativamente su afinidad, y mucho menos las secuencias específicas de CDRs que podrían ser incluidas en dicha humanización. En vista de lo anterior, los mencionados argumentos demostraron el nivel inventivo que conlleva la solicitud en estudio frente a las enseñanzas del arte más próximo.

6. Anexo me permito presentar:

- (i) Copia de las páginas **83** a **86** correspondientes al nuevo capítulo reivindicatorio constituido por **14** reivindicaciones, en el cual se han realizado las modificaciones expuestas anteriormente;
- (ii) Copia del documento Clark, Mike. *Immunology Today* 21, 8, 397-402, 2000, referenciado en el texto;
- (iii) Copia de la página <http://clinicaltrials.gov/ct2/show/NCT00622609> que presenta los ensayos clínicos Fase I con el anticuerpo de la presente invención; y
- (iv) Copia de la página <http://clinicaltrials.gov/ct2/show/NCT00833989> que muestra el ensayo Fase II actualmente en desarrollo con el anticuerpo de la invención.

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JOSE LLOREDA CAMACHO & CO.

Calle 72 No. 5-83 Piso 5, Bogotá, D.C. Tel: 6069700 Fax 6069701

7. En vista de lo anterior, ruego a usted proceder a estudiar nuevamente la solicitud a la luz de las argumentaciones y las modificaciones presentadas y conceder el privilegio de patente solicitado

Señor Superintendente,

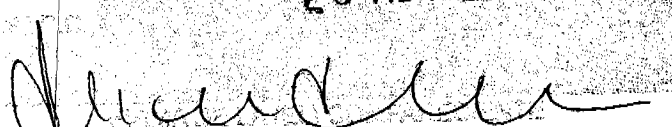

ALICIA LLOREDA RICAURTE

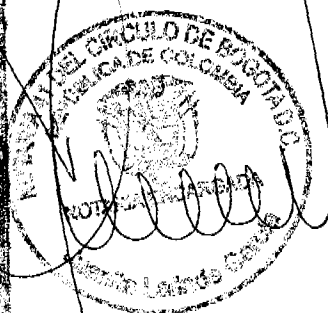
T.P. 53.215

NOTARIA 41 DEL CÍRCULO DE BOGOTÁ D.C.
DEPARTAMENTO DE CUNDINAMARCA
REPÚBLICA DE COLOMBIA

El Notario 41 del Círculo de Bogotá D.C. de la fecha de comparecencia
personalmente a la Srta. Alicia Lloreda Ricaurte, identificada (C) con el
profesorado de abogados No. 39.690.713 del Consejo Superior de la Judicatura, quien autentica la firma puesta por ella
(a) mismo en este escrito.
(Art. 84 C. P. C. Decreto 2202/89 mod. 36)

28 ABR. 2010


ALICIA LLOREDA RICAURTE



C.C. No. 39.690.713 de Usaqué

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REIVINDICACIONES

1. Un anticuerpo anti-MAG humanizado que comprende:

un dominio variable de cadena pesada (V_H) que comprende
5 regiones hipervariables CDRH1, como se define en SEC ID No:4,
CDRH2, como se define en SEC ID No:5 y CDRH3 como se define
en SEC ID No:6, y un dominio variable de cadena ligera (V_L) que
comprende regiones hipervariables en secuencia CDRL1 como se
define en SEC ID No:1, CDRL2 como se define en SEC ID No:2, y
10 CDRL3 como se define en SEC ID No:3.

2. Un anticuerpo de acuerdo con la reivindicación 1, el cual
es monoclonal.

15 3. Un anticuerpo de acuerdo con las reivindicaciones 1 ó 2,
que comprende una región variable de cadena pesada que
comprende una de las siguientes secuencias de aminoácidos: SEC
ID No: 13, SEC ID No: 14, SEC ID No: 15.

20 4. Un anticuerpo de acuerdo con la reivindicación 3, que
además comprende una región variable de cadena ligera que
comprende una de las siguientes secuencias de aminoácidos: SEC
ID No: 16, SEC ID No: 17, SEC ID No: 18 o SEC ID No:19.

25 5. Un anticuerpo de acuerdo con cualquiera de las
reivindicaciones precedentes, que comprende:

un fragmento variable de cadena pesada que comprende
SEC ID No: 13, 14 ó 15

30 y una parte constante o fragmento del mismo de una cadena
pesada humana

y un fragmento variable de cadena ligera que comprende



SEC ID No: 16, 17, 18 ó 19 y una parte constante o fragmento del mismo de una cadena ligera humana.

6. Un anticuerpo humanizado de acuerdo con cualquiera de las reivindicaciones precedentes seleccionado de los anticuerpos que comprenden:

Una región variable de cadena pesada que comprende SEC ID No: 13 y una región variable de cadena ligera que comprende SEC ID No: 16;

Una región variable de cadena pesada que comprende SEC ID No: 13 y una región variable de cadena ligera que comprende SEC ID No: 17;

Una región variable de cadena pesada que comprende SEC ID No: 13 y una región variable de cadena ligera que comprende SEC ID No: 18;

Una región variable de cadena pesada que comprende SEC ID No: 13 y una región variable de cadena ligera que comprende SEC ID No: 19;

Una región variable de cadena pesada que comprende SEC ID No: 14 y una región variable de cadena ligera que comprende SEC ID No: 16;

Una región variable de cadena pesada que comprende SEC ID No: 14 y una región variable de cadena ligera que comprende SEC ID No: 17;

Una región variable de cadena pesada que comprende SEC ID No: 14 y una región variable de cadena ligera que comprende SEC ID No: 18;

Una región variable de cadena pesada que comprende SEC ID No: 14 y una región variable de cadena ligera que comprende SEC ID No: 19;

Una región variable de cadena pesada que comprende SEC

ID No: 15 y una región variable de cadena ligera que comprende
SEC ID No: 16;

Una región variable de cadena pesada que comprende SEC
ID No: 15 y una región variable de cadena ligera que comprende
5 SEC ID No: 17;

Una región variable de cadena pesada que comprende SEC
ID No: 15 y una región variable de cadena ligera que comprende
SEC ID No: 18;

10 Una región variable de cadena pesada que comprende SEC
ID No: 15 y una región variable de cadena ligera que comprende
SEC ID No: 19.

15 7. Una Secuencia de polinucleótidos de SEC ID No: 20
codificando la secuencia de aminoácidos SEC ID No: 13.

8. Una Secuencia de polinucleótidos de SEC ID No: 21
codificando la secuencia de aminoácidos SEC ID No: 14.

20 9. Una Secuencia de polinucleótidos de SEC ID No: 22
codificando la secuencia de aminoácidos SEC ID No: 15.

10. Una Secuencia de polinucleótidos de SEC ID No: 23
codificando la secuencia de aminoácidos SEC ID No: 16.

25 11. Una Secuencia de polinucleótidos de SEC ID No: 24
codificando la secuencia de aminoácidos SEC ID No: 17.

12. Una Secuencia de polinucleótidos de SEC ID No: 25
codificando la secuencia de aminoácidos SEC ID No: 18.

13. Una Secuencia de polinucleótidos de SEC ID No: 26
codificando la secuencia de aminoácidos SEC ID No: 19.

5 (14) Una composición farmacéutica que comprende un
anticuerpo anti-MAG humanizado o un fragmento funcional del
mismo de acuerdo con las reivindicaciones 1-6 junto con un
diluyente o portador farmacéuticamente aceptable.

- 48 Posey, J.A. *et al.* (1999) A pilot trial of GM-CSF and MDX-H210 in patients with erbB-2-positive advanced malignancies. *J. Immunother.* 22, 371-379
- 49 Valone, F.H. *et al.* (1995) Phase Ia/Ib trial of bispecific antibody MDX-210 in patients with advanced breast or ovarian cancer that overexpresses the proto-oncogene HER-2/neu. *J. Clin. Oncol.* 13, 2281-2292
- 50 Weiner, L.M. *et al.* (1995) Phase I trial of 2B1, a bispecific monoclonal antibody targeting c-erbB-2 and FcγRIII. *Cancer Res.* 55, 4586-4593
- 51 Pfister, D.G. *et al.* (1999) A phase I trial of the epidermal growth factor receptor-directed bispecific antibody MDX-447 in patients with solid tumors. *Proc. Am. Soc. Clin. Oncol.* 1667
- 52 De Cramont, A. *et al.* (2000) Adoptive immunotherapy in ovarian cancer with monocyte derived activated killer cells mixed with anti HER-2/neu x anti FcγRI bispecific antibody. *Proc. Am. Soc. Clin. Oncol.* 1795
- 53 Wu, J. *et al.* (1999) An activating immunoreceptor complex formed by NKG2D and DAP10. *Science* 285, 730-732
- 54 Takeuchi, O. *et al.* (1999) Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. *Immunity* 11, 443-451
- 55 Tomizuka, K. *et al.* (2000) Double trans-chromosomal mice: maintenance of two individual human chromosome fragments containing Ig heavy and χ loci and expression of fully human antibodies. *Proc. Natl. Acad. Sci. U. S. A.* 97, 722-727



Antibody humanization: a case of the 'Emperor's new clothes'?

Mike Clark

The antiglobulin response is perceived as a major problem in the clinical development of therapeutic antibodies. Successive technical developments such as chimeric, humanized and, now, fully human antibodies claim to offer improved solutions to this problem. Although there is clear evidence that chimeric antibodies are less immunogenic than murine monoclonal antibodies, little evidence exists to support claims for further improvements as a result of more elaborate humanization protocols.

In the fable of the 'Emperor's New Clothes', a tailor tricks the Emperor into believing that he has a new and magical set of clothes, when in fact he does not. Most of the Emperor's subjects are equally taken in by the charade, too gullible, or too frightened to question what they are told. One small boy says what he actually sees and the trickery is eventually exposed. Without taking the analogy too far, I argue that antibody engineering has provided therapeutic antibodies with many new sets of clothes, and there are many competing claims as to which are better than the others. Not all these claims have a sound scientific basis and there is little evidence of rigorous scientific proof in support of some of these claims, particularly regarding immunogenicity of antibody variable regions.

It is 25 years since Kohler and Milstein published their work on the use of cell fusion for the production of monoclonal antibodies from immunized mice¹. The technique was rapidly and widely adopted and has provided an enormous repertoire of useful research reagents (see Little *et al.*, this issue). In turn, these reagents have formed a key element in the rapid progress of our understanding of biology. Although murine-derived monoclonal antibodies have been widely applied in clinical diagnostics, they have had limited success in human therapy (see Glennie and Johnson, this issue, p. 403). Two major problems in the therapeutic

use of murine monoclonal antibodies were identified early on. First, although murine antibodies had exquisite specificity for therapeutic targets, they did not always trigger the appropriate human effector systems of complement and Fc receptors². Second, even when murine antibodies were identified that did work *in vivo*, the patient's immune system normally cut short the therapeutic window, as murine antibodies were recognized by a human anti-murine-antibody immune response (HAMA)^{3,4}. An obvious exception to these generalizations has been the success of the mouse monoclonal antibody, orthoclone OKT3, used in prevention of organ graft rejection and which, in 1986, was the first monoclonal antibody to be approved by the FDA for clinical use (see Glennie and Johnson, this issue).

However, in parallel with the advances in the production of monoclonal antibodies from hybridomas, other major technological advances were happening in recombinant DNA technology. More was being understood about how genes for immunoglobulins were organized and expressed by B cells and about how germline immunoglobulin genes were rearranged and mutated, to form the repertoire of functional immunoglobulin genes^{5,6}. Inevitably, the techniques of monoclonal antibody production and recombinant DNA technology were combined to try to resolve the problems that had arisen in the application of murine antibodies to

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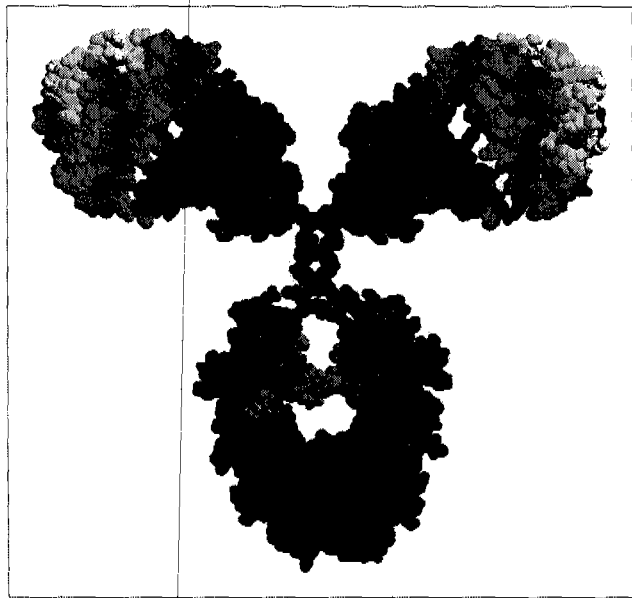


Fig. 1. Model of an IgG molecule. A spacefilling model of an IgG molecule coloured according to the structural features. Constant regions are in blue, carbohydrate in purple, variable region frameworks are in red and complementarity determining regions in yellow.

human therapy⁷⁻⁹. Thus, the field of antibody engineering was initiated, and today many of the products currently in clinical development by the biotechnology industry are recombinant monoclonal antibodies, or their derivatives (Glennie and Johnson, this issue, p. 403). It seems clear that recombinant DNA technology, as applied to antibody constant (C) regions, has provided solutions to the problems of antibody functions *in vivo*^{10,11}. But was this the right way to go for antibody variable (V) regions, or is it a case of the 'Emperor's new clothes'? Did enthusiasm to find ever more elaborate solutions to the HAMA response blind scientists to a more obvious shortcoming in the whole approach - namely human anti-human antibody

responses (HAMA), in particular to the idiotype¹²? In addition, is an anti-idiotype response only a function of the V-region sequence of the antibody or is immunogenicity more likely to depend on other factors^{12,13}?

IgG is the preferred class for therapeutic antibodies for several practical reasons¹¹. IgG antibodies are very stable, and easily purified and stored. *In vivo* they have a long biological half-life that is not just a function of their size but is also a result of their interaction with the so-called Brambell receptor (or FcRn)^{14,15}. This receptor seems to protect IgG from catabolism within cells and recycles it back to the plasma. In addition, IgG has subclasses that are able to interact with and trigger a whole range of humoral and cellular effector mechanisms. These mechanisms are initiated through immune complex formation, activation of complement, and through binding to cellular Fc receptors and complement receptors¹¹.

Figure 1 shows the basic structural features of a human IgG1. Figure 2 is a cartoon that illustrates how recombinant DNA technology can be used to transform a murine antibody into a human antibody for therapeutic applications. The immunoglobulin molecule is made up of a set of globular domains. Thus, antibody engineering can be applied in a modular fashion to generate a chimeric antibody with murine V-regions and human C-regions. Humanized antibodies can be generated where the antigen-binding complementarity determining regions (CDRs) are murine, while the rest of the antibody, including the V-region framework regions (FRs), is human.



Chimeric antibodies with human effector functions

The overall functions of an antibody are related to the constant regions, which determine the class and subclass. Clearly, human effector functions have evolved alongside human immunoglobulins. Very early during the development of rodent monoclonal antibodies for human therapy, it became clear that most monoclonal antibodies were ineffective in situations where cell destruction was a desired outcome (e.g., in cancer therapy). In some situations where several similar antibodies were used to target the same antigen, cell depletion

was dependent on the antibody class and subclass¹⁶. Several studies have shown that human IgG1 is the preferred choice for chimeric antibodies in situations where activation of effector functions is the desired outcome⁷⁻¹¹.

There might be other occasions in therapy when the effector activity of IgG1 is not required. In this situation it is possible to make use of the human subclasses IgG2 and IgG4, which lack some of the effector functions¹¹. However, all four human IgG subclasses mediate at least some biological functions. Antibody engineering has again been applied to this problem and antibodies with altered Fc regions, and thus altered activity, have been produced. For example, removal of the conserved N-linked glycosylation site

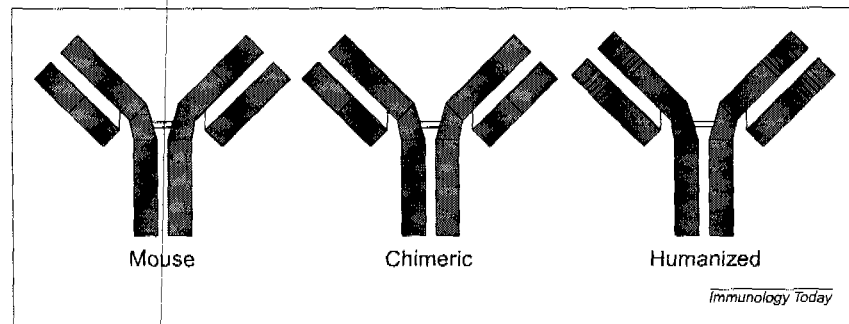


Fig. 2. Humanization of an IgG molecule. The blue represents mouse sequences and the red human sequences. In a chimeric antibody, the mouse heavy- and light-chain variable region (V-region) sequences are joined onto human heavy-chain and light-chain constant regions (C-regions). In humanized antibodies, the mouse complementarity determining regions sequences (three from the heavy-chain V-region and three from the light-chain V-region) are grafted onto human V-region framework regions and expressed with human C regions.

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on the constant region of a CD3 antibody reduces the unwanted biological side effects associated with Fc receptor binding, while retaining efficacy in blocking T-cell function¹⁷. An alternative strategy has recently been described whereby potent blocking antibodies can be generated by making antibodies with sequences derived from a combination of IgG1, IgG2 and IgG4 (Ref. 18).

Humanization to avoid the immune response

Immunogenicity of therapeutic antibodies is a significant problem and severely limits their widespread and repeated application to treat many diseases^{12,13}. Factors likely to be important in immunogenicity are listed in Box 1. Many believe that the immunogenicity of an immunoglobulin is mainly a function of its 'foreignness' to the human patient (top three items in Box 1). Most strategies for engineering antibodies to reduce immunogenicity are based upon the immunological concept that we are tolerant of all self-proteins and respond only to foreign proteins. Most immunologists would, I hope, recognize that this basic idea is flawed, particularly for immunoglobulins. By definition, every clone of B cells with a unique specificity has a unique V-region sequence: it possesses a unique 'idiotype'. New clones of B cells are generated every day throughout life by the processes of somatic gene recombination and somatic mutation^{5,6}. It seems implausible that tolerance to each B-cell clone is generated as soon as every new sequence emerges. Indeed, an equally familiar immunological concept is the opposite extreme: that every clone of B cells provokes an anti-idiotype response, which in turn provokes another anti-idiotype, thus forming an antibody network that regulates immune responses¹⁹. The true situation is probably somewhere between these two extremes.

Four basic antibody engineering strategies have been adopted to tackle the immunogenicity of therapeutic antibodies. In chimeric antibodies, the murine constant regions are replaced with human constant regions, on the basis that the constant region contributes a significant component to the immunogenicity^{7,8,12}. As a further step on from chimeric antibodies, V-region humanization, or reshaping, involves changing some of the FR residues of the V-regions to sequences considered more 'human', while retaining the CDR sequences necessary for antigen binding (see Fig. 2; Refs 9,20-22). Two additional strategies provide what some call 'fully human V-regions': human antibody V-regions can be selected from phage libraries by affinity selection on antigen^{23,24}; transgenic mice have been constructed which have had their own immunoglobulin genes replaced with human immunoglobulin genes so that they produce human antibodies upon immunization^{25,26}.

Immunogenicity versus antigenicity of therapeutic antibodies

Before discussing just how sensible it is to promulgate the idea that 'V-region humanization' or 'fully human V-regions' are the way ahead, we need to consider the immunological difference between the concepts of 'antigenicity' and 'immunogenicity'. Antigenicity is defined in terms of the ability of a molecule to be recognized by

Box 1. Factors likely to influence immunogenicity of therapeutic antibodies

- Murine constant regions
- V-region sequences
- Human Ig allotypes
- Unusual glycosylation
- Method of administration
- Frequency of administration
- Dosage of antibody
- Patients' disease status
- Patients' immune status
- Patients' MHC haplotype
- Specificity of antibody
- Cell-surface or soluble antigen?
- Formation of immune complexes with antigen
- Complement activation by antibody
- Fc receptor binding by antibody
- Inflammation and cytokine release

All of these factors are likely to have a bearing on the immunogenicity of a therapeutic antibody. Many of them might be an inevitable consequence of the treatment and of the desired mode of action of the antibody. Only the first three on the list are altered by humanization and the fourth is likely to depend on the expression system used for antibody production.

preformed antibodies. A given antigen might be recognized by many different antibody specificities and these define the antigenic 'epitopes'. Immunogenicity refers to the ability of an antigen to provoke an immune response. It is possible for an antigen to be highly immunogenic, weakly immunogenic, nonimmunogenic or tolerogenic²⁷. For example, high doses of deaggregated human IgG given intravenously to mice can be tolerogenic, but the same antigen given in aggregated form subcutaneously can be highly immunogenic. It is the immunogenicity that is important for therapy, not the antigenicity^{12,13,27}.

For protein antigens such as immunoglobulins the immune response is usually T-cell dependent and there are many factors that might have a significant influence on the immunogenicity, including the need for appropriate antigen processing and presentation, and 'secondary signals'. How might recombinant antibodies influence these requirements? We should not lose sight of the fact that antibodies have evolved to play a key role in the immune response to infection and thus have features that often enhance immunogenicity of antigens bound up in immune complexes with the antibodies. It seems unlikely that at the same time as enhancing immunogenicity of the bound antigen the antibody is completely inert with regard to its own immunogenicity (particularly for its idiotype). Binding to Fc receptors²⁸, and the activation of the complement cascade^{29,30}, are two obvious ways that antibodies can enhance immune responses. In addition, antibodies that directly bind to cell surfaces might be internalized and processed for presentation²⁷, whereas antibodies that

Table 1. Percentage homology of humanized and chimeric antibody V-regions^{a,b}

Antibody comparisons	FR	CDR	Complete V-region
Humanized V_H (i.e., complementarity determining regions grafted) versus human myeloma V_H^c			
Campath-1H versus NEW	98%	82%	74%
Anti-Tac versus EU	86%	78%	72%
OKT3 versus KOL	85%	75%	69%
Chimeric V_H (i.e., murine) versus human germline V_H^d			
Campath-1G	78%	71%	63%
Anti-Tac	77%	69%	65%
OKT3	77%	56%	72%
Human myeloma V_H versus human germline V_H^e			
NEW	87%	50%	78%
EU	95%	77%	91%
KOL	91%	81%	88%

^aAbbreviations: CDR, complementarity determining region; FR, framework region; V-region, variable region.

^bThe percentage amino acid homology for several antibody heavy-chain V-regions is shown from Ref. 34.

^cHumanized antibodies (Campath-1H, anti-Tac, OKT3) shown compared with the human myeloma protein sequences used as acceptor sequences for the humanization (NEW, EU, KOL).

^dMurine sequences compared to the closest matching human germline immunoglobulin sequences found in the databases, i.e., the homology for chimeric versions of the antibodies.

^eComparison of the three human myeloma sequences with human germline immunoglobulin sequences.

cause cell killing and destruction might give rise to what are sometimes referred to as 'danger' signals³¹. In support of these ideas, it has been reported that the presence of the murine Fc region might make antibodies more immunogenic and also that Fab fragments are much less immunogenic than are F(ab')₂ or whole antibodies¹². Additionally, antibodies that have been engineered so as not to activate effector functions are less immunogenic³². Unfortunately, those properties of antibodies that result in enhanced antigen presentation are likely to be related to the desired properties of the antibodies that have been deliberately chosen for their therapeutic effect^{9,10,16}. It might thus prove very difficult to generate tolerance even to humanized or fully human antibodies that cause such effects *in vivo*.

There is some hope from strategies whereby tolerance can be induced to a non-cell-binding, non-inflammatory antibody that has a slightly modified but otherwise similar idiootype to a cell-binding therapeutic antibody^{13,27,33}. However, the therapeutic antibody will still have a unique idiootype even compared with the deliberately engineered tolerizing antibody and so at best, this strategy might reduce or delay the probability of an anti-idiootype response, but is unlikely to eliminate it in all therapeutic circumstances. Other strategies involve identifying and engineering out the major peptide sequences that would activate helper T cells when presented on major histocompatibility complex (MHC) Class II molecules. The problem

here is that there are so many different alleles of MHC Class II in the human population that it would only be possible to do this for a limited number of the more common epitopes.

Humanized versus chimeric antibodies

So are 'humanized' antibodies, or even so called 'fully human' antibodies, likely to be better than 'chimeric' antibodies for human therapy? This has implications at several levels. Humanization of the V-regions might be considered to try to reduce potential problems of immunogenicity^{9,20}. However, during the humanization process, the antibody affinity is frequently reduced⁹. This reduction in affinity might be minimized by careful selection of human FRs that are homologous to the starting antibody^{21,22,34} or by reintroduction of important murine FR residues back into the engineered antibody^{9,21,34}. This strategy could result in a humanized antibody that is itself very homologous to a chimeric form of the same antibody. This can be easily seen in Table 1, where the murine V-regions of the antibodies anti-Tac and OKT3 have a similar overall percentage homology to a closest match human germline gene (75% and 72%) compared

with the homology of the humanized antibodies and the acceptor myeloma protein sequences (72% and 69%). Indeed, I am almost tempted to argue that humanization of these two antibodies has reduced their homology. The humanization methods used have maximized the homology for FRs at the expense of the homology for CDRs, however, the overall percentage homologies for chimeric and humanized versions of these antibodies are similar. Furthermore, the homology of chimeric antibodies compared with the human germline is only slightly less than some human myeloma protein sequences compared with the germline. This is presumably a consequence of somatic mutations in the myeloma protein sequences.

The similar homology of humanized and chimeric forms of an antibody as shown in Table 1 arises because V-regions are often much more homologous between species than are constant (C) regions. Although this seems counter-intuitive, the explanation is simple: there are many different human V-regions³⁵⁻³⁷ to compare with any one chosen murine V-region, but there are only four human IgG C-regions. However, there is little scientific evidence to support a substantial reduction in immunogenicity when the penalty is an antibody of perhaps lower affinity^{9,21,22} and one where the commercial viability is reduced further by the need to pay out numerous royalties for licences that cover the antibody engineering process. Humanization might increase the production costs of a therapeutic

antibody substantially. If these cost increases are passed on to patients, some healthcare providers might consider the treatment too expensive to be used routinely. Then, ironically, although the percentage of patients showing antiglobulin responses might be reduced, the total numbers of patients treated will also be reduced. My recommendation is that, at present, it is well worth carrying out a cost-benefit analysis for humanization on every new antibody. If humanization means that the costs outweigh the benefit, then it might well be better to produce and use a chimeric form of the antibody for therapy.

Testing the efficacy of V-region humanization

What would constitute a 'proper scientific test' of humanization of V-regions as a means of reducing the immunogenicity? At a minimum, this would probably require the generation of humanized and chimeric forms of several different antibodies with different idiotypes. These pairs of antibodies would then have to be used in similar clinical studies where the number of patients is high enough to establish reliable immunogenicity frequencies. A significant result would indicate that all the humanized antibodies are less immunogenic than their chimeric counterparts. Such an experiment is unlikely to be contemplated on commercial or on ethical grounds. Furthermore, there is already considerable evidence for a wide variability in measured immunogenicity of chimeric and humanized antibodies to different antigens and used in different diseases^{12,13,38}. Thus, it can already be concluded that other factors listed in Box 1 might be more important than the V-region sequences.

So what can be done? We can return to animal experiments. It is possible to make anti-idiotypic antibodies to rodent antibodies by immunizing the same strains from which the original monoclonal antibodies were derived³⁹. Thus, rodents are clearly not tolerant of antibodies from the same species and strain. However, deliberately generating an anti-idiotypic is often not easy, and usually requires the use of adjuvant or coupling to 'carrier' proteins such as keyhole limpet haemocyanin (KLH)³⁹. By contrast, it is also possible to generate tolerance to foreign proteins in rodents, including human immunoglobulins, as mentioned earlier^{13,27,33}. Animal models suggest that the nature of the target antigen is more important than the sequence of the antibody V-regions, with cell-binding antibodies being the most difficult with which to achieve tolerance²⁷.

Alternative strategies for producing 'human' antibodies

Other strategies for the production of 'fully human' antibodies include phage libraries^{23,24} or transgenic mice^{25,26}, both of which make use of repertoires of human V-regions. A third technique consists of reconstituting severe combined immunodeficient (SCID) mice with human peripheral blood lymphocytes, immunizing these animals with antigen, followed by rescuing the immune B cells by fusion with myeloma cells⁴⁰. However, antibodies generated using these methods still have unique idiotypes, and in the case of antibodies from transgenic mice (and of course antibodies from reconstituted

SCID mice), the somatic mutations are in FRs as well as in the CDRs (Ref. 26). Some like to claim that chimeric antibodies are overall about 75% human in sequence (% homology of whole IgG), whereas humanized antibodies are 95% human and antibodies from transgenic mice or phage display are 100% human. These figures can only be derived if murine and human antibodies are thought of as being totally different (Fig. 2). In fact, as illustrated in Table 1, there is considerable sequence homology between mouse and human immunoglobulin sequences, as would be obvious to anyone who looks at the sequence databases^{22,34}. The Kabat database shows clearly that many V-region sequences, particularly for FRs, are conserved between species⁴¹. This, combined with the large repertoire and diversity of sequences in each species, makes it likely that most rodent sequences have a homologous human counterpart (see Table 1; Refs 22,34–37,41). Equally, somatic mutations in antibodies from transgenic and reconstituted mice, particularly during affinity maturation, means that they are no longer 100% identical to inherited human germline genes, as is the case with human myeloma proteins (see Table 1; Ref. 34). The other problem with use of human antibodies generated by these methods is that it might still be necessary to engineer them as chimeric constructs to provide them with a suitable human C-region for therapy, particularly where novel C-regions are contemplated^{11,17,18}. Again, this has cost implications as it might then require royalty licence payments to cover both technologies.

Concluding remarks

For some therapeutic antibodies it seems likely that the problems of immunogenicity are likely to remain whatever the strategies chosen for their production. However, the anti-idiotypic response is generally only a problem where repeated treatments are required for chronic and relapsing diseases such as in therapy of autoimmune disease^{12,13}. Indeed for many therapeutic applications in acute disease situations, the possibility of an anti-idiotypic response is not likely to have a major impact on their efficacy in the vast majority of patients^{13,38}.

I would not want to imply that there is an absolute correlation between the fable of the Emperor's New Clothes and antibody engineering, but I think the scientific question of how the immunogenicity of an antibody V-region relates to its sequence must be addressed. It is unlikely, I argue, to be a simple matter of percentage homology. It is interesting to reflect on a comment written by Alan Munro to accompany the publication of the first descriptions of recombinant 'chimeric' antibodies⁴². Although he used the term 'chimaeric' where today we would refer to 'humanized', with some foresight, he wrote, 'by using the techniques of genetic engineering, it might be possible to obtain antibodies in which the antigen-binding site is defined by sequences from a rodent monoclonal antibody of the right specificity whereas the rest of the molecule is as "human" in structure as possible...Possibly the human immune system will not recognize such chimaeric molecules as foreign, but there are good reasons to think that they will'. Yet, what was obvious to Munro in 1984 seems to have been forgotten or is ignored by many today.

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I thank my colleagues over the years for many stimulating discussions on antibody therapy and immunological tolerance. More importantly, I acknowledge the contributions of the undergraduates to whom I have taught immunology. Their probing questions bring home the shortcomings that still remain in our understanding of immunological problems.

Mike Clark (mrc7@cam.ac.uk) is in the Immunology Division, Dept of Pathology, Cambridge University, Tennis Court Road, Cambridge, UK CB2 1QP.

References

- Kohler, G. and Milstein, C. (1975) Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 256, 495-497
- Clark M. *et al.* (1984) Advantages of rat monoclonal antibodies. *Immunol. Today* 4, 100-101
- Schroff, R.W. *et al.* (1985) Human anti-murine responses in patients receiving monoclonal antibody therapy. *Cancer Res.* 45, 879-885
- Shawler, D.L. *et al.* (1985) Human immune responses to multiple injections of murine monoclonal immunoglobulins. *J. Immunol.* 135, 1530-1535
- Tonegawa, S. (1983) Somatic generation of antibody diversity. *Nature* 302, 575-581
- Alt, F.W. *et al.* (1992) VDJ recombination. *Immunol. Today* 13, 306-314
- Boulianne, G.L. *et al.* (1984) Production of functional chimaeric mouse/human antibody. *Nature* 312, 643-646
- Morrison, S.L. *et al.* (1984) Chimeric human antibody molecules: mouse antigen-binding domains with human constant region domains. *Proc. Natl. Acad. Sci. U. S. A.* 81, 6851-6855
- Riechmann, L. *et al.* (1988) Reshaping human antibodies for therapy. *Nature* 332, 323-327
- Hale, G. *et al.* (1988) Remission induction in non-Hodgkin lymphoma with reshaped human monoclonal antibody CAMPATH-1H. *Lancet* 2, 1394-1399
- Clark, M.R. (1997) IgG effector mechanisms. *Chem. Immunol.* 65, 88-110
- Kuus-Reichel, K. *et al.* (1994) Will immunogenicity limit the use, efficacy, and future development of therapeutic monoclonal antibodies? *Clin. Diagn. Lab. Immunol.* 1, 365-372
- Isaacs, J.D. (1990) The antoglobulin response to therapeutic antibodies. *Semin. Immunol.* 2, 449-456
- Chetie, V. *et al.* (1996) Abnormally short serum half-lives of IgG in beta-2-microglobulin-deficient mice. *Eur. J. Immunol.* 26, 690-696
- Junghans, R.P. and Anderson, C.L. (1996) The protection receptor for IgG catabolism is the beta2-microglobulin-containing neonatal intestinal transport receptor. *Proc. Natl. Acad. Sci. U. S. A.* 93, 5512-5516
- Dyer, M.J.S. *et al.* (1989) Effects of CAMPATH-1 antibodies *in vivo* in patients with lymphoid malignancies: influence of antibody isotype. *Blood* 73, 1431-1439
- Bolt, S. *et al.* (1993) The generation of a humanised, non-mitogenic CD3 monoclonal antibody which retains *in vitro* immunosuppressive properties. *Eur. J. Immunol.* 23, 403-411
- Armour, K.L. *et al.* (1999) Recombinant human IgG molecules lacking Fc-gamma receptor I binding and monocyte triggering activities. *Eur. J. Immunol.* 29, 2613-2624
- Macias, A. *et al.* (1999) Novel cross-reactive anti-idiotype antibodies with properties close to human intravenous immunoglobulin (IVIg). *Hybridoma* 18, 263-272
- Jones, P.T. *et al.* (1986) Replacing the complementarity-determining regions in a human antibody with those from a mouse. *Nature* 321, 522-525
- Queen, C. *et al.* (1989) A humanized antibody that binds to the interleukin 2 receptor. *Proc. Natl. Acad. Sci. U. S. A.* 86, 10029-10033
- Gorman, S.D. *et al.* (1991) Reshaping a therapeutic CD4 antibody. *Proc. Natl. Acad. Sci. U. S. A.* 88, 4181-4185
- Winter, G. *et al.* (1994) Making antibodies by phage display technology. *Annu. Rev. Immunol.* 12, 433-435
- Low, N.M. *et al.* (1996) Mimicking somatic hypermutation: affinity maturation of antibodies displayed on bacteriophage using a bacterial mutator strain. *J. Mol. Biol.* 260, 359-368
- Bruggemann, M. *et al.* (1991) Human antibody expression in transgenic mice: expression from 100kb of the human Igh locus. *Eur. J. Immunol.* 21, 1323-1326
- Mendez, M.J. *et al.* (1997) Functional transplant of megabase human immunoglobulin loci recapitulates human antibody response in mice. *Nat. Genet.* 15, 146-156
- Benjamin, R.J. *et al.* (1986) Tolerance to rat monoclonal antibodies: implications for serotherapy. *J. Exp. Med.* 163, 1539-1552
- van-Vugt, M.J. *et al.* (1999) The Fc-gammaRIa (CD64) ligand binding chain triggers major histocompatibility complex class II antigen presentation independently of its associated FcR gamma-chain. *Blood* 94, 808-817
- Fearon, D.T. (1998) Non-structural determinants of immunogenicity and the B-cell co-receptors, CD19, CD21 and CD22. *Adv. Exp. Med. Biol.* 452, 181-184
- Lou, D. and Kohler, H. (1998) Enhanced molecular mimicry of CEA using photaffinity crosslinked C3d peptide. *Nat. Biotechnol.* 16, 458-462
- Matzinger, P. (1994) Tolerance, danger, and the extended family. *Annu. Rev. Immunol.* 12, 991-1045
- Routledge, E.G. *et al.* (1995) The effect of aglycosylation on the immunogenicity of a humanized therapeutic CD3 monoclonal antibody. *Transplantation* 60, 847-853
- Gilliland, L.K. *et al.* (1999) Elimination of the immunogenicity of therapeutic antibodies. *J. Immunol.* 162, 3663-3671
- Routledge, E.G. *et al.* (1993) Reshaping antibodies for therapy. In *Protein Engineering of Antibody Molecules for Prophylactic and Therapeutic Applications in Man* (Clark, M., ed.), pp. 13-44, Academic Titles
- Cook, G.P. and Tomlinson, I.M. (1995) The human immunoglobulin VH repertoire. *Immunol. Today* 16, 237-242
- Williams, S.C. *et al.* (1996) Sequence and evolution of the human germline V lambda repertoire. *J. Mol. Biol.* 264, 220-232
- Tomlinson, I.M. *et al.* (1995) The structural repertoire of the human V kappa domain. *EMBO J.* 14, 4628-4638
- Rebello, P.R. *et al.* (1999) Anti-globulin responses to rat and humanized CAMPATH-1 monoclonal antibody used to treat transplant rejection. *Transplantation* 68, 1417-1420
- Cobbold, S.P. *et al.* (1990) A simple method for measuring patient antiglobulin responses against isotypic or idiotypic determinants. *Methods Enzymol.* 127, 19-24
- Lubin, I.H. *et al.* (1994) Engraftment of human peripheral blood lymphocytes in normal strains of mice. *Blood* 83, 2368-2381
- Johnson, G. and Wu, T.T. (2000) Kabat database and its applications: 30 years after the first variability plot. *Nucleic Acids Res.* 28, 214-218
- Munro, A. (1984) Uses of chimaeric antibodies. *Nature* 312, 597

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This study has been completed.

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Sponsor:	GlaxoSmithKline
Information provided by:	GlaxoSmithKline
ClinicalTrials.gov Identifier:	NCT00622609

Purpose

GSK249320 is a monoclonal antibody directed against myelin associated glycoprotein (MAG), a protein that inhibits axonal regeneration. GSK249320 acts as a MAG antagonist, and through this activity it is hypothesised that it will enhance recovery from neuronal degeneration following acute axonal injury, which occurs in spinal cord injury or stroke.

Condition	Intervention	Phase
Chronic Diseases Associated With Axonal Damage	Drug: GSK249320 Drug: Placebo	Phase I

Study Type: **Interventional**
Study Design: **Allocation: Randomized**
Endpoint Classification: Safety Study
Intervention Model: Parallel Assignment
Masking: Single Blind (Subject)
Primary Purpose: Treatment

Official Title: **A Single-blind, Single Dose, Placebo Controlled, Parallel Group, First Time in Human Study to Assess the Safety, Tolerability, Pharmacokinetics and Immunogenicity of Ascending IV Doses of GSK249320 in Healthy Volunteers**

Resource links provided by NLM:

MedlinePlus related topics: [Coping with Chronic Illness](#)

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Further study details as provided by GlaxoSmithKline:

Primary Outcome Measures:

- Safety and tolerability of GSK249320 including: adverse events,vital signs,ECG,continuous lead II ECG monitoring,safety laboratory assessments,clinical assessment of peripheral nerve function,nerve conduction testing [Time Frame: Throughout study]
[Designated as safety issue: No]

Secondary Outcome Measures:

- Pharmacokinetic parametersAnti-GSK249320 antibody titres [Time Frame: At various timepoints from pre-dose to Week 52 follow-up] [Designated as safety issue: No]

- Pharmacokinetic parameters of ascending single IV doses of GSK249320: C_{inf}, AUC(0-24), AUC(0-t) and AUC(0-inf) [Time Frame: At various timepoints from pre-dose to Week 52 follow-up] [Designated as safety issue: No]
- Anti-GSK249320 Antibody titres: serum samples will be collected for anti-drug (anti-GSK249320) antibody confirmation, titration and neutralization testing [Time Frame: At various timepoints from pre-dose to Week 52 follow-up] [Designated as safety issue: No]
- Novel candidate biomarkers and subsequently discovered biomarkers of the biological response associated with the action of GSK249320 may be identified by application of: [Time Frame: At various timepoints from pre-dose to Week 52 follow-up] [Designated as safety issue: No]
- RNA transcriptome analysis of blood samples [Time Frame: At various timepoints from pre-dose to Week 52 follow-up] [Designated as safety issue: No]
- Proteome analysis of plasma samples [Time Frame: At various timepoints from pre-dose to Week 52 follow-up] [Designated as safety issue: No]
- Non-compartmental pharmacokinetic parameters of GSK249320 : T_{inf} , I_z, T_{1/2}, CL and V_{ss} [Time Frame: At various timepoints from pre-dose to Week 52 follow-up] [Designated as safety issue: No]

Enrollment: 46
 Study Start Date: August 2007
 Study Completion Date: April 2009
 Primary Completion Date: April 2009 (Final data collection date for primary outcome measure)

Arms	Assigned Interventions
GSK249320: Experimental	Drug: GSK249320 IV Infusion
Placebo: Placebo Comparator	Drug: Placebo Placebo Comparator

► Eligibility

Ages Eligible for Study: 18 Years to 60 Years
 Genders Eligible for Study: Both
 Accepts Healthy Volunteers: Yes

Criteria

Inclusion Criteria:

- Healthy adult men or women of non-child bearing potential (i.e. post-menopausal or surgically sterile e.g. hysterectomy or bilateral oophorectomy). If necessary, postmenopausal status will be confirmed by serum FSH and oestradiol concentrations at screening. Surgical sterility will be defined as females who have had a documented hysterectomy or bilateral oophorectomy.
- Between 18 and 60 years of age inclusive.
- Body weight >/ 60 kg and BMI within the range 19-29.9 kg/m2 inclusive.
- Healthy as judged by responsible physician with no clinically significant abnormality identified on the medical or laboratory evaluation. A subject with a clinical abnormality or laboratory parameters outside the reference range for this age group may be included only if the Investigator considers that the finding will not introduce additional risk factors and will not interfere with the study procedures.
- A 12-lead ECG at pre-study screening which in the opinion of the Investigator or his/her designee has no abnormalities that will compromise safety in this study.
- Normal clinical neurological exam, normal QST results, and normal pattern of conduction latencies in medial, ulnar, sural, tibial and peroneal nerves at screening.
- Signed and dated written informed consent prior to admission to the study.
- The subject is able to understand and comply with protocol requirements, instructions and protocol-stated restrictions.

Exclusion Criteria:

- Positive drug/ alcohol screen at screening or baseline.
- Positive pre-study HIV, Hepatitis B surface antigen or positive Hepatitis C antibody result at screening.

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- History of acute neuronal injury (central or peripheral) within the previous 12 months, peripheral neuropathy or neuritis, neurodegenerative disorders or other neurological diseases, including conditions which are known or hypothesised to be associated with disruption in the blood-brain barrier (BBB).
 - Considered to be at a high risk of developing a stroke including a history of carotid artery disease or surgery, transient ischaemic attacks, reversible ischaemic neurological deficits or other abnormalities of the brain vessels, including but not limited to berry aneurysms or arteriovenous malformations.
 - History of regular alcohol consumption averaging >7 drinks/week for women or >14 drinks/week for men. One drink is equivalent to (12 g alcohol) = 5 ounces (150 mL) of wine or 12 ounces (360 mL) of beer or 1.5 ounces (45 mL) of 80 proof distilled spirits) within 6 months of screening.
 - Subjects who smoke 10 or more cigarettes per day. Subjects who smoke <10 cigarettes per day may be admitted into the study but will be asked to refrain from smoking for at least 24 hours before the planned day of admission into the unit. They will NOT be allowed to smoke during their stay in the unit.
 - The subject is unable to abstain from strenuous physical activity for 72 h prior to each clinic visit where safety lab tests are conducted.
 - The subject has participated in a clinical trial and has received an investigational product within the following time period prior to the dosing day in the current study: 30 days, 5 half-lives or twice the duration of the biological effect of the investigational product (whichever is longer).
 - Use of prescription or non-prescription drugs, including vitamins, herbal and dietary supplements (including St John's Wort) within 7 days or 5 half-lives (whichever is longer) prior to the first dose of study medication.
 - Where participation in study would result in donation of blood in excess of 500 mL within a 56 day period.
 - Previous exposure to humanised antibody therapy for any reason.
 - Unwillingness of the male subjects to abstain from sexual intercourse with pregnant or lactating women from the time of the first dose of study medication until five half lives following administration of the dose of study medication. This may be increased depending on PK analysis during the study.
 - Unwillingness of the male subject to use a condom/spermicide in addition to having their female partner use another form of contraception such as an intra-uterine device (IUD), diaphragm with spermicide, oral contraceptives, injectable progesterone, subdermal implants or a tubal ligation if the woman could become pregnant from the time of the first dose of study medication until five half lives following administration of the last dose of study medication. The duration of the period during which contraception must be practiced may be modified during the study, depending on data emerging from PK analysis. The mean half-life predicted for GSK249320 is 30 days
 - Female subjects with positive urine/serum pregnancy test result at screening or prior to first dose
 - Status as a "vulnerable" subject, defined by the US Food and Drug Administration (FDA) Code of Federal Regulations; 45 CFR, Section 46, Subparts B and C.

Contacts and Locations

Please refer to this study by its ClinicalTrials.gov identifier: NCT00622609

Locations

United States, Maryland

GSK Investigational Site
Baltimore, Maryland, United States, 21225

Australia, New South Wales

GSK Investigational Site
Randwick, Sydney, New South Wales, Australia, 2031

Sponsors and Collaborators

GlaxoSmithKline

Investigators

Study Director: GSK Clinical Trials GlaxoSmithKline

More Information

No publications provided

Responsible Party: GSK (Study Director)
Study ID Numbers: MAG103114
Study First Received: February 13, 2008
Last Updated: March 17, 2010
ClinicalTrials.gov Identifier: [NCT00622609](#) [History of Changes](#)
Health Authority: United States: Food and Drug Administration

Keywords provided by GlaxoSmithKline:

first time in human,
MAG
monoclonal antibody,
GSK249320,
healthy subjects,

Additional relevant MeSH terms:

Disease Attributes
Pathologic Processes
Chronic Disease

ClinicalTrials.gov processed this record on April 26, 2010

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Study 4 of 6 for search of: **MAG antibody**

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[No Study Results Posted](#)

[Related Studies](#)

Safety Escalating Repeat IV, in Stroke Patients (MAG111539)

This study is currently recruiting participants.

Verified by GlaxoSmithKline, March 2010

First Received: January 29, 2009 Last Updated: March 31, 2010 [History of Changes](#)

Sponsor:	GlaxoSmithKline
Information provided by:	GlaxoSmithKline
ClinicalTrials.gov Identifier:	NCT00833989

Purpose

The purpose of this study is to test increasing repeat doses of GSK249320 compared to placebo in patients with stroke.

Condition	Intervention	Phase
Stroke	Drug: PLACEBO Drug: GSK249320	Phase II

Study Type: Interventional
Study Design: Allocation: Randomized
Endpoint Classification: Safety Study
Intervention Model: Parallel Assignment
Masking: Single Blind (Subject)

Official Title: A Single-Blind Study of the Safety, Pharmacokinetics and Pharmacodynamics of Escalating Repeat Doses of GSK249320 in Patients With Stroke

Further study details as provided by GlaxoSmithKline:

Primary Outcome Measures:

- Adverse events (AEs), vital signs, physical examination (incl. full neurological exam.), 12-lead ECGs, nerve conduction tests (NCTs), magnetic resonance imaging (MRI) and clinical laboratory tests [Time Frame: 16 weeks] [Designated as safety issue: No]

Secondary Outcome Measures:

- Presence of **antibodies** to GSK249320 will be assessed in serum samples using immunoelectro-chemiluminescent (ECL) assay [Time Frame: At least 16 weeks] [Designated as safety issue: No]
- PK parameters: AUC(0-), Cmax, Tmax, T1/2 after second dose, clearance, volume of distribution [Time Frame: At least 16 weeks] [Designated as safety issue: No]
- Gait velocity, Berg Balance, Fugl-Meyer Motor Assessment, Box and Blocks, grip strength (dynamometer), modified Rankin, Barthel, NIHSS, Transcranial Magnetic Stimulation (TMS), and MRI [Time Frame: At least 16 weeks] [Designated as safety issue: No]

- To explore linear or non-linear relationships between exposure parameters and one or a combination of PD endpoints. Possible extension to a disease progression model, if feasible [Time Frame: At least 16 weeks] [Designated as safety issue: No]
- Exploratory biomarker levels, such as S100β [Time Frame: At least 16 weeks] [Designated as safety issue: No]

Estimated Enrollment: 40
 Study Start Date: July 2009
 Estimated Study Completion Date: April 2011
 Estimated Primary Completion Date: April 2011 (Final data collection date for primary outcome measure)

Arms	Assigned Interventions
ACTIVE: Experimental	Drug: GSK249320 I.V. infusion
PLACEBO: Placebo Comparator	Drug: PLACEBO Placebo

Detailed Description:

GSK249320 is a humanised monoclonal antibody (mAb) that binds with high specificity to myelin-associated glycoprotein (MAG) and antagonises or neutralises MAG-mediated inhibition and has been shown to improve functional recovery after stroke in pre-clinical models, possibly by promoting neuroregeneration and plasticity. The present study is the first in patients with stroke. The main aim of this study is to select tolerated doses of GSK249320 that can be used in future trials to evaluate its efficacy in improving clinical function in patients recovering from stroke. This clinical trial is designed as a placebo-controlled, single-blind, multicenter study to investigate the safety, pharmacokinetics (PK) and pharmacodynamics (PD) of escalating repeat IV doses of GSK249320. Three sequential dose escalation cohorts (1, 5 and 15 mg/kg) are planned, with 8 patients on placebo and 8 on active in cohort 1 and 4 patients on placebo and 8 on active in cohorts 2 and 3. Each patient will receive 2 repeat IV doses 9 ± 1 days apart and assessments will extend to at least 16 weeks.

► Eligibility

Ages Eligible for Study: 18 Years to 90 Years
 Genders Eligible for Study: Both
 Accepts Healthy Volunteers: No

Criteria

Inclusion Criteria:

- Have a confirmed diagnosis of stroke
- Stroke onset must be within the last 24-72 hours.
- Have a stroke that is either:
 - radiologically confirmed to be ischaemic and supratentorial. The diameter of the ischemic lesion is >15mm in any single direction or the volume is >4cc. OR
 - radiologically confirmed to be an intracerebral hemorrhage that is supratentorial, deep (i.e., blood must not directly contact cerebral cortex) and with minimal or no intraventricular extension. The Intracerebral Hemorrhage (CH) score must be 0-2 and is calculated based on age, Galsgow coma Scale score ad the initial CT or MRI findings for the index stroke. See the SOM for the full calculation procedure.
- Have a total NIHSS score of 3-21.
- Have an upper and/or lower limb deficit defined as:
 - Score of 1-3 on the NIHSS Motor Arm question, and palpable and observable voluntary extension or flexion of the fingers. AND/OR b. Score of 1-3 on the NIHSS Motor Leg question
- Aged 18-90, inclusive.
- Male subjects and females of non-child-bearing potential are allowed to participate in this study.
- Females of child-bearing potential are also allowed to participate in this study provided they are using a contraceptive method with a failure rate of <1%.

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Exclusion Criteria:

- History of a previous symptomatic stroke within 3 months prior to study entry.
- Presence of significant disability prior to the current stroke. Significant disability is defined as having a pre-stroke Rankin score of >2.
- Presence of depression that is active and not adequately controlled such that it interfered with major activities of daily living immediately prior to the current stroke.
- Subjects who are not alert or are unresponsive as defined by a score of 2 or 3 on the NIHSS Level of Consciousness question (question #1a).
- Presence of significant aphasia as likely to confound or interfere with completion of the study assessments.
- Presence of peripheral neuropathy, including diabetic neuropathy, which is clinically active and symptomatic at time of screening.
- Presence of neurological or psychiatric disease, such as dementia or mild cognitive impairment, prior to study entry that is likely to confound clinical evaluations.
- Presence of a demyelinating disease, such as multiple sclerosis.
- Evidence of other chronic co-morbid conditions or unstable acute systemic illnesses which, in the opinion of the investigator, could shorten the subject's survival or limit his/her ability to complete the study.
- History of sensitivity to heparin or heparin-induced thrombocytopenia.
- Presence of QTcB > 500 msec; or uncorrected QT >600msec (machine or manual over-read) on baseline ECG.
- Contraindication to TMS, such as:
 - have metal present, such as hardware or plate on the scalp in the area to which TMS will be applied, implanted cardiac pacemaker, implanted prosthetic heart valve, medication pump or line, metallic implant or clip in the head/neck, electrical, mechanical or magnetic implants, neuro-stimulation device, or orthodontic work involving ferromagnetic materials
 - occupation or activity that may cause accidental lodging of ferromagnetic materials or embedded metal fragments in the head. Subjects can be cleared by a head computed tomography scan.
 - concomitant use of drugs that substantially lower seizure threshold (e.g., tricyclic antidepressants and neuroleptics)
 - known history of seizures or epilepsy
 - brain tumor, recent brain injury (within 5 years) associated with definite loss of consciousness, or any history of brain surgery
- Contraindication to MRI, such as:
 - have metal present, such as implanted cardiac pacemaker, implanted prosthetic heart valve, medication pump or line, metallic implant or clip in the head/neck, electrical, mechanical or magnetic implants, neuro-stimulation device, or orthodontic work involving ferromagnetic materials, permanent tattooed metallic eye-liner
 - occupation or activity that may cause accidental lodging of ferromagnetic materials or embedded metal fragments in the head. Subjects can be cleared by a head computed tomography scan.
 - claustrophobia
- Participation in any investigational rehabilitation paradigm targeting stroke recovery during the duration of this study.
- The subject has participated in a clinical trial and has received an investigational product within the following time period prior to the first dosing day in the current study: 30 days, 5 half-lives or twice the duration of the biological effect of the investigational product (whichever is longer).

Pregnant or lactating females.

- Subjects considered unwilling or unable to comply with the procedures and study visit schedule outlined in the protocol.

► Contacts and Locations

Please refer to this study by its ClinicalTrials.gov identifier: NCT00833989

Contacts

Contact: US GSK Clinical Trials Call Center 877-379-3718

Locations

United States, California

GSK Investigational Site **Not yet recruiting**
Los Angeles, California, United States, 90095

GSK Investigational Site **Recruiting**
Orange, California, United States, 92868-4280

United States, Colorado

GSK Investigational Site **Recruiting**
Fort Collins, Colorado, United States, 80524

United States, Michigan

GSK Investigational Site **Not yet recruiting**
Detroit, Michigan, United States, 48201

United States, Oregon

GSK Investigational Site **Recruiting**
Portland, Oregon, United States, 97239

United States, West Virginia

GSK Investigational Site **Suspended**
Morgantown, West Virginia, United States, 26506-9180

Canada, Ontario

GSK Investigational Site **Not yet recruiting**
Toronto, Ontario, Canada, M4N 3M5

Canada, Quebec

GSK Investigational Site **Not yet recruiting**
Montreal, Quebec, Canada, H3T 1E2

Germany, Baden-Wuerttemberg

GSK Investigational Site **Recruiting**
Ulm, Baden-Wuerttemberg, Germany, 89081

Germany, Niedersachsen

GSK Investigational Site **Not yet recruiting**
Hannover, Niedersachsen, Germany, 30625

Germany, Nordrhein-Westfalen

GSK Investigational Site **Not yet recruiting**
Essen, Nordrhein-Westfalen, Germany, 45122

Germany, Sachsen

GSK Investigational Site **Recruiting**
Leipzig, Sachsen, Germany, 04103

Sponsors and Collaborators

GlaxoSmithKline

Investigators

Study Director: GSK Clinical Trials GlaxoSmithKline

► More Information

No publications provided

Responsible Party: GSK (Study Director)

Study ID Numbers: 111539

Study First Received: January 29, 2009

Last Updated: March 31, 2010
ClinicalTrials.gov Identifier: [NCT00833989](#) [History of Changes](#)
Health Authority: United States: Food and Drug Administration

Additional relevant MeSH terms:

Cerebral Infarction
Nervous System Diseases
Stroke
Vascular Diseases
Brain Ischemia

Central Nervous System Diseases
Cardiovascular Diseases
Brain Infarction
Brain Diseases
Cerebrovascular Disorders

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**SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIRECCIÓN DE NUEVAS CREACIONES**

Radicación N° 05-8403

Clasificación internacional (IPC): A61K 39/395

Concepto Técnico N° 1992

Patente de Invención	☒	Patente de Modelo de Utilidad	☐
Vía Nacional	☐		
Vía PCT (fase nacional)	☒	Cap. I	☐
		Cap. II	☒
No. Sol. Internacional	PCT/EP2003/008749		
Fecha de Presentación Internal.	05/08/2003		

Como resultado del examen definitivo de fondo, realizado con fundamento en el artículo 48 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica:

1. Documentos estudiados

<u>Descripción:</u>	Folios: 22 a 104 como se radicó inicialmente.
<u>Reivindicaciones:</u>	Folios: 105 a 114 como se radicó inicialmente. Folios: 163 a 166 modificadas el 28/05/2008 Folios: 177 a 180 modificadas el 30/04/2010
<u>Figuras:</u>	Folios: 115 a 123 como se radicó inicialmente.
<u>Respuesta del solicitante:</u>	Folios: 146 a 161 radicada el 28/05/2008 Folios: 172 a 176 radicada el 30/04/2010
<u>Prioridad:</u>	06/08/2002, Gran Bretaña, GB0218230.1 06/08/2002, Gran Bretaña, GB0218232.7 06/08/2002, Gran Bretaña, GB0218234.3 06/08/2002, Gran Bretaña, GB0218229.3

2. Objeto de la invención

Título de la solicitud: "Anticuerpos de glicoproteína asociada con anti-mielina (MAG)".

El objeto de la presente solicitud de patente de invención consiste en anticuerpos alterados para glicoproteína asociada con Mielina (MAG) y formulaciones farmacéuticas que los contienen para tratamiento y/o profilaxis de enfermedades / trastornos neurológicos.

El problema planteado en esta solicitud es la necesidad de métodos novedosos conducentes a la disminución de la muerte celular que se presenta luego del cese de flujo sanguíneo en los eventos de derrame cerebral.

