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DIVISION DE NUEVAS CREACIONES
Superintendencia de Industria y Comercio
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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 02-008005-00006-0000

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Evento : 378

Actuación : 444

Referencia : Expediente No. **02.008.005**
Asunto : Solicitud de patente para: **MOLÉCULAS DE ANTICUERPO QUE TIENEN ESPECIFICIDAD POR EL FACTOR DE NECROSIS TUMORAL ALFA HUMANO, Y USO DE LAS MISMAS**
Solicitante : CELLTECH R&D LIMITED
Fecha presentación : 1 de febrero de 2002

1. Yo, Emilio FERRERO, obrando como apoderado del solicitante en el asunto antes identificado, me refiero al Oficio No. **8514** notificado por fijación en lista del 13 de julio de 2007.

2. Mediante el Oficio No. **8514** se puso en conocimiento del solicitante el informe que contiene las observaciones hechas por su Despacho sobre la presente solicitud de patente.

Atiendo a las observaciones de la manera siguiente:

2.1 CLARIDAD DE LA SOLICITUD Y REIVINDICACIONES DE USO.

Para subsanar las objeciones que sobre claridad hizo su Despacho, presento nuevo pliego reivindicatorio con 59 reivindicaciones en el cual se han hecho cambios que permiten apreciar claramente el objeto reivindicado en la presente solicitud de patente, es decir, anticuerpos derivados de un nuevo anticuerpo monoclonal producido por el solicitante (hTNF40), el cual está dirigido contra el TNF α humano. Así, las reivindicaciones se refieren a anticuerpos que comprenden uno o más de los CDR de este anticuerpo monoclonal de murino. Las modificaciones al capítulo reivindicatorio fueron las siguientes:

- El nuevo pliego reivindicatorio define los anticuerpos de acuerdo con el resto de sus secuencias de región variable, las composiciones que comprenden estos anticuerpos, las secuencias de ADN y los vectores que codifican los anticuerpos, evitando la multiplicidad de reivindicaciones independientes.

- Se dejaron solamente 5 reivindicaciones independientes y se eliminaron reivindicaciones que podrían repetir materia revelada anteriormente en otras reivindicaciones.

- Como se anotó anteriormente, las reivindicaciones están unificadas por los CDR que se obtienen del nuevo anticuerpo monoclonal de murino hTNF40. Sin embargo, aunque el solicitante considera que las reivindicaciones 1 y 2 están unificadas y perfectamente claras, estas reivindicaciones se han eliminado con el fin de evitar confusión. La reivindicación previa 3 es ahora la reivindicación principal.

A este respecto, respetuosamente ponemos de presente que la nueva reivindicación 1 se relaciona ahora con una molécula de anticuerpo que comprende por lo menos un CDR de cadena pesada del hTNF40 y por lo menos un CDR de cadena ligera del hTNF40. La persona versada en el arte consideraría importante que fuera posible hacer un anticuerpo contra el hTNF α al utilizar solamente un CDR de cadena pesada del hTNF40, dos CDR de cadena pesada de otro anticuerpo anti hTNF α y un CDR de cadena ligera del hTNF40 y dos CDR de cadena ligera de otro anticuerpo anti hTNF α . Lo cual esta protegido por el alcance de las reivindicaciones y no representa una ampliación en la materia inicialmente reivindicada.

- La nueva reivindicación 2 se relaciona con un anticuerpo que tiene todos los 6 CDR del hTNF40 (aunque se da la opción para el CDRH2 a ser un CDR modificado).

- Los compuestos de las reivindicaciones 36 a 41 se refieren ahora al compuesto de la reivindicación 24 y por lo tanto, hacen refieren a los anticuerpos de la invención.

- Las reivindicaciones 61 a 64 que reclamaba el uso de los anticuerpos han sido eliminadas de éste pliego reivindicatorio.

- Con respecto a las SEQ ID NOs: 11, 113 y 115, respetuosamente ponemos de presente que la manifestación del señor examinador a este respecto no es completamente cierta, toda vez que la SEQ ID NO: 11 se relaciona con la secuencia Vh injertadas del hTNF40.4 como se encuentra en la Figura 11. La Figura 11 de la publicación PCT es idéntica a la Figura 11 del documento de prioridad. Por lo tanto, contrario a la aseveración del examinador, esta secuencia aparece revelada en la solicitud de la cual se reivindica prioridad en la presente solicitud de patente. De otro lado y a pesar de que las SEQ ID NOs: 113 y 115 fueron nuevas secuencias agregadas en la solicitud PCT, las mismas no habían sido reveladas por el estado del arte y en consecuencia, su patentabilidad no se encuentra afectada. La SEQ ID NO:113 se relaciona con la cadena L injertada para el Fab y el Fab modificado, mientras que la SEQ ID NO:115 se relaciona con la cadena H injertada para el Fab modificado.

Finalmente, respetuosamente ponemos de presente al Despacho que si bien comprendemos que el mismo es autónomo en cuanto al estudio de patentabilidad se refiere, el pliego reivindicatorio de la presente invención ha sido objeto de patente en países como Gran Bretaña (GB2366800) y Estados Unidos (US7012135 y US7186820), entre otros.

De esta manera es claro que con las anteriores modificaciones del capítulo reivindicatorio quedan subsanadas las objeciones que por claridad y por incluir reivindicaciones de uso hizo su Despacho, ya que ahora, dicho pliego reivindica un producto y un procedimiento en los términos sugeridos por su Despacho y permite determinar el alcance de la protección reivindicada y compararla frente al estado del arte, de acuerdo con las disposiciones de los artículos 14 y 30 de la Decisión 486.

2.2 EVALUACIÓN DE LA UNIDAD DE INVENCION

Su Despacho considera que la solicitud carece de unidad de invención, ya que afirma que hace referencia a anticuerpos caracterizados mediante estructuras o secuencias diferentes.

A este respecto, respetuosamente ponemos de presente que las nuevas reivindicaciones poseen un único concepto inventivo, toda vez que las mismas se refieren a los anticuerpos que comprenden los CDR del hTNF40 y a los polipéptidos que comprenden las secuencias CDR específicas.

En vista de la relación con el hTNF40, el cual es en sí mismo un anticuerpo novedoso e inventivo, se considera que teniendo en cuenta las modificaciones realizadas al pliego reivindicatorio, este ahora cumple con el requisito de unidad de invención ya que involucra un único concepto inventivo, por lo tanto queda subsanada la objeción que sobre unidad de invención hizo ese Despacho.

2.3 EXCEPCIONES DE PATENTABILIDAD

Ese Despacho considera que las reivindicaciones 61 a 64 de la presente invención se encuentran incursas en la excepción de patentabilidad del Art. 20, literal (d) de la Decisión 486. Con el fin de subsanar dicha objeción las mismas han sido eliminadas del pliego reivindicatorio.

2.4 NOVEDAD Y NIVEL INVENTIVO

Aunque en la Acción Oficial No. **8514** no se efectuaron objeciones sobre la falta de novedad o de nivel inventivo de la presente invención, el presente asunto reviste especial importancia para mi representada y por lo tanto con el fin de agilizar su trámite a continuación se presentan los argumentos necesarios que permiten comprobar la novedad y el nivel inventivo de la presente solicitud frente a los documentos WO98/25971, WO92/11383, WO99/64460 y EP0380068, que fueron citados como posibles anterioridades en el reporte de búsqueda internacional.

El documento WO98/25971 describe la producción de fragmentos de anticuerpo monovalente que están ligados a moléculas de polímero, lo cual es muy diferente de las moléculas de anticuerpo completas y de los fragmentos que se describen en la presente solicitud de patente, toda vez que las moléculas de anticuerpo y los fragmentos de la presente solicitud se definen mediante secuencias específicas que no habían sido descritas previamente.

Por su parte la materia revelada en WO99/64460 es similar a aquella de WO98/25971, excepto que este documento se refiere a los fragmentos de anticuerpo divalente unidos a un polímero. Estos fragmentos consisten de dos cadenas pesadas y una molécula de polímero, mientras que la presente invención revela tanto las moléculas de anticuerpo completas como los fragmentos. Adicionalmente, estos anticuerpos y los fragmentos se definen por secuencias específicas que no han sido previamente descritas. Por lo tanto, las secuencias especificadas objeto de la presente solicitud no habrían podido ser descritas por la anterioridad en cuestión.

EP-A-0380068 se refiere a un sistema de expresión para producir anticuerpos quiméricos. Aunque el documento dice que es posible producir anticuerpos anti-TNF α utilizando este sistema, no hay ninguna descripción de las secuencias de ningunos anticuerpos anti-TNF α y no existe ciertamente ninguna descripción de las secuencias CDR citadas en la reivindicación 1 de la presente solicitud.

Las secuencias CDR específicas mencionadas en las reivindicaciones de la solicitud presente no habían sido nunca reveladas con anterioridad al público y por lo tanto las reivindicaciones de la presente solicitud deben considerarse como novedosas a la luz de las anterioridades citadas.

Aunque es cierto que el término "hTNF40" se puede encontrar en WO98/25971 (ver página 13) y en WO99/64460 (ver página 26), no existe descripción en estos documentos sobre cuál hTNF40 se está hablando, diferente de la mención sobre que es un anticuerpo trabajado por ingeniería que reconoce el TNF α humano. La única mención referente al proceso de producción del hTNF40 se encuentra en la página 13, líneas 16-19 de WO98/25971, donde se menciona que el hTNF40 se hizo utilizando métodos "similares a aquellos encontrados en WO92/01059". Teniendo en cuenta esta referencia tan general, no se puede concluir bajo ninguna circunstancia que esta descripción destruya la novedad del hTNF40 o de los CDR reivindicados en la presente solicitud de patente. Si bien el nombre hTNF40 pudo haber sido revelado al público, ni el anticuerpo ni de esta manera las secuencias específicas lo estaban.

En conclusión, aunque la técnica anterior previera la producción de un anticuerpo contra TNF α (de hecho la técnica anterior que se relaciona con los anticuerpos, en teoría, permitiría la producción de un anticuerpo contra casi cualquier molécula), ninguno de éstos documentos describe los anticuerpos específicos citados en la solicitud o su proceso de producción. Es por lo tanto claro que como el anticuerpo hTNF40 no era conocido, las reivindicaciones son novedosas.

Por otra parte, el problema resuelto por la solicitud de mi representada es el suministro de un anticuerpo humanizado específico para el TNF α humano y el suministro de tal anticuerpo con una vida media larga y baja inmunogenicidad.

El documento WO92/11383 describe métodos para hacer anticuerpos humanizados que son específicos para TNF α humano y podrían por lo tanto ser considerados como el estado

del arte más cercano. Sin embargo, en ninguna parte este documento describe los CDR específicos reivindicados en la solicitud, o discute cómo se pueden producir los mismos.

Este documento enseña una jerarquía de residuos que debe ser cambiada del aceptador al donador con el fin de lograr la mejor afinidad del anticuerpo. Los residuos de estructura que se deben cambiar son dados tanto para la cadena pesada como ligera. Cuando se consideran las reivindicaciones 8-11, se debe tener en cuenta que en ninguna parte de WO92/11383 aparece la combinación de los residuos cambiados del aceptador al donador tal y como se menciona en la presente solicitud.

Existen por lo tanto un número de deficiencias en WO92/11383 que fueron solucionadas por la invención de mí representada en ausencia de cualquier enseñanza del estado del arte sobre el hTNF40 o su forma detallada de producción.

De la misma forma, cuando se consideran los meritos inventivos de la presente invención se debe tener en cuenta el hecho de que estos no son cambios arbitrarios que se hagan para crear estos anticuerpos, que si hubiera una fórmula específica que pudiera ser seguida para producir anticuerpos con una probabilidad razonable de éxito cada vez, entonces el campo de la producción de anticuerpo estaría mucho más avanzado de lo que esta, y habría mucho más amplio rango de anticuerpos disponibles que serían adecuados para uso en el tratamiento de enfermedades.

Sin embargo, este no es el caso, toda vez que es claro que WO92/11383 se publicó en julio de 1992 dando una jerarquía de residuos para ser alterada proveniente del aceptador al donador con el fin de producir anticuerpos con buena afinidad, mientras que la presente solicitud se presentó en junio de 2001 (casi 9 años más tarde) y diferentes residuos fueron alterados del aceptador al donador. Esto demuestra que no había métodos obvios disponibles para las personas expertas que posibilitaran la producción de estos anticuerpos.

Es así como el problema a ser resuelto por la presente invención es hacer anticuerpos alternativos específicos para $TNF\alpha$. Sin embargo, no podría haber una expectativa razonable de que tales anticuerpos, con tan buena afinidad, pudieran haber sido obtenidos, y ciertamente ninguna expectativa de que los anticuerpos específicamente mencionados en la solicitud tuvieran tan buena afinidad. Es importante resaltar que los CDR comprenden un número diferente de amino ácidos. El CDR más corto es el CDRH1 (de acuerdo a Kabat) el cual es de 5 amino ácidos en longitud. Esto significa que este CDR podría tener 20^5 combinaciones posibles de amino ácidos naturales. Así, no puede ser razonable esperar que una persona experta en la técnica pruebe todas las posibles combinaciones con el objeto de encontrar un CDR, dejar solo un anticuerpo completo, que tenga una mejor afinidad para $TNF\alpha$ aunque sea de baja inmunogenicidad.

Los CDR escogidos en la presente solicitud fueron cuidadosamente investigados, diseñados y producidos con el fin de crear un anticuerpo específico para $TNF\alpha$ que tuviera una alta afinidad. Se puede notar en la página 10, líneas 26-29 que los inventores han tenido éxito en su objetivo en razón a que el hTNF40 tiene una afinidad de aproximadamente 0.85×10^{-10} y el anticuerpo humanizado injertado con CDR de la

invención tiene una afinidad de aproximadamente 0.5×10^{-10} . Los CDR (y los residuos de estructura cambiados del aceptador al donador) claramente no representan una simple selección arbitraria de los residuos.

Los anticuerpos de la presente invención son particularmente efectivos y muestran baja inmunogenicidad como se evidencia no solamente en los ejemplos de la solicitud, sino también en los resultados publicados del ensayo clínico Fase III para el CDP870.

A este respecto, respetuosamente solicitamos al Señor examinador referirse a los siguientes documentos, de los cuales adjuntamos copia para su más fácil conocimiento.

Schreiber et al. (2005) *Gastroenterology*, 129:807-818 discute los ensayos clínicos Fase II exitosos del CDP870 en la Enfermedad de Crohn y también confirma el nexo entre el nombre aprobado "certolizumab pegol" y el código de propietario interno "CDP870", que se emplea en la solicitud (ver por ejemplo, página 10, línea 6 y página 41, línea 8 de la solicitud).

Schreiber et al. (2005) *Gut*, 54 (Suppl VII), A82 es un reporte preliminar de los ensayos clínicos Fase III exitosos del CDP870 en la Enfermedad de Crohn (el estudio "PRECISE"). Los ensayos clínicos Fase III están en este momento en proceso para artritis reumatoide y los ensayos clínicos para otras indicaciones también están en proceso.

Choy et al. que relaciona los ensayos clínicos fase II para tratamiento con CDP870 contra artritis reumatoide. Este documento lleva a cabo comparaciones contra dos productos que bloquean el hTNF α en el mercado, infliximab (un anticuerpo monoclonal anti-hTNF α) y Etanercept (una construcción de inmunoglobulina G1 humana de receptor TNF soluble en p75 humana recombinante).

Este documento concluye (ver discusión en la página 1137) que el CDP870 suministra una mejora clínica más adecuada en pacientes que el etanercept o infliximab (75% comparado con el 60% o el 50%) aunque manteniendo una vida media larga y una respuesta antigénica baja. La mejora clínica resulta de la afinidad del anticuerpo que es un resultado directo de las secuencias de CDR.

La vida media larga de los anticuerpos de la invención se confirma en la solicitud en la página 41, línea 10.

La baja inmunogenicidad de los anticuerpos de la invención se establece en la solicitud en la página 46, línea 2 ("CDP870 generando solo muy bajos niveles de respuesta de anticuerpo"). También se nota en Schreiber et al. (*Gastroenterology* – ver página 816, último párrafo completo) que "el número de pacientes positivos al anticuerpo en el grupo de 400 mg de certolizumab era bajo".

Así, no es posible objetar la presente invención con base en los documentos citados en el informe de búsqueda por cuanto el anticuerpo monoclonal de murino hTNF40 no había sido revelado al público antes de la fecha de prioridad. Por lo tanto cualquier argumento

basado en esta presunción no puede tener éxito pues no se deriva de las enseñanzas, expresas o tácitas, del estado del arte, tal y como lo exige el método problema- solución.

Adicionalmente, se ha demostrado que los anticuerpos de la invención tienen una vida media larga, tienen una alta afinidad, muestran baja inmunogenicidad y son particularmente útiles en terapia, por lo cual los mismos representan un avance en la ciencia y de esta forma gozan de nivel inventivo.

3. Por lo tanto, le solicito se sirva continuar con el trámite de la presente solicitud pues tanto la descripción como las reivindicaciones se presentan de una forma clara y en consecuencia, con fundamento en las mismas una persona versada en la materia podría realizar el examen de patentabilidad del producto objeto de esta invención. A pesar que las objeciones que sobre claridad planteó ese Despacho en el oficio No. **8514** se deben tener por superadas, en el evento que el Despacho considere que sea necesario, respetuosamente le solicito hacer uso de la facultad que le otorga el artículo 45 inciso 2. de la Decisión 486 de la Comisión de la Comunidad Andina a efecto de requerir nuevamente de nuestra parte cualquier complementación o aclaración que considere necesaria.

4. Puesto que se han atendido a cabalidad las observaciones hechas por su Despacho sobre la presente solicitud de patente, atentamente solicito que se continúe su tramitación.

5. **Anexos:**

- 5.1. Nuevo pliego reivindicatorio con 59 reivindicaciones;
- 5.2. Formulario para modificaciones;
- 5.3. Recibo por valor de \$99.000 por concepto de modificaciones en solicitud pendiente;
- 5.4. Schreiber et al. (2005) Gastroenterology, 129:807-818;
- 5.5. Choy et al. (2002) Rheumatology, 41:1133-1137;
- 5.6. Schreiber et al. (2005) Gut, 54 (Suppl VII), A82.

Señora Jefe,


EMILIO FERRERO
T.P.A. No. 31.929

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No. M-2007-109831\NNJ



Industria y Comercio
SUPERINTENDENCIA

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FORMULARIO UNICO DE CORRECCIONES Y MODIFICACIONES

1

SOLICITUD DE:
☐ Corrección de Error Material ☒ Modificación a una solicitud

2

SOLICITANTE

Nombre: CELLTECH R&D LIMITED
Sociedad constituida y existente bajo las leyes de Reino Unido
Dirección: 208 Bath Road,
Slough,
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E-mail: clientes@camyp.com

IDENTIFICACION
C.C. ☐ **NIT** ☐
C.E. ☐ **Otro** ☐
Número: _____

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REPRESENTANTE O APODERADO

Nombre: Emilio FERRERO
Dirección: Carrera 4ª No. 72-35
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IDENTIFICACION
C.C. ☒ **NIT** ☐
C.E. ☐ **Otro** ☐
Número: 438.019 TP 31.929

4

Número de expediente: 02.008.005

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Descripción de la corrección o modificación solicitada:
Presento nuevo capítulo reivindicatorio compuesto por 59 reivindicaciones con el fin de subsanar las objeciones hechas por Su Despacho a la presente solicitud de Patente y solicito que el Examen de Patentabilidad sea efectuado sobre estas nuevas reivindicaciones.

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Comprobante de pago No. 07-80,662 **Fecha:** 1 de octubre de 2007

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Anexos
☒ Comprobante de pago de la tasa por \$99.000.00
☐ Poderes, si fuere el caso
☐ Documento que acredita la existencia y representación legal de las partes, cuando sean personas jurídicas.

8

NOMBRE: EMILIO FERRERO **FIRMA:** 

C.C. 438.019 de Usaquén T.P.A. 31.929

NNJ

REIVINDICACIONES

1. Una molécula de anticuerpo que tiene especificidad para TNF α humano, que comprende una cadena pesada en donde el dominio variable comprende un CDR que tiene la secuencia dada en la SEQ ID NO: 1 para CDRH1, la SEQ ID NO:2 o SEQ ID NO:7, para CDRH2 o SEQ ID NO: 3 para CDRH3 y una cadena ligera en donde el dominio variable comprende un CDR que tiene la secuencia dada en la SEQ ID NO:4 para CDRL1, la SEQ ID NO:5 para CDRL2 o la SEQ ID NO:6 para CDRL3.
2. La molécula de anticuerpo de la reivindicación 1, que comprende la SEQ ID NO:1 para CDRH1, la SEQ ID NO:2 o la SEQ ID NO:7 para CDRH2, la SEQ ID NO:3 para CDRH3, la SEQ ID NO:4 para CDRL1, la SEQ ID NO:5 para CDRL2 y la SEQ ID NO:6 para CDRL3.
3. La molécula de anticuerpo de la reivindicación 1 o reivindicación 2, que comprende la SEQ ID NO:2 para CDRH2.
4. La molécula de anticuerpo de una cualquiera de las reivindicaciones 1 a 3, que es una molécula de anticuerpo injertada con CDR.
5. La molécula de anticuerpo de la reivindicación 4, en donde el dominio variable comprende regiones de estructura aceptadoras humanas y los CDR de donadores no humanos.
6. La molécula de anticuerpo de la reivindicación 5, en donde las regiones de estructura aceptadoras humanas del dominio variable de la cadena pesada están basadas en una secuencia de consenso del grupo humano 1 y comprende residuos de donador no humanos en las posiciones 28, 69 y 71.
7. La molécula de anticuerpo de la reivindicación 5, en donde las regiones de estructura aceptadoras humanas del dominio variable de la cadena pesada están basadas en una secuencia de consenso del grupo humano 1 y comprende residuos de donador no humanos en las posiciones 28, 38, 46, 67, 69 y 71.
8. La molécula de anticuerpo de la reivindicación 5, en donde las regiones de estructura aceptadoras humanas del dominio variable de la cadena pesada están basadas en una secuencia de consenso del grupo humano 3 y comprende residuos de donador no humanos en las posiciones 27, 28, 30, 48, 49, 69, 71 73, 76 y 78.

9. La molécula de anticuerpo de una cualquiera de las reivindicaciones 5 a 8, en donde las regiones de estructura aceptadoras humanas del dominio variable de la cadena ligera están basadas en una secuencia de consenso del grupo humano 1 y comprende los residuos de donador no humanos en las posiciones 46 y 60.

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10. La molécula de anticuerpo de una cualquiera de las reivindicaciones 1 a 9, que comprende la región variable de cadena ligera hTNF40-gL1 (SEQ ID NO:8) y la región variable de cadena pesada gh3hTNF40.4 (SEQ ID NO:11).

10 11. La molécula de anticuerpo de una cualquiera de las reivindicaciones 1 a 10 la cual es un fragmento Fab.

12. La molécula de anticuerpo de las reivindicaciones 10 y 11, que es un fragmento Fab que comprende una cadena pesada que tiene la secuencia dada en la SEQ ID NO:111 y una cadena ligera que tiene la secuencia dada en la SEQ ID NO:113.

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13. La molécula de anticuerpo de una cualquiera de las reivindicaciones 1 a 10, que es un fragmento Fab modificado que tiene en el extremo C-terminal de su cadena pesada uno o más amino ácidos para permitir la unión de un efector o una molécula indicadora.

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14. La molécula de anticuerpo de la reivindicación 13, en donde los amino ácidos adicionales forman una región de pivoteo modificada que contiene uno o dos residuos de cisteína a los cuales se puede unir la molécula efectora o indicadora.

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15. La molécula de anticuerpo de la reivindicación 10, que es un fragmento Fab modificado que comprende una cadena pesada que tiene la secuencia dada en la SEQ ID NO:115 y una cadena ligera que tiene la secuencia dada en la SEQ ID NO:113.

30 16. Una molécula de anticuerpo de acuerdo a la reivindicación 1, que tiene especificidad para TNF α humano, que tienen una cadena ligera que comprende la secuencia dada en la SEQ ID NO:113.

35 17. Una molécula de anticuerpo de acuerdo a la reivindicación 1, que tiene especificidad para TNF α humano, que tienen una cadena ligera que consiste de la secuencia dada en la SEQ ID NO:113.

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18. Una molécula de anticuerpo de acuerdo a la reivindicación 1, que tiene especificidad para TNF α humano, que tienen una cadena pesada que comprende la secuencia dada en la SEQ ID NO:115.
- 5 19. Una molécula de anticuerpo de acuerdo a la reivindicación 1, que tiene especificidad para TNF α humano, que tienen una cadena pesada que consiste de la secuencia dada en la SEQ ID NO:115.
- 10 20. Una molécula de anticuerpo de acuerdo a la reivindicación 1, que tiene especificidad para TNF α humano, que tienen una cadena ligera que comprende la secuencia dada en la SEQ ID NO:113 y una cadena pesada que comprende la secuencia dada en la SEQ ID NO:115.
- 15 21. Una molécula de anticuerpo de acuerdo a la reivindicación 1, que tiene especificidad para TNF α humano, que tienen una cadena ligera que consiste de la secuencia dada en la SEQ ID NO:113 y una cadena pesada que consiste de la secuencia dada en la SEQ ID NO:115.
- 20 22. El anticuerpo de la reivindicación 1 que es un anticuerpo monoclonal anti-TNF α de murino hTNF40.
- 25 23. La molécula de anticuerpo de la reivindicación 1, que es una molécula de anticuerpo quimérica que comprende los dominios variables de cadena ligera y pesada del anticuerpo monoclonal de la reivindicación 22.
- 30 24. Un compuesto que comprende la molécula de anticuerpo de una cualquiera de las reivindicaciones 13 a 21 que tiene covalentemente unido a un amino ácido en o hacia el extremo C-terminal de su cadena pesada una molécula efectuada o indicadora.
- 25 25. El compuesto de la reivindicación 24, que comprende una molécula efectuada.
26. El compuesto de la reivindicación 25, en donde la molécula efectuada comprende uno o más polímeros.
- 35 27. El compuesto de la reivindicación 26, en donde el uno o más polímeros es/son un polialquileno de cadena recta o ramificada opcionalmente sustituido, un polímero de polialquilenilo o polioalquilenilo o un polisacárido ramificado o no ramificado.

28. El compuesto de la reivindicación 27, en donde el uno o más polímeros es/son un metoxipoli(etilenoglicol).

29. Una compuesto que comprende la molécula de anticuerpo de la reivindicación 15 que tiene unido a uno de los residuos de cisteína en el extremo C-terminal de la cadena pesada un grupo lisil-maleimida en donde cada grupo amino del residuo lisil está covalentemente ligado a éste un residuo metoxipoli(etilenoglicol) tiene un peso molecular de aproximadamente 20,000 Da.

30. Un compuesto de acuerdo a la reivindicación 24 que comprende una molécula de anticuerpo que tiene especificidad por TNF α humano, que tiene una cadena ligera que comprende la secuencia dada en la SEQ ID NO:113 y una cadena pesada que comprende la secuencia dada en la SEQ ID NO:115, que tiene unido a uno de los residuos de cisteína en el extremo C-terminal de la cadena pesada uno o más polímeros de ocurrencia sintética o natural.

31. Un compuesto de acuerdo a la reivindicación 24 que comprende una molécula de anticuerpo que tiene especificidad por TNF α humano, que tiene una cadena ligera que consiste de la secuencia dada en la SEQ ID NO:113 y una cadena pesada que consiste de la secuencia dada en la SEQ ID NO:115, que tiene unido a uno de los residuos de cisteína en el extremo C-terminal de la cadena pesada uno o más polímeros de ocurrencia sintética o natural.

32. Un compuesto de acuerdo a la reivindicación 24 que comprende una molécula de anticuerpo que tiene especificidad por TNF α humano, que tiene una cadena ligera que comprende la secuencia dada en la SEQ ID NO:113, que tiene unido a uno de los residuos de cisteína en el extremo C-terminal de la cadena pesada un grupo lisil-maleimida en donde cada grupo amino del residuo lisil tiene covalentemente ligado a éste un residuo metoxipoli(etilenoglicol) que tiene un peso molecular de aproximadamente 20,000 Da.

33. Un compuesto de acuerdo a la reivindicación 24 que comprende una molécula de anticuerpo que tiene especificidad por TNF α humano, que tiene una cadena ligera que consiste de la secuencia dada en la SEQ ID NO:113, que tiene unido a uno de los residuos de cisteína en el extremo C-terminal de la cadena pesada un grupo lisil-maleimida en donde cada grupo amino del residuo lisil tiene covalentemente ligado a éste un residuo metoxipoli(etilenoglicol) que tiene un peso molecular de aproximadamente 20,000 Da.

34. Un compuesto de acuerdo a la reivindicación 24 que comprende una molécula de anticuerpo que tiene especificidad por TNF α humano, que tiene una cadena pesada que comprende la secuencia dada en la SEQ ID NO:115, que tiene unido a uno de los residuos de cisteína en el extremo C-terminal de la cadena pesada un grupo lisil-maleimida en donde cada grupo amino del residuo lisil tiene covalentemente ligado a éste un residuo metoxipoli(etilenoglicol) que tiene un peso molecular de aproximadamente 20,000 Da.

35. Un compuesto de acuerdo a la reivindicación 24 que comprende una molécula de anticuerpo que tiene especificidad por TNF α humano, que tiene una cadena pesada que consiste de la secuencia dada en la SEQ ID NO:115, que tiene unido a uno de los residuos de cisteína en el extremo C-terminal de la cadena pesada un grupo lisil-maleimida en donde cada grupo amino del residuo lisil tiene covalentemente ligado a éste un residuo metoxipoli(etilenoglicol) que tiene un peso molecular de aproximadamente 20,000 Da.

36. Un compuesto de acuerdo a la reivindicación 24 que comprende una molécula de anticuerpo que tiene especificidad por TNF α humano, que tiene una cadena ligera que comprende la secuencia dada en la SEQ ID NO:113 y una cadena pesada que comprende la secuencia dada en la SEQ ID NO:115, que tiene unido a uno de los residuos de cisteína en el extremo C-terminal de la cadena pesada un grupo lisil-maleimida en donde cada grupo amino del residuo lisil tiene covalentemente ligado a éste un residuo metoxipoli(etilenoglicol) que tiene un peso molecular de aproximadamente 20,000 Da.

37. Un compuesto de acuerdo a la reivindicación 24 que comprende una molécula de anticuerpo que tiene especificidad por TNF α humano, que tiene una cadena ligera que consiste de la secuencia dada en la SEQ ID NO:113 y una cadena pesada que consiste de la secuencia dada en la SEQ ID NO:115, que tiene unido a uno de los residuos de cisteína en el extremo C-terminal de la cadena pesada un grupo lisil-maleimida en donde cada grupo amino del residuo lisil tiene covalentemente ligado a éste un residuo metoxipoli(etilenoglicol) que tiene un peso molecular de aproximadamente 20,000 Da.

38. Una molécula de anticuerpo de acuerdo a una cualquiera de las reivindicaciones 1-23, que comprende un CDR híbrido que comprende una secuencia CDR donadora truncada en donde la porción faltante del CDR donador se reemplaza por una secuencia diferente y forma un CDR funcional.

39. La molécula de anticuerpo de la reivindicación 38, en donde la parte faltante de la secuencia de CDR es del anticuerpo del cual las regiones de estructura de la molécula de anticuerpo se derivan.

40. La molécula de anticuerpo de la reivindicación 39, en donde la parte faltante de la secuencia de CDR se deriva de un anticuerpo de línea germinal que tiene regiones de estructura de consenso.

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41. La molécula de anticuerpo de una cualquiera de las reivindicaciones 38 a 40, en donde el CDRH2 de la cadena pesada es híbrido en la molécula de anticuerpo.

42. La molécula de anticuerpo de una cualquiera de las reivindicaciones 38 a 41, en donde el truncamiento del CDR donador es de 1 a 8 amino ácidos.

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43. La molécula de anticuerpo de la reivindicación 42, en donde el truncamiento es de 4 a 6 amino ácidos.

44. La molécula de anticuerpo de una cualquiera de las reivindicaciones 38 a 43, en donde el truncamiento se hace en el terminal C del CDR.

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45. Una secuencia de ADN que codifica la cadena pesada y/o ligera de la molécula de anticuerpo de una cualquiera de las reivindicaciones 1 a 23 y 38 a 44.

20

46. La secuencia de ADN de la reivindicación 45 que comprende la secuencia mostrada en la SEQ ID NO:8 o 10.

47. La secuencia de ADN de la reivindicación 45 que comprende la secuencia mostrada en la SEQ ID NO:10 o 11.

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48. La secuencia de ADN de la reivindicación 45 que comprende la secuencia mostrada en la SEQ ID NO:110, 112 o 114.

49. Un vector de clonación o de expresión que contiene una secuencia de ADN de una cualquiera de las reivindicaciones 45 a 48.

30

50. Un vector de expresión de *E. coli* que comprende la secuencia de ADN de una cualquiera de las reivindicaciones 44 a 48.

35

51. El vector de expresión de *E. coli* de la reivindicación 50 que es pTTO(CDP870).

52. Una célula huésped transformada con el vector de una cualquiera de las reivindicaciones 49 a 51.

53. Un proceso para la producción de la molécula de anticuerpo de una cualquiera de las reivindicaciones 1 a 23 y 38 a 44, que comprende cultivar la célula huésped de la reivindicación 52 y aislar la molécula de anticuerpo.

54. Un proceso para la producción de la molécula de anticuerpo de una cualquiera de las reivindicaciones 1 a 23 y 38 a 44, que comprende cultivar *E. coli* que comprende un vector de expresión de *E. coli* que comprende la secuencia de ADN de una cualquiera de las reivindicaciones 49 a 51 y aislar la molécula de anticuerpo.

55. El proceso de la reivindicación 54 en donde la molécula de anticuerpo es apuntada al periplasma.

56. Una composición terapéutica o de diagnóstico que comprende la molécula de anticuerpo de una cualquiera de las reivindicaciones 1 a 23 y 38 a 44 o el compuesto de una cualquiera de las reivindicaciones 24 a 37.

57. El vector pADNbEng-G1.

58. El vector pTTO(CDP870).

59. Un polipéptido que tiene la secuencia de amino ácido dada en una cualquiera de la SEQ ID NO:1 a 7.

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OP-G-355 CERTOLIZUMAB PEGOL, A HUMANISED ANTI-TNF PEGYLATED FAB[®] FRAGMENT, IS SAFE AND EFFECTIVE IN THE MAINTENANCE OF RESPONSE AND REMISSION FOLLOWING INDUCTION IN ACTIVE CROHN'S DISEASE: A PHASE III STUDY (PRECISE)

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INTRODUCTION: The efficacy and tolerability of sc certolizumab pegol (certolizumab) 400 mg 4-weekly in the maintenance of clinical response following induction therapy were assessed in patients with moderate to severe Crohn's disease (CD). **AIMS & METHODS:** Adult patients (n=668) with active CD (CDAI 220-450) who responded (decrease in baseline CDAI score ≥ 100 points) at Week (Wk) 6 to open induction therapy with certolizumab 400 mg by sc injection at Wk 0, 2 and 4 were randomised to maintenance therapy with certolizumab 400 mg or placebo every 4 wks up to Wk 24. Patients were stratified according to baseline serum C-reactive protein (CRP; <10 mg/L or ≥ 10 mg/L) and by corticosteroid and immunosuppressant use. Primary endpoint was the percentage of patients at Wk 26 in the CRP ≥ 10 mg/L stratum who maintained a clinical response after successful induction. Major secondary endpoints included remission (CDAI ≤ 150) in the CRP ≥ 10 mg/L stratum, and response and remission in the overall ITT population.

RESULTS: 428 (64%) of these 668 patients (mean age 37.7 [18-69 yrs]; 53% female) responded to open label induction and were randomised (216 certolizumab, 212 placebo). At Wk 26, the clinical response rate in the CRP ≥ 10 mg/L stratum (112 certolizumab, 101 placebo) was 61.6% for certolizumab vs 33.7% for placebo ($p < 0.001$). The proportion of patients who achieved remission in the CRP ≥ 10 mg/L stratum was 42.0% for certolizumab vs 25.7% for placebo ($p < 0.01$). The clinical response rate at Wk 26 in the overall ITT population was 62.8% for certolizumab vs 36.2% for placebo ($p < 0.001$). Overall ITT population remission rates were 47.9% for certolizumab vs 28.6% for placebo ($p < 0.001$). Patients mainly reported mild to moderate adverse events, the most common was headache (12.6% during induction; 6.9% vs 6.6% in the certolizumab and placebo groups, respectively). Serious non-CD-related infections were observed with certolizumab (3 events) and placebo (2 events). One patient (certolizumab) died from a fentanyl overdose.

CONCLUSION: Certolizumab 400 mg so demonstrated significant efficacy in the maintenance of clinical response and remission following induction in moderate to severe CD compared with placebo, independently of baseline CRP levels. No major erosion of efficacy was observed over time. Certolizumab was safe and well tolerated.

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A Randomized, Placebo-Controlled Trial of Certolizumab Pegol (CDP870) for Treatment of Crohn's Disease

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See editorial on page 1114.

Ongoing phase III trials are necessary to establish the clinical efficacy of certolizumab.

Background & Aims: To investigate the efficacy and safety of certolizumab pegol (a polyethylene-glycolated Fab' fragment of anti-tumor necrosis factor, CDP870) in Crohn's disease. **Methods:** In a placebo-controlled, phase II study, 292 patients with moderate to severe Crohn's disease received subcutaneous certolizumab 100, 200, or 400 mg or placebo at weeks 0, 4, and 8. The primary end point was the percentage of patients with a clinical response at week 12 (a Crohn's Disease Activity Index decrease of ≥ 100 points or remission [Crohn's Disease Activity Index ≤ 150 points]) in the intent-to-treat population. **Results:** All certolizumab doses produced significant clinical benefit over placebo at week 2 (placebo, 15.1%; certolizumab 100 mg, 29.7% [$P = .033$]; 200 mg, 30.6% [$P = .026$]; 400 mg, 33.3% [$P = .010$]). At all time points, the clinical response rates were highest for certolizumab 400 mg, greatest at week 10 (certolizumab 400 mg, 52.8%; placebo, 30.1%; $P = .006$) but not significant at week 12 (certolizumab 400 mg, 44.4%; placebo, 35.6%; $P = .278$). Patients with baseline C-reactive protein levels of 10 mg/L or greater ($n = 119$) showed clearer separation between active treatment and placebo (week 12 clinical response: certolizumab 400 mg, 53.1%; placebo, 17.9%; $P = .005$; post hoc analysis) owing to a lower placebo response rate than patients with C-reactive protein levels of less than 10 mg/L. Adverse events were similar among groups. **Conclusions:** Certolizumab 400 mg may be effective and is well tolerated in patients with active Crohn's disease. High placebo response rates in the large patient subgroup with low C-reactive protein levels may have obscured statistical separation between certolizumab and placebo.

The proinflammatory cytokine tumor necrosis factor (TNF) is a key mediator of the inflammation associated with Crohn's disease.¹⁻⁶ It can be detected at high concentrations in diseased areas of the bowel wall,^{2,4,6} and in the blood and feces of patients with the disease.^{1,3}

Several biologic products targeted toward the neutralization of TNF have shown clinical efficacy in patients with Crohn's disease.⁷⁻¹² Infliximab is the only anti-TNF agent currently approved for use in the management of the disease.^{13,14} Infliximab is administered by intravenous infusion and has proven efficacy in the treatment of both refractory luminal and fistulizing Crohn's disease.^{8,12,15,16} The use of infliximab has been associated with a number of potentially serious adverse events (AEs), in addition to the development of human antichimeric antibodies, which can lead to infusion reactions and may reduce the duration of response.¹⁷⁻²⁰

Certolizumab pegol (CDP870) hereafter referred to as certolizumab is a polyethylene glycolated Fab' fragment of a humanized anti-TNF- α monoclonal antibody intended for subcutaneous administration. Subcutaneous delivery of the drug has cost and convenience advantages compared with intravenous dosing. Certolizumab has been constructed by grafting the short hypervariable complementarity-determining regions derived from the murine monoclonal antibody HTNF40 onto an other-

Abbreviations used in this paper: AE, adverse event; CI, confidence interval; CRP, C-reactive protein; IBDQ, Inflammatory Bowel Disease Questionnaire; ITT, intent to treat; TNF, tumor necrosis factor.

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wisc virtually human Ig Fab' fragment (IgG γ 1 κ). The engineered Fab' fragment retains the biologic potency of the original antibody. The Fab' fragment is linked via a maleimide to 2 cross-linked chains of polyethylene glycol that each have a molecular weight of 20 kilodaltons. This site-specific polyethylene glycolation increases the half-life of the antibody fragment to approximately 2 weeks in plasma, thereby reducing the frequency of required dosing. In phase II studies in patients with rheumatoid arthritis, certolizumab has been shown to be clinically effective and well tolerated.^{21,22} We have assessed the efficacy and safety of subcutaneous administration of certolizumab in a phase II, randomized, double-blind, placebo-controlled, dose-response study in patients with moderate to severe Crohn's disease

Materials and Methods

Patient Selection

Eligible patients were at least 18 years old with a clinical diagnosis of Crohn's disease as confirmed by radiologic, endoscopic, or histologic evidence following established diagnostic criteria. Patients had moderate to severe disease, defined by a score of 220–450 points on the Crohn's Disease Activity Index (CDAI)²³ over a 7-day screening period before the first study dose was administered.

Patients were excluded from the study if they had a suspected or diagnosed abscess at screening, a bowel perforation or evidence of noninflammatory obstruction during the 6 months before screening, extensive bowel resection, a functional colostomy or ileostomy, a positive stool culture for enteric pathogens, or a known history of tuberculosis. Other exclusion criteria included treatment for Crohn's disease with sodium cromoglycate, mycophenolate, or cyclosporin within 4 weeks of study entry, or receipt of other anti-TNF therapy with a biologic agent within 12 weeks of screening. Patients were also excluded from the study if they had been treated previously with any anti-TNF agent and either had experienced an infusion reaction that was suspected or confirmed to be associated with an immune response, or had showed a lack of clinical response to the first dose. Any patients who had participated in another clinical trial with certolizumab were ineligible to take part in the study, as were those who had been involved in any other clinical drug trial within the 4 weeks before screening.

All patients had an anteroposterior chest radiograph for tuberculosis at screening (or within the 12 weeks before screening) and at the final study visit.

Concomitant Medication

Concomitant medication was permitted if the patient was on a stable dose that could be continued throughout the 12-week duration of the double-blind phase of the study. The minimum stable treatment periods required before screening were as follows: 2 weeks for steroids (≤ 9 mg/day budesonide, ≤ 24 mg/day methylprednisolone, or ≤ 30 mg/day prednisone or prednisolone) and

any topical anorectal treatment; 4 weeks for long-term anti-infectives and mesalamine or mesalamine analogs (eg, sulfasalazine, olsalazine, or balsalazide); and 8 weeks for the immunosuppressants azathioprine, 6-mercaptopurine, and methotrexate.

Study Medication

Because certolizumab and placebo did not have the same color or viscosity, full blinding was not possible. Consequently, patients received their treatment from a nurse or physician who was not involved in the study. All other staff involved in the study remained blind to treatment. Certolizumab 100 mg, 200 mg, 400 mg, or placebo was administered subcutaneously. Certolizumab was supplied as a clear, colorless to pale yellow solution at a nominal concentration of 200 mg/mL in 50 mmol/L sodium acetate buffer and 125 mmol/L sodium chloride solution. Saline was used for dilution and for placebo injections. Each patient received 2 subcutaneous injections (1 mL each) at separate injection sites in the lateral abdominal wall or the outer upper thigh. Patients remained at the study site for 30 minutes after drug administration and any AEs were reported. Patients received the first dose at week 0 and further doses of the same medication at weeks 4 and 8.

Study Design

This was a phase II, multicenter, randomized, double-blind, placebo-controlled, parallel-group, dose-response study, recruiting patients from tertiary referral centers. The study was designed to assess the efficacy and safety of subcutaneous administration of certolizumab. It was conducted between February 15, 2001, and March 12, 2002, at 58 centers in a total of 10 countries (the number of centers in each country is shown in parentheses): Belgium (4), Canada (16), Denmark (6), Germany (8), Ireland (3), Russia (5), Serbia (5), South Africa (2), Sweden (4), and the United Kingdom (3). The 3 active treatment groups (certolizumab 100, 200, or 400 mg) were compared with placebo.

At screening, patients were stratified into 1 of 2 groups according to whether or not they were receiving concomitant steroids, immunosuppressants, or long-term anti-infectives. They then were randomized to 1 of the 4 treatment groups (1:1:1:1 certolizumab 100 mg:certolizumab 200 mg:certolizumab 400 mg:placebo). The randomization code was prepared by an independent statistician and patients were assigned to treatment by the use of a randomization allocation schedule managed via an interactive voice response system. Efficacy assessments were performed every 2 weeks up until week 12, with a further 8-week follow-up evaluation for safety.

Before dosing, demographic data were recorded for each patient, together with any significant past medical history and all concomitant diseases. Disease activity (by using the CDAI) was assessed prospectively in the week before screening, after 0, 2, 4, 6, 8, 10, and 12 weeks of treatment, and on withdrawal from the study (if appropriate). Patients kept a daily diary of their symptoms throughout the study. At weeks 0, 2, 4, 6, 8, 10, 12, and study end point, the patients' quality of life was

assessed using the Inflammatory Bowel Disease Questionnaire (IBDQ).²⁴ Our own translations were used in Russian, Serbian, and Afrikaans.

Standard laboratory tests, including hematology, biochemistry, and urinalysis, together with measurement of serum C-reactive protein (CRP) levels, were performed at each visit up until week 20 or on withdrawal from the study. Investigators were blinded to the results of the CRP analyses. Certolizumab plasma concentrations were measured using an enzyme-linked immunosorbent assay with a lower limit of sensitivity of .11 µg/mL.

AEs were monitored throughout the study. The duration and intensity of each event were recorded by the investigator, together with its putative relationship to the study drug, and its outcome and seriousness. Any patient requiring a change in baseline therapy or hospitalization to manage an exacerbation of the disease was considered to be a treatment failure and was withdrawn from the study.

Written informed consent to participate in the trial was obtained from each patient and the independent ethics committee/institutional review board at each study center approved the protocol before commencement. The trial was performed in accordance with the International Conference on Harmonization Guidelines for Good Clinical Practice, which have their origins in the Declaration of Helsinki.

Outcomes and Primary Statistical Analysis

A sample size of 260 patients was considered adequate for this study, with 65 patients being allocated to each treatment group. This would give approximately 83% power to detect a true difference of 23% between treatment groups for the primary end point (clinical response) based on a placebo response rate of 12%. The efficacy was analyzed for the intent-to-treat (ITT) population. This included all patients recruited who received at least 1 injection and had at least 1 efficacy measurement after the first injection. Patients who terminated the trial prematurely were advanced to the end-of-study visit.

A clinical response was defined as a decrease in CDAI score of 100 points or greater or remission (CAI score, ≤150 points). The primary efficacy end point was the percentage of patients achieving a clinical response at week 12. The percentage of patients who showed a clinical response at weeks 2, 4, 6, 8, and 10 was a secondary efficacy variable, as was the percentage of patients in remission at weeks 2, 4, 6, 8, 10, and 12. Statistical analyses of these outcomes were performed using the χ^2 test to compare among the treatment groups and 95% confidence intervals (CI) were calculated.

Mean CDAI scores and mean IBDQ scores, summed over all categories, were summarized at weeks 0, 2, 4, 6, 8, 10, and 12. Serum CRP concentrations (geometric means) at all time points were analyzed using descriptive statistics. (The geometric mean is the *n*th root of the product of *n* values.)

All treatment comparisons for efficacy were made using a 2-sided significance level of .05. A closed testing procedure²⁵ was used for the analysis of the primary end point. Results from patients receiving certolizumab 400 mg were compared

with placebo. If the comparison was not significant at the 5% level, no further analysis was performed. If significance was apparent, comparisons of certolizumab 200 mg vs placebo and certolizumab 100 mg vs placebo were performed simultaneously. For the analysis of secondary end points, there was no adjustment for multiple efficacy end points. Additional subgroup analyses (including effect of stratum) were for exploratory purposes only and therefore no adjustments for multiplicity were made.

The safety analysis (safety population) included all patients who received at least 1 dose of study medication and subsequently had a postbaseline safety evaluation. Actual values and changes from baseline relating to vital signs and laboratory data were analyzed using descriptive statistics.

Post Hoc Analyses

In an exploratory analysis, changes from baseline in IBDQ total score for each treatment group were compared with the placebo group by using a comparison of least-squares means. Least-squares means were adjusted for stratum, and pooled country and baseline IBDQ scores.

In addition, patients were stratified according to baseline CRP concentrations of less than 10 mg/L or 10 mg/L or greater in an exploratory analysis. The percentage of patients with a clinical response and the percentage of patients in remission in the certolizumab- and placebo-treated groups were analyzed according to baseline concentrations of CRP. A further series of analyses was also performed using breakpoint baseline CRP concentrations of 5, 6, 7, 8, and 9 mg/L. The χ^2 test was used to compare treatment groups.

Results

Patients

The trial profile for the participating patients is shown in Figure 1. A total of 292 patients were enrolled in the study. A total of 291 patients were included in the ITT population and received either certolizumab 100 mg (*n* = 74), 200 mg (*n* = 72), or 400 mg (*n* = 72), or placebo (*n* = 73). One patient who received certolizumab 400 mg did not provide any postinjection efficacy measurements and therefore was excluded from the ITT population before breaking the blind. The safety population comprised all 292 enrolled patients. The demographic characteristics of the patients at screening are shown in Table 1. The majority of the patients (96.6%) were Caucasian. Patient characteristics were generally well balanced among treatment groups, although the mean age of patients receiving certolizumab 200 mg (40.1 y) was slightly higher than in the other groups, in which the mean ages of patients ranged from 33.5 to 35.9 years. The greatest difference among the groups was a small preponderance of women in the placebo group (67.1%) in comparison with both the certolizumab 100



mg (52.7%) and 400 mg (55.6%) groups. The concomitant medications with which the patients were being treated were broadly similar in both the placebo and the certolizumab treatment groups (Table 2). The most notable difference was observed for overall glucocorticoid use: 39.7% for the placebo group vs 30.6% for the certolizumab 400-mg group. In the ITT population, 15.5% of the patients had concomitant medication with

both immunomodulators and steroids, and 21.6% of the patients had received prior anti-TNF therapy with a biologic agent.

Eighteen patients (24.7%) in the placebo group withdrew from the study by week 12. The corresponding numbers for the certolizumab 100-, 200-, and 400-mg treatment groups were 22 (29.7%), 15 (20.8%), and 20 (27.4%) patients, respectively. In all groups, the major-

Table 1. Baseline Demographic Characteristics of Patients (ITT Population)

Variable	Placebo (n = 73)	Certolizumab		
		100 mg (n = 74)	200 mg (n = 72)	400 mg (n = 72)
Mean age, y (range)	35.8 (19-64)	33.5 (18-56)	40.1 (19-71)	35.9 (18-67)
Sex, n (% of patients)				
Male	24 (32.9)	35 (47.3)	22 (30.6)	32 (44.4)
Female	49 (67.1)	39 (52.7)	50 (69.4)	40 (55.6)
Mean body mass index, kg/m ² , (range)	23.03 (16.3-38.6)	23.06 (15.0-37.3)	24.14 (16.2-40.7)	22.73 (13.5-38.1)
Mean duration of disease, y, (range)	7.95 (1-27.6)	7.73 (0.0-31.8)	8.84 (0.0-30.7)	8.43 (1.2-26.5)
Previous anti-TNF therapy, n (% of patients)	16 (21.9)	18 (24.3)	17 (23.6)	12 (16.7)
CRP concentrations				
Geometric mean, mg/L (range)	7.3 (1.3-86.1)	6.2 (1.2-141.0)*	6.5 (1.2-127.0)	7.7 (1.4-128.2)
≥10 mg/L, n (% of patients)	28 (38.4)	31 (42.5)*	28 (38.9)	32 (44.4)
Disease site, n (% of patients)				
Duodenum	1 (1.4)	5 (6.8)	2 (2.8)	3 (4.2)
Ileum	54 (74.0)	57 (77.0)	61 (70.8)	56 (77.8)
Cecum	37 (50.7)	45 (60.8)	32 (44.4)	41 (56.9)
Ascending colon	31 (42.5)	35 (47.3)	35 (48.6)	36 (50.0)
Transverse colon	33 (45.2)	32 (43.2)	33 (45.8)	37 (51.4)
Descending colon	42 (57.5)	39 (52.7)	40 (55.6)	47 (65.3)
Rectum	36 (49.3)	36 (48.6)	33 (45.8)	32 (44.4)
Perianal	23 (31.6)	20 (27.0)	23 (31.9)	21 (29.2)
Other	5 (6.8)	9 (12.2)	4 (5.6)	7 (9.7)
Resection performed, n (% of patients)	27 (37.0)	27 (36.5)	25 (34.7)	27 (37.5)

*Data missing for 1 patient.

Table 2. Number (%) of Patients Receiving Concomitant Medication (ITT Population)

Medication type	Placebo (n = 73)	Certolizumab		
		100 mg (n = 74)	200 mg (n = 72)	400 mg (n = 72)
Aminosalicylates	29 (39.7)	37 (50.0)	32 (44.4)	28 (38.9)
Antidiarrheals	10 (13.7)	19 (25.7)	16 (22.2)	12 (16.7)
Anti-infectives	7 (9.6)	6 (8.1)	7 (9.7)	6 (8.3)
Metronidazole	6 (8.2)	4 (5.4)	4 (5.6)	5 (6.9)
Ciprofloxacin	5 (6.8)	4 (5.4)	5 (6.9)	3 (4.2)
Codeine and derivatives	6 (8.2)	5 (6.8)	5 (6.9)	2 (2.8)
Immunomodulators	26 (35.6)	26 (35.1)	29 (40.3)	27 (37.5)
Azathioprine	17 (23.3)	13 (17.6)	23 (31.9)	22 (30.6)
6-mercaptopurine	4 (5.5)	9 (12.2)	2 (2.8)	2 (2.8)
Methotrexate	5 (6.8)	4 (5.4)	4 (5.6)	3 (4.2)
Glucocorticoids (overall)	29 (39.7)	24 (32.4)	29 (40.3)	22 (30.6)
Systemic glucocorticoids	20 (27.4)	15 (20.3)	22 (30.6)*	16 (22.2)
Budesonide	9 (12.3)	9 (12.2)	6 (11.1)*	6 (8.3)

*One patient received both systemic glucocorticoids and budesonide.

ity of withdrawals were a consequence of disease progression.

Efficacy

Primary end point: clinical response and dose-ranging results. The percentage of patients reaching the primary end point (a decrease in CDAI score of ≥ 100 points or remission at week 12) in the ITT population is shown in Figure 2. An onset of effect was evident at 2 weeks postinjection, when all doses of certolizumab produced a statistically significant benefit compared with placebo (week 2: placebo, 15.1% [95% CI, 6.2–24.0] vs certolizumab 100 mg, 29.7% [95% CI, 18.6–40.8; $P = .033$]; 200 mg, 30.6% [95% CI, 19.2–41.9; $P = .026$]; 400 mg, 33.3% [95% CI, 21.8–44.9; $P = .010$]). Certolizumab also showed significantly higher efficacy compared with placebo at weeks 4 (400 and 200 mg), 8 (400 and 100 mg), and 10 (400 mg). Patients receiving certolizumab 400 mg showed the highest response rate at all time points, with the highest response observed at week 10 (52.8% [95% CI, 40.6–65.0] vs 30.1% [95% CI, 18.9–41.3] in the placebo group [$P = .006$]). At all time points, the rate of response among patients receiving certolizumab was higher than in those receiving placebo. However, at week 12 (the primary end point) the difference between the certolizumab- and placebo-treated groups did not reach statistical significance (week 12: certolizumab 400, 200, and 100 mg 44.4% [95% CI, 32.3–56.6], 36.1% [95% CI, 24.3–47.9], and 36.5% [95% CI, 24.8–48.1], respectively, vs placebo 35.6% [95% CI, 23.9–47.3]). There was no effect of sex or stratum (steroid, immunosuppressant, and anti-inflammatory use) on the rate of clinical response at week 12 (data not shown).

Remission. At week 4, all certolizumab treatment groups showed statistically significant higher per-

centages of patients achieving remission (CAI score, ≤ 150 points) compared with placebo (Figure 3). Significant benefit over placebo treatment was also seen at week 8 for both the certolizumab 400-mg and 100-mg treatment groups. At week 12, the difference between the certolizumab- and placebo-treated groups did not reach statistical significance (week 12 remission rates: certolizumab 400, 200, and 100 mg 26.4% [95% CI, 15.5–37.3], 19.4% [95% CI, 9.6–29.3], and 27.0% [95% CI, 16.2–37.8], respectively, vs placebo 23.3% [95% CI, 12.9–33.7]).

Biologic response. CRP concentrations (geometric means) in the 4 treatment groups are shown in Figure 4. At week 2, CRP concentrations were reduced in all active treatment groups. At this time point, the 95% CIs for the mean CRP concentrations in the placebo group did not overlap with those of the certolizumab 100-mg or 400-mg treatment groups, giving an indication of a significant difference between these groups and placebo. The data support the anti-inflammatory effect of certoli-

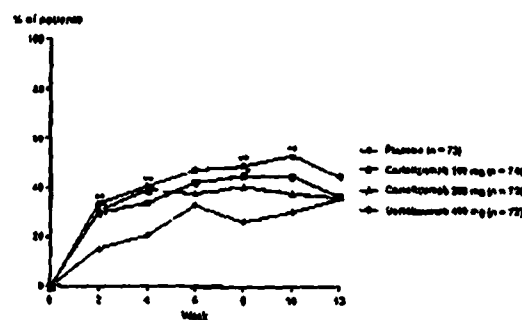


Figure 2. The percentage of patients with a decrease in CDAI score of 100 points or greater or who achieved remission (CAI score, ≤ 150 points) (ITT population—primary end point). * $P \leq .05$, ** $P \leq .01$ compared with placebo.

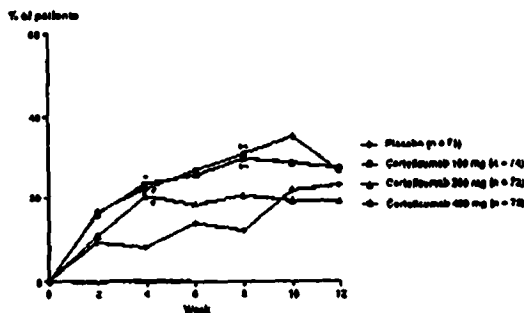


Figure 3. The percentage of patients achieving remission (CDAI score, ≤ 150 points) (ITT population). * $P \leq .05$, ** $P \leq .01$ compared with placebo.

zumab. The lowest geometric mean concentration of CRP was measured at week 2 in patients being treated with certolizumab 400 mg (2.8 mg/L). The corresponding value for the placebo group at this time point was 7.1 mg/L. The results over the 12-week study period suggest that treatment with certolizumab 200 mg and 400 mg may be more effective at maintaining a biologic response than treatment with certolizumab 100 mg. A statistically significant relationship between the extent of reduction in CRP from weeks 0 to 2 and clinical outcome at week 12 in the ITT population was not detected.

Antibodies. In this patient population with various concomitant medications (only 37.5% of the patients from the certolizumab 400-mg group were on concomitant immunomodulators at baseline), we found that 12.3% ($n = 9$ of 73) of the patients receiving certolizumab 400 mg had at least 1 positive result for anticertolizumab antibodies (period, 0–12 weeks). The plasma concentrations of certolizumab appeared to be lower in the antibody-positive patients (data not shown), however, no decrease in efficacy was seen—the proportion of responder patients at week 12 was similar in antibody-positive and antibody-negative patients, with 44% of patients responding in both subgroups.

IBDQ. Baseline IBDQ total scores (mean $\pm$ SD) were 122.9 ± 26.60 , 132.2 ± 30.60 , 122.9 ± 27.07 , and 126.5 ± 25.20 for the placebo and the 100-mg, 200-mg, and 400-mg certolizumab groups, respectively. For all treatment groups, there was an increase in IBDQ total scores within 2 weeks of treatment that paralleled the changes in CDAI scores. The largest change from baseline at week 2 (22.8 points) was observed in the certolizumab 400-mg group, compared with 10.6 points for patients receiving placebo ($P = .002$). From weeks 2 to 12, patients receiving 400 mg certolizumab had greater changes from baseline at any time point than patients receiving placebo ($P < .05$). In addition, pa-

tients receiving 200 mg certolizumab had significantly greater changes from baseline at weeks 2 and 4 than those receiving placebo ($P < .05$). At week 12, IBDQ total scores (mean $\pm$ SD) were 156.4 ± 37.36 in the certolizumab 400-mg group compared with 140.5 ± 35.88 in the placebo group.

Post hoc analyses according to baseline CRP concentration. Baseline CRP measurements. Baseline CRP measurements were obtained from 290 of the 291 patients in the ITT population. Of these patients, 119 (41.0%) had a baseline serum CRP concentration of 10 mg/L or greater and 171 (59.0%) had serum levels less than 10 mg/L.

Efficacy of certolizumab according to a CRP breakpoint of 10 mg/L. In the initial post hoc analysis, in patients with baseline CRP levels of 10 mg/L or greater, treatment with certolizumab 400 mg resulted in a statistically significant benefit at all time points throughout the 12-week study in terms of both rates of clinical response (a decrease in CDAI score of ≥ 100 points or remission [CDAI score, ≤ 150 points]) (Figure 5A) and remission (CDAI score, ≤ 150 points) (Figure 5B). There was clearer separation between the certolizumab treatment groups and the placebo group in this subanalysis than in the overall ITT population.

In patients with baseline CRP levels less than 10 mg/L, none of the active treatments achieved a statistical difference from placebo for the primary end point of clinical response at week 12. A high placebo response was observed in these patients. At week 12, 46.7% of the patients who had received placebo showed a clinical response, compared with 38.1%, 38.6%, and 37.5% for the certolizumab 100-, 200-, and 400-mg groups, respectively. The percentages of certolizumab-treated patients achieving remission were not significantly different from placebo and, as with the primary variable, relatively high rates of placebo response (up to 31.1% at week 12) were evident.

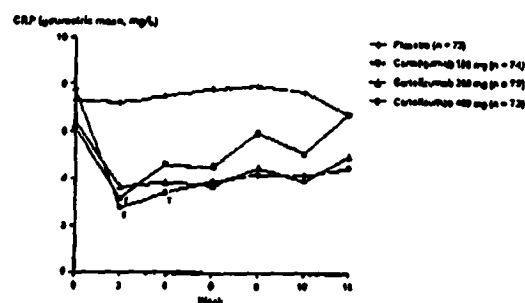


Figure 4. Mean concentrations of CRP (geometric means) (ITT population). *Nonoverlapping 95% CIs compared with placebo.

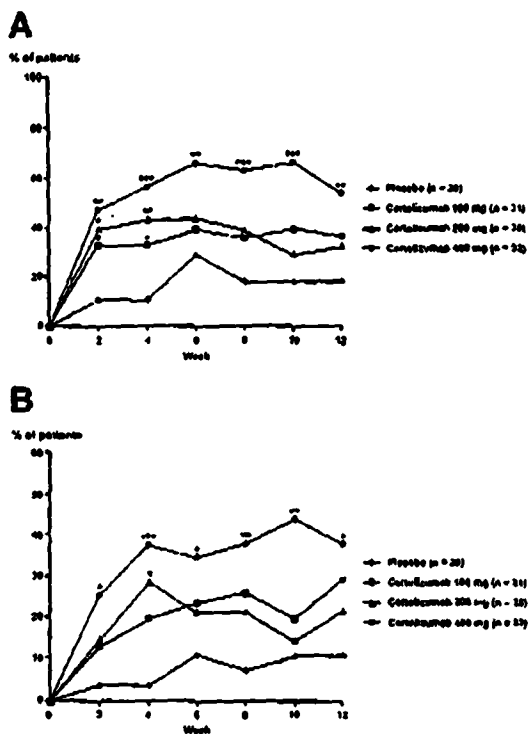


Figure 6. The percentage of patients with baseline concentrations of CRP of 10 mg/L or greater who (A) showed a decrease in CDAI score of 100 or more points or achieved remission (CDAI score, ≤ 150 points), or (B) achieved remission. * $P \leq .05$, ** $P \leq .01$, *** $P \leq .001$ compared with placebo.

Efficacy of certolizumab in patients with baseline CRP breakpoint concentrations between ≥ 5 and ≥ 10 mg/L. Further post hoc analyses, showing the primary end point of clinical response at week 12 for patients with baseline CRP breakpoint concentrations between ≥ 5 and ≥ 10 mg/L, are shown in Figure 6. For each of the CRP breakpoints analyzed, clinical response rates for patients in the certolizumab 400-mg treatment group were higher than those in the other treatment groups at this time point (50.0%, 50.0%, 51.4%, 51.4%, 51.5%, and 53.1% for certolizumab 400-mg treatment at baseline CRP concentrations of ≥ 5 mg/L, ≥ 6 mg/L, ≥ 7 mg/L, ≥ 8 mg/L, ≥ 9 mg/L, and ≥ 10 mg/L, respectively). In patients with baseline CRP levels of ≥ 7 –10 mg/L who received certolizumab 400 mg, the primary clinical response rates at week 12 showed statistically significant differences from placebo. Placebo response rates decreased with increasing baseline CRP concentration (34.9%, 31.6%, 28.6%, 25.0%, 20.0%, and 17.9% for baseline CRP concentrations of ≥ 5 mg/L, ≥ 6 mg/L, ≥ 7 mg/L, ≥ 8 mg/L, ≥ 9 mg/L, and ≥ 10 mg/L, respectively).

Safety

The incidence and pattern of AEs were similar in the active treatment and placebo groups (Table 3). The majority of AEs were of mild or moderate intensity. The most frequently reported events among patients treated with certolizumab during the double-blind treatment period were headache not otherwise specified (13.2% vs 16.4% for placebo), aggravation of Crohn's disease (11.9% vs 13.7% for placebo), nausea (11.4% vs 5.5% for placebo), nasopharyngitis (9.1% vs 4.1% for placebo), dizziness (excluding vertigo) (6.4% vs 4.1% for placebo), arthralgia (5.9% vs 2.7% for placebo), abdominal pain not otherwise specified (5.9% vs 5.5% for placebo), pharyngolaryngeal pain (5.0% vs 5.5% for placebo), and pyrexia (5.0% vs 4.1% for placebo). None of these AEs occurred with an incidence of 5% or more in certolizumab-treated patients during the safety follow-up period. The most common serious AE during both the double-blind and safety follow-up periods was aggravated Crohn's disease (23 reports). This was considered possibly related to the study drug in 4 instances. There were no deaths during the study; no cases of lymphoma, lupus, tuberculosis, or increases in opportunistic infections were recorded. During the double-blind period, 17 patients in the placebo group (23.3%) and 58 certolizumab-treated patients (26.5%) experienced AEs of infections. Over the safety follow-up period, 10 patients in the placebo group (13.7%) and 31 patients in the certolizumab group (14.2%) experienced AEs of infection. Details of AEs representing infectious complications are shown in Table 4.

The following serious adverse events were reported during the double-blind period: 4 placebo patients experienced aggravated Crohn's disease, and genital warts and arthralgia were experienced by 1 patient each. Two patients in the certolizumab 100-mg group experienced

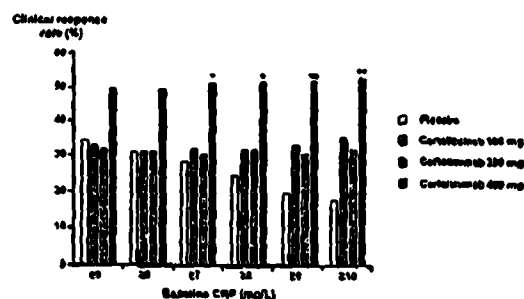


Figure 6. Clinical response rates (decrease in CDAI score ≥ 100 points or remission [CDAI score, ≤ 150 points]) to certolizumab 100, 200, and 400 mg and placebo at week 12 according to mean baseline CRP concentrations. * $P \leq .05$, ** $P \leq .01$ compared with placebo.

Table 3. Number (%) of Patients Experiencing AEs (Safety Population)

	Placebo (n = 73)	Certolizumab		
		100 mg (n = 74)	200 mg (n = 72)	400 mg (n = 73)
Weeks 0-12				
Any AE	51 (69.9)	57 (77.0)	55 (76.4)	48 (65.9)
Serious AE	6 (8.2)	7 (9.5)	10 (13.9)	6 (8.2)
AE leading to withdrawal	7 (9.6)	9 (12.2)	7 (9.7)	7 (9.6)
Relationship to study medication				
Unrelated	42 (57.5)	43 (58.1)	44 (61.1)	37 (50.7)
Possible	34 (46.6)	35 (47.3)	41 (56.9)	27 (37.0)
Probable	3 (4.1)	4 (5.4)	7 (9.7)	3 (4.1)
Definite	1 (1.4)	2 (2.7)	3 (4.2)	0 (0.0)
Weeks 13-20				
Any AE	37 (50.7)	28 (37.8)	31 (43.1)	26 (35.6)
Serious AE	11 (15.1)	1 (1.4)	3 (4.2)	4 (5.5)
AE leading to withdrawal	0	0	0	0
Relationship to study medication				
Unrelated	34 (46.6)	24 (32.4)	25 (34.7)	21 (28.8)
Possible	7 (9.6)	5 (6.8)	9 (12.5)	9 (12.3)
Probable	0	0	0	0
Definite	0	0	0	0

NOTE. Patients experiencing more than 1 AE in any category were counted only once in that category.

aggravated Crohn's disease; another patient had nonspecific abdominal pain and aggravated Crohn's disease. Rectal hemorrhage, abdominal mass, and reduced visual acuity were experienced by 1 patient each in the certolizumab 100-mg group and 1 patient had abdominal pain, pyrexia, vomiting, and paralytic ileus. Five patients in the certolizumab 200-mg group experienced aggravated Crohn's disease, 1 patient had aggravated Crohn's disease and pyrexia, 2 patients had blurred vision, 1 patient had breast hyperplasia, and 1 patient had ankle ulcer, aphthous stomatitis, and thrombocytopenia. Two patients in the certolizumab 400-mg group experienced aggravated Crohn's disease and 1 patient had abdominal pain and aggravated Crohn's disease; 1 patient had abdominal pain, diarrhea, and pyrexia; pyrexia and perianal abscess were experienced by 1 patient each.

The number (%) of patients who experienced injection-site AEs was 2 (2.7%), 5 (6.8%), 4 (5.6%), and 2 (2.7%) in the placebo, 100-mg, 200-mg, and 400-mg certolizumab treatment groups, respectively. All of the injection-site reactions were classified as mild or moderate in intensity, and none resulted in withdrawal from the trial. The most common of the injection-site AEs occurring within 30 minutes of administration of the study medication was injection-site burning—occurring on 3 occasions in the same patient in the placebo group and twice each in patients who received certolizumab 100 and 200 mg. There were 2 reports of injection-site erythema (in 1 certolizumab-treated patient); 1 each of injection-site inflammation, pain, and rash; and 1 other injection-site reaction was not otherwise specified. There

were no incidences of anaphylaxis. Subcutaneous administration of certolizumab and placebo was generally well tolerated, with the majority of patients (78.1%) receiving all 3 injections of study medication.

Certolizumab treatment did not result in any clinically relevant effects on oral temperature, blood pressure, pulse rate, respiration rate, or electrocardiogram recordings. Serial hematologic and biochemical measurements, together with urinalysis, did not show the treatment to have any untoward effects.

Additional analyses of AEs, stratifying these according to a CRP breakpoint of 10 mg/L, did not show any differences among the groups (data not shown).

Discussion

The results of this phase II study suggest that the polyethylene glycolated Fab' fragment of a humanized anti-TNP monoclonal antibody, certolizumab, administered subcutaneously at 4-week intervals is clinically effective and well tolerated in the treatment of moderate to severe Crohn's disease. The onset of treatment effect was rapid, being evident 2 weeks after administration (when all doses produced significant benefit compared with placebo). In this dose-ranging study, certolizumab 400-mg treatment had the greatest efficacy, with patients in this treatment group showing the highest rates of clinical response at all time points. Statistical separation was evident at weeks 2, 4, 8, and 10, indicating the clinical effect of the study drug. Because the differences between the certolizumab 100-mg treatment group and

Table 4. AEs of Infection Occurring in ≥ 2 Patients (Safety Population)

		Certolizumab		
	Placebo (n = 73)	100 mg (n = 74)	200 mg (n = 72)	400 mg (n = 73)
Weeks 0-12				
Nasopharyngitis	3 (3, 4.1)	12 (9, 12.2)	7 (7, 9.7)	5 (4, 5.5)
Influenza	3 (3, 4.1)	4 (4, 5.4)	3 (3, 4.2)	1 (1, 1.4)
Fungal infection NOS	0	2 (2, 2.7)	1 (1, 1.4)	2 (2, 2.7)
Sinusitis NOS/acute	3 (3, 4.1)	2 (2, 2.7)	3 (2, 2.8)	0
Gastroenteritis NOS/ <i>E coli</i> / <i>Campylobacter</i>	0	1 (1, 1.4)	1 (1, 1.4)	1 (1, 1.4)
Bronchitis NOS/acute NOS	2 (2, 2.7)	2 (2, 2.7)	1 (1, 1.4)	0
UTI NOS	5 (5, 6.8)	1 (1, 1.4)	2 (1, 1.4)	0
Pharyngitis NOS	0	0	0	2 (2, 2.7)
Herpes zoster	0	0	1 (1, 1.4)	1 (1, 1.4)
Herpes simplex	1 (1, 1.4)	1 (1, 1.4)	0	1 (1, 1.4)
URTI NOS/Viral NOS	1 (1, 1.4)	1 (1, 1.4)	0	1 (1, 1.4)
Vaginitis fungal NOS	2 (1, 1.4)	0	2 (2, 2.8)	0
Oral candidiasis	1 (1, 1.4)	0	1 (1, 1.4)	0
Abscess NOS	0	2 (2, 2.7)	0	0
Tooth abscess/Infection	1 (1, 1.4)	2 (2, 2.7)	0	0
Weeks 13-20				
Nasopharyngitis	5 (4, 5.5)	4 (4, 5.4)	3 (3, 4.2)	3 (3, 4.1)
Sinusitis NOS	0	1 (1, 1.4)	2 (2, 2.8)	0
Bronchitis NOS	0	1 (1, 1.4)	1 (1, 1.4)	0
Gastrointestinal infection NOS	1 (1, 1.4)	0	0	1 (1, 1.4)
Influenza	2 (2, 2.7)	0	1 (1, 1.4)	0
Gastroenteritis NOS	2 (2, 2.7)	0	0	0

NOTE. AEs are shown as number of events (number of patients, % of patients).

NOS, not otherwise specified; URTI, upper respiratory tract infection; UTI, urinary tract infection.

placebo did not reach statistical significance at week 12 (the primary end point), phase III studies will be necessary to confirm the efficacy of certolizumab and to assess the extent of the difference compared with the placebo population. The clinical effect of certolizumab was corroborated by a secondary analysis of remission rates, together with improvements in patients' quality of life (as measured by the IBDQ) and a reduction in serum concentrations of CRP in the certolizumab groups.

Serum concentrations of CRP, an acute-phase protein, provide an objective criterion of inflammatory activity.²⁶ Patients with stable quiescent IBD are characterized by geometric mean CRP concentrations of approximately 1.5 mg/L.²⁷ CRP is induced by interleukin-6, TNF- α , and other proinflammatory cytokines that are produced within the intestinal lamina propria.⁴ Increased serum concentrations of CRP have been reported as a regular feature of patients with active Crohn's disease in both clinical cohorts^{28,29} and on a population level (M. Varn, personal communication, May 17, 2005). In asymptomatic patients, increased levels of this protein may indicate subsequent clinical relapse, and acute-phase proteins have been included in clinical indices formulated to predict relapse.³⁰

The clinical trial described here included a surprisingly high percentage of patients (59%) with low base-

line concentrations of CRP. In post hoc analyses of the certolizumab data, there was a clearer separation between active treatment and placebo among patients with increased baseline concentrations of CRP (41% of patients) than in the overall ITT population, in terms of both rates of clinical response and rates of remission. Dose separation was also clearly apparent. In the patients with baseline CRP levels of 10 mg/L or greater, subcutaneous dosing with certolizumab 400 mg every 4 weeks resulted in statistically significant rates of clinical response and remission at all time points up to week 12. Statistically significant primary clinical responses were associated with certolizumab 400-mg treatment at week 12 in patients with baseline CRP concentrations of 7 mg/L or greater, 8 mg/L or greater, 9 mg/L or greater, and 10 mg/L or greater. In contrast to the certolizumab 400-mg group, clinical response and remission rates for patients receiving certolizumab 200 mg and 100 mg were not generally significantly different from rates in patients receiving placebo, irrespective of baseline CRP levels. Certolizumab 400 mg therefore is the dose that should be evaluated in phase III studies.

The use of patients' baseline CRP measurements to show clear separation between active treatment and placebo has been reported in recent trials of a number of biologic agents for Crohn's disease. For example, in phase

433

III evaluation of the humanized monoclonal antibody to $\alpha 4$ -integrin, natalizumab, a large placebo effect was apparent in the ITT population and the primary end point of clinical response (a >70 -point reduction in CDAI score) at week 10 was not achieved. However, in a subanalysis of patients with increased baseline CRP levels, the placebo response was managed such that natalizumab treatment showed statistically significant greater rates of clinical response than placebo at week 10 and other time points.³¹

The therapeutic efficacy of infliximab was established in a population of Crohn's disease patients with high mean baseline CRP concentrations—up to 23.2 mg/L in one of the treatment groups.⁸ In the certolizumab clinical trial reported here, although all patients had symptoms indicating moderate to severe Crohn's disease activity (with CDAI scores of ≥ 220 points), only 119 of 290 (41.0%) patients had baseline serum CRP concentrations of 10 mg/L or greater. It is possible that the relatively low proportion of patients with biologic signs of disease activity may be a reflection of the consequent long-term use of immunosuppressive therapeutic agents (including infliximab) at tertiary referral centers, from where patients were recruited for this study. Alternatively, the patient selection may be a reflection of the availability of other therapies outside of this study. Both scenarios would influence a priori the availability and therefore the selection of clinically active patients for therapeutic study protocols.

Recent Crohn's disease clinical trials have often shown relatively high placebo response rates. In a systematic review of 21 randomized trials in Crohn's disease, the pooled estimate of the placebo response rate was 19%, with substantial heterogeneity among different trials.³² Further investigation of placebo response rates in clinical trials in Crohn's disease and, in particular, its relationship with CRP levels is warranted.

In this study of certolizumab, an overall placebo response rate of 35.6% was observed at week 12 in the ITT population. Patients with CRP concentrations less than 10 mg/L showed a particularly high placebo response rate (46.7% for the clinical response at week 12). The placebo response decreased with increasing baseline CRP concentrations. At week 12, the placebo response rate was 34.9% in patients with baseline CRP levels of 5 mg/L or greater and 17.9% for those with CRP levels of 10 mg/L or greater. A relationship between low CRP concentrations and high placebo response rates has also been found in a joint analysis of the primary data from 733 Crohn's disease patients collared from a total of 13 randomized controlled trials.³³ The lack of efficacy of anti-inflammatory drugs in populations of patients with low CRP

levels could be explained either by therapeutic effects being obscured by the placebo response, or by the fact that patients with low CRP levels may have problems that are not necessarily amenable to anti-inflammatory treatment. For example, in our study the week 12 response rates in the high-dose group were greater than 50% in those with CRP levels of 10 mg/L or greater at baseline and only 37.5% in those with CRP levels of less than 10 mg/L at baseline.

In patients with low CRP levels, placebo effects may drive responses in both the active-treatment and control arms of clinical trials. Consequently, the use of baseline CRP measurements may be a useful addition to the CDAI (which contains subjective components and may be susceptible to placebo influence) in the assessment of Crohn's disease patients entering clinical trials. Phase III clinical trials of certolizumab have been designed to address issues relating to high placebo response rates. In clinical practice, CRP levels might provide an objective indication of active inflammation and measurement of CRP levels may help to identify patients for whom anti-inflammatory therapy could be appropriate.

As previously shown in the treatment of patients with rheumatoid arthritis,³¹ subcutaneous administration of certolizumab was well tolerated in this study, with patients experiencing relatively few side effects. There were no deaths during the study, and no cases of malignancy, lupus, tuberculosis, lymphoma, or increases in opportunistic infections were recorded. Interestingly, the rate of AEs of infection was similar in the placebo- and certolizumab-treated patient populations (23.3% and 26.5% during the double-blind period, respectively). Although this finding has to be confirmed in the large phase III studies currently underway, it is possible that the subcutaneous route of drug administration could contribute to the good tolerability because peak serum concentrations are lower than would be achieved after intravenous delivery. Although complement-dependent cytotoxicity-effector mechanisms could potentially affect therapeutic safety, certolizumab is a Fab' fragment and has been designed specifically not to fix complement; this may contribute to its favorable safety profile.

As previously reported with the recombinant proteins infliximab³⁰ and adalimumab,³⁴ an immune response directed against certolizumab was observed in this phase II study. However, the number of antibody-positive patients in the certolizumab 400-mg group was low ($n = 9$ of 73), even in the presence of immunosuppressants in only one third of the population, and larger phase III studies will allow further exploration of this observation.

In conclusion, this phase II study is the first evidence suggesting that certolizumab, administered subcutane-

ously at a dose of 400 mg 4 times weekly, is effective in patients with moderate to severe Crohn's disease and is well tolerated. The study was powered under the assumption of a placebo response rate of 12%; the placebo response rate actually observed was far greater (15.1%–35.6% over the study period). This may have contributed to a lack of statistical difference in clinical response at the week 12 end point. Therefore, these findings need to be confirmed in a phase III study. It may prove beneficial to consider the use of CRP measurements in the future clinical development of certolizumab and other anti-TNF agents: compared with the overall ITT population, patients with increased baseline CRP levels showed the greatest difference between certolizumab treatment and placebo in terms of both rates of clinical response and remission.

Acknowledgments

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436

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Correction

Schreiber S, Rutgeerts P, Fedorak RN, Khalik-Kareemi M, Kamm MA, Bolvin M, Bernstein CN, Staun M, Thomsen O, Innes A, and the CDP870 Crohn's Disease Study Group. A randomized, placebo-controlled trial of certolizumab pegol (CDP870) for treatment of Crohn's disease. *Gastroenterology* 2005;129:807-818.

In the above article, on page 817, the sentence "In conclusion, this phase II study is the first evidence suggesting that certolizumab, administered subcutaneously at a dose of 400 mg 4 times weekly, is effective in patients with moderate to severe Crohn's disease and is well tolerated," should have appeared as "In conclusion, this phase II study is the first evidence suggesting that certolizumab, administered subcutaneously at a dose of 400 mg 4-weekly, is effective in patients with moderate to severe Crohn's disease and is well tolerated." A dose of 400 mg 4-weekly means 400 mg administered at 4-week intervals.

Efficacy of a novel PEGylated humanized anti-TNF fragment (CDP870) in patients with rheumatoid arthritis: a phase II double-blinded, randomized, dose-escalating trial

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Abstract

Objective. Biological products that neutralize tumour necrosis factor α (TNF- α) are beneficial in rheumatoid arthritis (RA). We studied the effects of CDP870, a novel anti-TNF- α antibody fragment modified to obtain a prolonged plasma half-life (~ 14 days).

Methods. Thirty-six patients were randomized in a double-blind, ascending-dose group study to a single intravenous infusion of placebo ($n = 12$) or 1, 5 or 20 mg/kg CDP870 (each $n = 8$). The patients were predominantly female (30/36), had a mean age of 56 yr and a mean duration of RA of 13 years. They had received a mean of five DMARDs or experimental therapies (with 1 month washout before the study started) and had active disease. Continuation of NSAIDs and up to 7.5 mg prednisolone daily was allowed. Following the blinded dosing period, 32 patients received a single open-label infusion of either 5 or 20 mg/kg CDP870.

Results. In the blinded dosing period, 6/12 placebo patients withdrew from the study (for deteriorating RA ≤ 4 weeks after dosing). Two of 24 CDP870-treated patients withdrew, both in the 1 mg/kg group (for deteriorating RA or lost to follow up > 4 weeks after dosing). The proportion of patients with ACR20 improvement for the per-protocol population with the last observation carried forward was 16.7, 50, 87.5 and 62.5% after 0, 1, 5 and 20 mg/kg CDP870 respectively (combined treatment effect, $P = 0.012$, primary analysis) at 4 weeks and 16.7, 25, 75 and 75% ($P = 0.032$) at 8 weeks. The proportion of patients with ACR50 improvement for the per-protocol population with the last observation carried forward was 0, 12.5, 12.5 and 50% after 0, 1, 5 and 20 mg/kg CDP870 respectively (combined treatment effect, $P = 0.079$) at 4 weeks and 0, 12.5, 12.5 and 50% ($P = 0.079$) at 8 weeks. Following the open-label dose of CDP870, similar beneficial effects were achieved.

Conclusion. CDP870 is effective, was very well tolerated in this small study, and has an extended duration of action following one or more intravenous doses.

Key words: Rheumatoid arthritis, Immunotherapy, Biologicals, Monoclonal antibodies, Tumour necrosis factor, Polyethylene glycol.

Tumour necrosis factor α (TNF- α) is a proinflammatory cytokine that plays a major role in the pathogenesis of rheumatoid arthritis (RA) [1]. Mice transgenic for human TNF- α produce high levels of TNF- α constitutively and develop a spontaneous inflammatory, destructive polyarthritis resembling RA [2]. Furthermore, in collagen-induced arthritis, disease can be treated effectively with anti-TNF- α monoclonal antibody

(mAb) [3]. In addition, increased levels of TNF- α are found both in the synovial joints and the peripheral blood of RA patients. When TNF- α -blocking agents are administered to RA patients, they reduce inflammation, improve symptoms and retard joint damage [4].

Two TNF- α -blocking agents, etanercept and infliximab, are currently licensed for the treatment of RA. Etanercept (Enbrel; Immunex Corporation, Seattle, WA, USA) is a recombinant human p75 soluble TNF receptor-human immunoglobulin G1 (IgG1) construct. It contains two TNF- α -binding domains linked to the Fc portion of human Ig. Infliximab (cA2; Centocor, Malvern, PA, USA) is a chimeric anti-TNF- α mAb that

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438

has a human Ig-γ1 Fc. Both are recombinant proteins manufactured by mammalian cell culture techniques.

CDP870 (Celltech Research and Development, Slough, Berkshire, UK) is a novel compound that neutralizes TNF-α *in vitro*. It comprises an engineered human anti-TNF-α antibody Fab' fragment that is linked chemically to polyethylene glycol (PEG). The Fab' fragment is made by microbial fermentation rather than in mammalian cell culture. The attachment of PEG increases the circulating half-life of Fab to approximately 14 days. We evaluated the safety and efficacy of intravenous CDP870 in a randomized double-blind, placebo-controlled, dose-escalating trial in patients with RA.

Methods

Patients

Patients aged 18–75 yr who satisfied the 1987 revised American College of Rheumatology (ACR) diagnostic criteria for RA [5] were recruited from out-patient rheumatology clinics in London, Cambridge, Norfolk and Norwich (UK). Patients were required to have clinically active disease, defined by having at least three of the following criteria: at least three swollen joints (28-joint count), at least six painful or tender joints (28-joint count), > 45 min of early morning stiffness and erythrocyte sedimentation rate (ESR) > 28 mm/h. They must have failed treatment with at least one disease-modifying anti-rheumatic drug (DMARD) and have been off treatment for at least 4 weeks. Corticosteroids were permitted if the dose was < 7.5 mg/day of prednisolone. Pregnant women, nursing women and women of child-bearing potential not using an effective method of contraception were excluded. Patients were also excluded if they had a history of malignancy, concomitant severe uncontrolled medical conditions, previous failure of TNF-α-neutralizing therapy or allergy to PEG. Written informed consent was obtained from each patient before enrolment. The study was approved by the research ethics committees of King's College Hospital, Addenbrooke's Hospital, Norfolk and Norwich Hospital as well as Middlesex Hospital and University College Hospitals London.

Treatment protocol

Thirty-six RA patients were divided into three groups, each of which were to receive an increasing dose of the trial drug (1, 5 or 20 mg/kg). Each group of 12 patients was randomly divided so that eight received CDP870 and four received placebo. CDP870 was given as a single intravenous infusion (100 ml in total) over 60 min. Placebo (sodium acetate buffer) was given similarly as a single intravenous infusion of 100 ml over 60 min. Treatment was given on an out-patient basis. After 8 weeks, patients had the opportunity to receive an open-label infusion of 1, 5 or 20 mg/kg of CDP870 within 2 weeks of the blinded dosing period if they tolerated the initial infusion.

Clinical assessment

RA disease activity was assessed based on the World Health Organization and International League of Associations for Rheumatology [6] and European League Against Rheumatism (EULAR) [7] core data sets. In brief, these include pain score, assessor and patient global assessment of disease activity measured by visual analogue scales (0–10 cm), tender and swollen joint count (max. 28), health assessment questionnaire (0–3), ESR (mm/h) and C-reactive protein (CRP, mg/dl). Changes in disease activity were assessed with the Disease Activity Score (DAS) [8] and the ACR response criteria [9]. Assessments were carried out before treatment and 1, 2, 4, 6 and 8 weeks after both blinded and open-label therapy. Patients were also assessed for safety and tolerance of the study drug. Haematology, biochemistry, anti-CDP870 antibodies and adverse events were assessed at each visit.

CDP870 plasma concentration and anti-CDP870 antibodies

CDP870 was measured by enzyme-linked immunosorbent assay (ELISA). Serial dilutions of patients' plasma were incubated in microtitre plates (Nunc, Rochester, NY, USA) coated with recombinant human TNF-α (Strathmann Biotech, Hanover, Germany). Captured CDP870 was revealed with horseradish peroxidase (HRP)-conjugated goat anti-human κ light chain (Cappel, ICN, Costa Mesa, CA, USA) followed by tetramethyl benzidine (TMB) substrate.

Antibodies to CDP870 were screened (at 1/10 plasma dilution) using a double-antigen sandwich ELISA with biotinylated CDP870 as the second layer. Bound antibodies were revealed using HRP-streptavidin and TMB substrate. The assay was calibrated using a hyper-immune rabbit IgG standard. A unit of activity is equivalent to 1 μg of the rabbit standard. Patients were considered positive for antibodies if there was a greater than two-fold increase in anti-CDP870 levels over a baseline cut-off and pre-infusion level.

Statistical analysis

The study was exploratory in nature and sample size was based on previous experience with similar agents. The efficacy of CDP870 was analysed by calculating the DAS28 score and ACR20/50 responses for intention to treat and per-protocol using a closed testing procedure. The DAS28 score was calculated as follows: $\text{DAS28} = 0.555 \times \text{square root of (28 tender joint count)} + 0.284 \times \text{square root of (28 swollen joint count)} + 0.7 \times \ln(\text{ESR}) + 0.0142 \times (\text{patient's global assessment})$. First, the pooled active groups were compared with patients treated with placebo. If this comparison was significant at the 5% level, each dosage group was compared with the placebo group. All comparisons were two-tailed with a significance level of 5%. All *P*-values were derived from exploratory analysis and should not be used for inferential interpretation.

TABLE 1. Demographic details of patients (mean $\pm$ s.d.)

Treatment	No. of patients	Sex (M, F)	Age (yr)	Duration of disease (yr)	Rheumatoid factor-positive	No. of previous DMARDs	DAS at baseline
Placebo	12	1, 11	51 $\pm$ 8	12 $\pm$ 8	8 (67%)	5 $\pm$ 1	5.9
CDP870							
1 mg/kg	8	1, 7	59 $\pm$ 7	12 $\pm$ 7	4 (50%)	4 $\pm$ 1	5.9
5 mg/kg	8	2, 6	54 $\pm$ 13	13 $\pm$ 5	5 (63%)	5 $\pm$ 2	6.6
20 mg/kg	8	2, 6	61 $\pm$ 11	14 $\pm$ 13	4 (50%)	4 $\pm$ 2	6.2

Results

Patient characteristics

Thirty-six patients with RA were recruited. Their demographic details are given in Table 1. The mean age was 56 yr and 30 patients were female. The mean duration of RA was 13 yr and 21 patients were positive for rheumatoid factor. Patients in the different groups had similar demographic characteristics. In the blinded dosing period, 6/12 placebo patients withdrew from the study for deteriorating RA $\leq$ 4 weeks after dosing. Two of 24 CDP870-treated patients withdrew, both in the 1 mg/kg group, for deteriorating RA or loss to follow-up $>$ 4 weeks after dosing. The difference was statistically significant ($P=0.009$, Fisher's exact test).

Clinical efficacy

The proportion of patients with ACR20 improvement for the per-protocol population with the last observation carried forward was 16.7, 50, 87.5 and 62.5% after placebo and 1, 5 and 20 mg/kg after CDP870 respectively (combined treatment effect, $P=0.012$) at 4 weeks and 16.7, 25, 75 and 75% ($P=0.032$) at 8 weeks. The proportion of patients with ACR50 improvement for the per-protocol population with the last observation carried forward was 0, 12.5, 12.5 and 50% after 0, 1, 5 and 20 mg/kg CDP870 respectively (combined treatment effect, $P=0.079$) at

4 weeks and 0, 12.5, 12.5 and 50% ($P=0.079$) at 8 weeks. Reduction in DAS28 scores (median) for the per-protocol population with the last observation carried forward was 0.15, 1.14, 1.91 and 1.95 after placebo, 1, 5 and 20 mg/kg CDP870 respectively (combined treatment effect, $P=0.001$) at 4 weeks and 0.31, 0.09, 2.09 and 1.76 ($P=0.008$) at 8 weeks (Fig. 1). Changes in individual components of the World Health Organization and International League of Associations for Rheumatology core data set are shown in Fig. 2.

Following the open-label dose of CDP870, similar beneficial effects were achieved. Of the 36 patients recruited into the study, 32 received a second infusion of CDP870. The proportion of patients with ACR20 improvement since before the first infusion was 72.2 and 55.6% after 5 and 20 mg/kg CDP870 respectively at 4 weeks and 55.6 and 66.7% at 8 weeks.

Adverse events

Treatment was well tolerated, with no infusion-related reaction. No allergic reaction or skin rash was reported. In the double-blind phase, there were 19, 38, 8 and 14 adverse events in the placebo, 1, 5 and 20 mg/kg groups respectively. The commonest was headache, with nine episodes in five patients (one placebo, three at 1 mg/kg, one at 20 mg/kg). One patient who received placebo and three patients who received CDP870 (one at 5 mg/kg and two at 20 mg/kg) developed lower respiratory tract infections. These were reported as mild or moderate. They were treated with oral antibiotics and resolved over a 1–2 week period. Three patients each in the 1 and 5 mg/kg groups and one in the 20 mg/kg group developed a urinary tract infection 1–2 months after CDP870 treatment. One adverse event was described as severe, which was an episode of neck pain occurring 3 days after infusion with 1 mg/kg. An increase in antinuclear antibody was seen in four patients: one in the placebo group (negative to 1/40), two in the 1 mg/kg group (negative to 1/40, negative to 1/80) and one in the 20 mg/kg group (negative to 1/40). No change was found in anti-DNA or anti-cardiolipin antibodies.

In the open phase, one patient who received 20 mg/kg CDP870 died from complications following rapid drainage of a large, chronic rheumatoid pericardial effusion. No infective agent was isolated from either the pericardial fluid or peripheral blood. In the opinion of the investigator, this event was unrelated to treatment with CDP870.

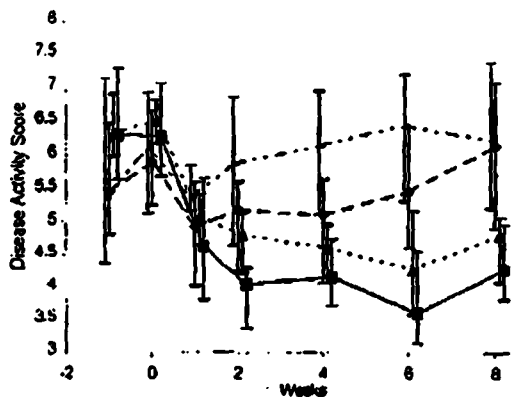


FIG. 1. DAS in patients treated with different doses of CDP870 and placebo. Median and interquartile range are presented for the per-protocol population with the last observation carried forward.

440

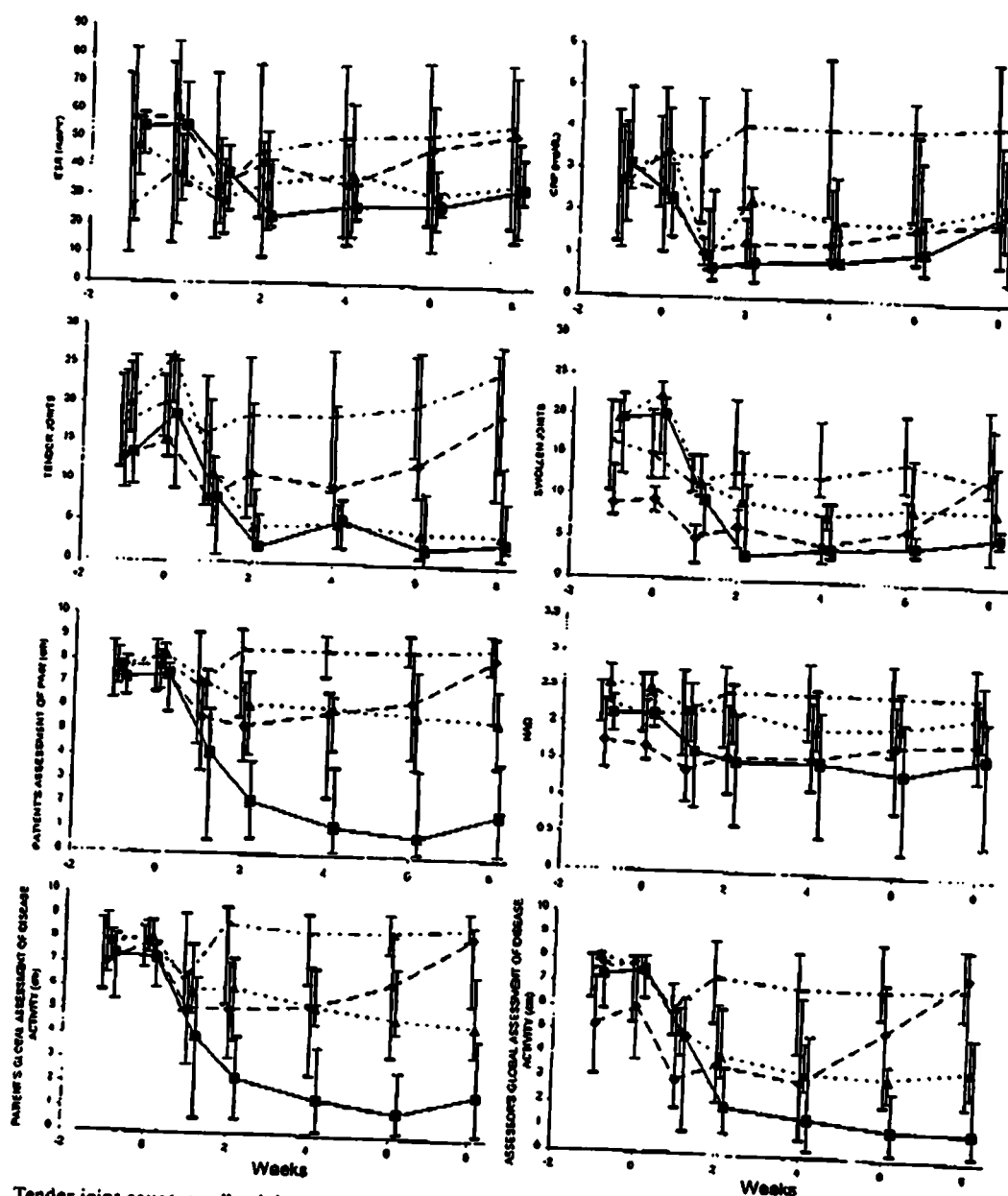


FIG. 2. Tender joint count, swollen joint count, pain score (cm), patient and assessor's global assessment of disease activity (cm), modified Health Assessment Questionnaire (HAQ) score, C-reactive protein (CRP) concentration and ESR in patients treated with different doses of CDP870 and placebo. Median and interquartile range are presented for the per-protocol population with the last observation carried forward.

CDP870 plasma concentration and anti-CDP870 levels

As expected, for all dose levels of CDP870, the peak plasma concentration occurred at the end of infusion and was proportional to dose; the plasma concentration declined slowly thereafter. The plasma concentration profile of CDP870 appeared very similar to that

previously observed in volunteers in whom the half-life was calculated to be ~14 days. On redosing, a profile similar to that obtained with single-dose infusion was observed.

Following a single intravenous infusion, anti-CDP870 levels were low or undetectable. Following a second

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cycle of treatment, antibodies were detected in all treatment groups. The incidence varied with the dose, but was lower in the higher dose groups.

Discussion

Neutralizing TNF- α is an effective treatment strategy in RA. Currently, this requires the use of biological agents, such as chimeric mAbs and soluble receptor-human Fc fusion protein, which are expensive to manufacture. An optimal TNF- α -neutralizing agent needs to bind TNF- α with high affinity and have a long plasma half-life, low antigenicity and high tolerability and safety. It also needs to be accessible to all patients with RA who would benefit from TNF- α blockade. One technology to achieve these objectives is the conjugation with PEG of a TNF- α -binding antibody fragment made in *E. coli*. In this preliminary study, we found that the PEGylated anti-TNF- α Fab' CDP870 was effective and well tolerated by patients with RA.

In vitro studies have shown that CDP870 has similar TNF- α -neutralizing activity to the murine anti-TNF- α parent antibody. This study confirms that CDP870 reduced inflammation and improved symptoms in RA. Clinical improvement, as measured by the ACR20 response criteria, in the 5 and 20 mg/kg groups (75%, 75%) was comparable to that of etanercept (60%) [10] and infliximab (50%) [11]. At the middle and highest dose levels tested, the therapeutic effect lasted 8 weeks, which is comparable to previous mAbs [12, 13]. A previous study has shown that the therapeutic effect of anti-TNF- α antibody is related to its plasma half-life and the generation of circulating antibodies [14]. Our study showed that CDP870 has a plasma half-life of 14 days, which is that of a whole antibody [13] and much longer than the half-life of unconjugated Fab' fragments. At higher doses, CDP870 generated only very low levels of antibody response. A longer study with repeat dosing is currently being undertaken to investigate this more thoroughly.

One of the important objectives of this study was to examine the tolerability and safety of administering this PEGylated Fab'. In our study CDP870 appeared well tolerated, but further study will be needed to assess long-term safety, especially the risks of demyelinating disease, infection and skin rashes, which have been reported with etanercept and infliximab.

In summary, CDP870 is a novel PEGylated anti-TNF- α Fab' fragment that is therapeutically effective in RA and was well tolerated in this short-term study. A larger study with repeated dosing is currently under way to assess its long-term safety and therapeutic effect in RA.

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442

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176089-2

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 02-008005- -00006-0000

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FECHA : OCTUBRE 1 DE 2007

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
CAVELIER ABOGADOS	CONSIGNACION	BANCO POPULAR	050-00110-6	63769926	01/10/2007	45.675.000.00

***** CONCEPTO *****

CONCEPTO	TOTAL CONCEPTO
12 MARCAS Y LENAS COMERCIALES	99.000.00
TOTAL :	\$ 99.000.00

SUN: NOVENTA Y NUEVE MIL PESOS

RESPONSABLE :

Cdo BIL-841001-2

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



443
D1

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07K 16/00	A1	(11) International Publication Number: WO 98/25971 (43) International Publication Date: 18 June 1998 (18.06.98)
(21) International Application Number: PCT/GB97/03400 (22) International Filing Date: 10 December 1997 (10.12.97) (30) Priority Data: 9625640.9 10 December 1996 (10.12.96) GB (71) Applicant (for all designated States except US): CELLTECH THERAPEUTICS LIMITED [GB/GB]; 216 Bath Road, Slough, Berkshire SL1 4EN (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): KING, David, John [GB/GB]; 69 Watchetts Drive, Camberley, Surrey GU15 2PF (GB). CHAPMAN, Andrew, Paul [GB/GB]; 48 Hanworth Road, Hampton, Middlesex TW12 3DL (GB). (74) Agent: MERCER, Christopher, Paul; Carpmaels & Ransford, 43 Bloomsbury Square, London WC1A 2RA (GB).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: MONOVALENT ANTIBODY FRAGMENTS (57) Abstract Monovalent antibody fragments are described, each of which has one or more polymer molecules site-specifically attached through a sulphur atom of a cysteine residue located outside of the variable region domain of the antibody. The polymers include synthetic or naturally occurring polymers such as polyalkylenes, polyalkenylenes, polyoxyalkylenes or polysaccharides. Each fragment may be attached to one or more effector or reporter molecules, and is of use in therapy or diagnostics where it has markedly improved binding and/or pharmacokinetic properties when compared to other antibody fragments which have the same number and type of polymer molecules, but in which the polymer molecules are randomly attached.		

are non-covalently associated. Where desired, however, the chains may be covalently coupled either directly, for example via a disulphide bond between the two variable domains, or through a linker, for example a peptide linker, to form a single chain domain.

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The variable region domain may be any naturally occurring variable domain or an engineered version thereof. By engineered version is meant a variable region domain which has been created using recombinant DNA engineering techniques. Such engineered versions include those created for example from natural antibody variable regions by insertions, deletions or changes in or to the amino acid sequences of the natural antibodies. Particular examples of this type include those engineered variable region domains containing at least one CDR and optionally one or more framework amino acids from one antibody and the remainder of the variable region domain from a second antibody.

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The variable region domain will generally be covalently attached to at least one cysteine residue or in particular two or three cysteine residues each covalently linked through its sulphur atom to a polymer molecule.

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The location of each cysteine residue may be varied according to the size and nature of the antibody fragment required. Thus, in one extreme example a cysteine residue linked through its sulphur atom to a polymer may be attached directly to a C-terminal amino acid of the variable region domain. This may be for example the C-terminus of a V_H or V_L chain as described above. If desired, in this example, further amino acids, including further cysteine-linked polymers, may be covalently linked to the C-terminus of the first cysteine residue.

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In practice however, it is generally preferable that the variable region domain is covalently attached at a C-terminal amino acid to at least one other antibody domain or a fragment thereof which contains, or is attached to one, two, three or more cysteine residues, each covalently linked through its sulphur atom to a polymer molecule. Thus, for example where a V_H domain is present in the variable region domain this may be linked to an immunoglobulin C_H1 domain or a fragment thereof. Similarly a V_L

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Therapeutic and diagnostic uses of fragments according to the invention typically comprise administering an effective amount of the antibody fragment to a human subject. The exact amount to be administered will vary according to the use of the antibody and on the age, sex and condition of the patient but may typically be varied from about 0.1mg to 1000mg for example from about 1mg to 500mg. The antibody may be administered as a single dose or in a continuous manner over a period of time. Doses may be repeated as appropriate. Typical doses may be for example between 0.1-50mg/kg body weight per single therapeutic dose, particularly between 0.1-20 mg/kg body weight for a single therapeutic dose.

The following Examples illustrate the invention. In the Examples, the following antibody fragments are used and are identified in each case by the abbreviated name given below. In each instance the antibody was prepared from a mouse monoclonal antibody as described in International Patent Specification No. WO92/01059 (A5B7) or by using similar methods (hTNF40 and cTN3):

hA5B7 - This is an engineered human antibody which recognises carcinoembryonic antigen. The antibody fragment used here has one cysteine residue available for pegylation and located in its hinge region after activation with β -mercapto-ethylamine.

hTNF40 - This is an engineered human antibody which recognises human TNF α . Two hTNF40 antibody fragments are used in the Examples, one (Example 2) which has a single cysteine residue in the hinge region (see hA5B7 above), and a second (Example 3) which has two hinge cysteine residues, available for pegylation.

cTN3 - This is a chimeric hamster/mouse antibody which recognises mouse TNF α and has a mouse IgG2a constant region. The

EXAMPLE 2**Site-specific attachment of PEG to hTNF40 Fab'****Purification of hTNF40 Fab'**

- 5 hTNF40 Fab' was expressed in *E. coli* W3110 cells grown in a 1.5 litre fermenter and a cell extract prepared as described in Example 1. The cell extract was diluted to a conductivity of 3.5µS/cm, adjusted to pH4.5 and applied to a column of Streamline® SP (Pharmacia) equilibrated with 50mM acetate buffer pH4.5. After washing with equilibration buffer, the
- 10 Fab' was eluted with 200mM sodium chloride in 50mM acetate buffer pH4.5. The pH of the eluted material was adjusted to 6 and the Fab' was purified further by applying to a column of protein G-sepharose equilibrated in PBS. After washing with PBS, the Fab' was eluted with 0.1M glycine-hydrochloric acid pH2.7 and immediately the pH was re-
- 15 adjusted to 6. The purified Fab' was then concentrated to >10mg/ml by ultrafiltration.

Preparation of hTNF40 Fab'-PEG(25kDa) and hTNF40 Fab'-PEG(40kDa) through hinge thiol PEG attachment

- 20 Purified Fab' was buffer exchanged into 0.1M phosphate buffer pH6 containing 2mM EDTA. The hinge thiol group was then activated by reduction with 5mM β-mercaptoethylamine as described in Example 1. This resulted in an average of 1.1 thiols per Fab' as determined by titration with DTDP. Fab'-PEG samples were then produced with both 25kDa and
- 25 a 40kDa PEG using PEG-maleimide derivatives supplied by Shearwater Polymers Inc. Huntsville, AL, USA. The 25kDa PEG-maleimide derivative was a single PEG chain linked directly to a maleimide group, and the 40kDa PEG-maleimide derivative was prepared from a branched structure comprising two 20kDa PEG chains linked through a lysine derivative to a maleimide group. Freshly reduced and desalted Fab' was incubated with a three-fold molar excess of 25kDa PEG-maleimide or a nine-fold molar excess of 40kDa PEG-maleimide overnight at room temperature and the resulting Fab'-PEG conjugates purified by gel-filtration HPLC using a Zorbax GF-250 column run in 0.2M phosphate buffer pH7.0. For
- 30 comparative purposes, a randomly PEG-modified hTNF40 Fab' was also prepared with 25kDa PEG as described for 5kDa PEG attachment to



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : C12P 21/08, C07K 15/28 C12N 15/13, 15/62, A61K 39/395	A1	(11) International Publication Number: WO 92/11383 (43) International Publication Date: 9 July 1992 (09.07.92)
(21) International Application Number: PCT/GB91/02300 (22) International Filing Date: 20 December 1991 (20.12.91) (30) Priority data: PCT/GB90/02017 21 December 1990 (21.12.90) WO (34) Countries for which the regional or international application was filed: 9109645.3 3 May 1991 (03.05.91) GB et al. GB (71) Applicant (for all designated States except US): CELLTECH LIMITED [GB/GB]; 216 Bath Road, Slough, Berkshire SL1 4EN (GB). (72) Inventors; and (75) Inventors/Applicants (for US only) : ADAIR, John, Robert [GB/GB]; 23 George Road, Stokenchurch, High Wycombe, Bucks HP14 3RN (GB). ATHWAL, Diljeet, Singh [GB/GB]; Flat 35, Knollys House, Tavistock Square, London WC1 7CHX (GB). EMTAGE, John, Spencer [GB/GB]; 49 Temple Mill Island, Temple, Marlow, Bucks SL7 1SQ (GB). BODMER, Mark, William [GB/GB]; Rose Cottage, 5 Manor Road, South Hinksey, Oxford OX1 5AS (GB).		(74) Agent: MERCER, Christopher, Paul; Carpmaels & Ransford, 43 Bloomsbury Square, London WC1A 2RA (GB). (81) Designated States: AT, AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), CH, CH (European patent), CI (OAPI patent), CM (OAPI patent), CS, DE, DE (European patent), DK, DK (European patent), ES, ES (European patent), FI, FR (European patent), GA (OAPI patent), GB, GB (European patent), GN (OAPI patent), GR (European patent), HU, IT (European patent), JP, KP, KR, LK, LU, LU (European patent), MC (European patent), MG, ML (OAPI patent), MN, MR (OAPI patent), MW, NL, NL (European patent), NO, PL, RO, SD, SE, SE (European patent), SN (OAPI patent), SU*, TD (OAPI patent), TG (OAPI patent), US. Published <i>With international search report.</i>
(54) Title: RECOMBINANT ANTIBODIES SPECIFIC FOR TNF α (57) Abstract Recombinant, in particular humanised, e.g. humanised chimeric and CDR-grafted humanised, antibody molecules having specificity for human TNFα, are provided for use in diagnosis and therapy. In particular the antibody molecules have antigen binding sites derived from murine monoclonal antibodies CB0006, CB0010, hTNF3 or 101.4. Preferred CDR-grafted humanised anti-hTNFα antibodies comprise variable region domains comprising human acceptor framework and donor antigen binding regions and wherein the frameworks comprise donor residues at specific positions. The antibody molecules may be used for therapeutic treatment of human patients suffering from or at risk of disorders associated with undesirably high levels of TNF, in particular for treatment of immunoregulatory and inflammatory disorders or of septic, endotoxic or cardiovascular shock.		

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISIÓN DE NUEVAS CREACIONES

2020

Bogotá, D.C., 6 de noviembre de 2007

Abogado

Emilio Ferrero

Apoderado

CELLTECH R&D LIMITED.

ASUNTO

Radicación

02-8005

Trámite

002

Evento

001

Actuación

430

Oficio

14687

Folios

Patente de Invención

5

Patente de Modelo de Utilidad

7

Vía Nacional

11

Vía PCT (fase nacional)

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Cap.I

11

Cap. II

11

No. Sol. Internacional

PCT/GB01/02477

Fecha de Presentación Internal.

05/06/2001

Como resultado del examen de fondo, realizado con fundamento en el artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica:

1. Documentos estudiados

- | | | |
|------|-----------------------------------|---|
| 1.1. | <u>Descripción:</u> | Folios: 4 a 90 como se radicó inicialmente.
Folios: 307 a 393 (Según modificación presentada). |
| 1.2. | <u>Reivindicaciones:</u> | Folios: 91 a 105 como se radicó inicialmente.
Folios: 415 a 421 (Según modificación presentada). |
| 1.3. | <u>Figuras:</u> | Folios: 106 a 191 como se radicó inicialmente. |
| 1.4. | <u>Prioridad:</u> | 6/07/2000, Gran Bretaña, GB00138107. |
| 1.6 | <u>Respuesta del solicitante:</u> | Folios: 407 a 441. |

2. Análisis de la Prioridad

Se mantiene la decisión de no reconocer prioridad para las secuencias no incluidas en el documento prioritario, los anticuerpos correspondientes a éstos y su ADN codificante, así como sus compuestos derivados y procedimiento de obtención (Se excluye de esto la SEQ. No. 11 según los argumentos presentados por el solicitante).

3. Objeto de la invención

Título de la solicitud: "Moléculas de anticuerpo que tienen especificidad por el factor de necrosis tumoral alfa humano y uso de las mismas".

El objeto de la presente solicitud de patente de invención consiste en la producción de un anticuerpo con afinidad y especificidad por el factor de necrosis tumoral humano. El anticuerpo producido está caracterizado por tener una cadena pesada que puede presentar los fragmentos CDR con secuencias SEQ ID No. 1, 2, 7 y 3. El anticuerpo producido, a su vez, cuenta con una cadena ligera que puede presentar los fragmentos SEQ ID No. 4, 5 y 6. Igualmente se reclaman variaciones de este anticuerpo y el intercambio de estas secuencias por otras, así como el cambio en diferentes posiciones de los aminoácidos de los restos donadores y aceptores. Además de éstos se reivindica el anticuerpo monoclonal hTNF40.

Del mismo modo, se reivindica el compuesto que contenga el anticuerpo unido a otras moléculas como receptores y efectores.

De manera independiente se reivindica un anticuerpo híbrido y algunos cambios estructurales referentes a secuencias CDR de diferentes procedencias.

El ADN codificante de estos anticuerpos también es reclamado, al igual que varios vectores de clonación y expresión y la célula huésped.

El procedimiento *in Vitro* para la producción del ADN y del (los) anticuerpo(s) también se reclama.

Sucesivamente se define una composición que contiene el (los) anticuerpo(s) y un polipéptido con una secuencia de aminoácidos que incluya cualquiera de las SEQ Nos. 1 a 7.

El problema planteado en esta solicitud es la necesidad de producir fácil y eficazmente un anticuerpo para tratar enfermedades inflamatorias crónicas, que pueda ser utilizado repetidamente. Igualmente este anticuerpo debe presentar alta afinidad por el factor TNF α y baja inmunogenicidad en seres humanos.

Para solucionar este problema técnico la solicitud en estudio proporciona un(os) anticuerpo(s) producidos mediante técnicas *in Vitro* de última generación con especificidad y afinidad por el TNF α humano.

- El objeto de la solicitud definida anteriormente se clasificó en la IPC C12 N05/013.

4. De la solicitud de Patente (según el caso)

4.1. Descripción (artículo 28 D. 486)

El desarrollo experimental y los resultados presentados son insuficientes al no sustentar toda la materia reclamada, no demostrar las ventajas técnicas de la invención, ni evidenciar el efecto sorprendente asociado con la misma. Adicionalmente, en la información presentada no se encuentra el sustento del desarrollo de un anticuerpo como el reivindicado en la cláusula 1, ni de una indicación sobre su aporte cualitativo al estado de la técnica.

En este mismo sentido, según el problema técnico planteado en la solicitud no aparece a lo largo de todo el capítulo descriptivo una evidencia sobre la solución positiva de esta problemática.

De esta manera, la descripción presentada no cumple con los requisitos establecidos en el art. 28 de la Decisión 486.

4.2. Reivindicaciones (artículo 30 D 486)

El capítulo reivindicatorio en general carece de concisión y claridad por cuánto:

- 1) se incluye materia que no es caracterizante del anticuerpo, el compuesto y los métodos reclamados, entre otros, al realizar la reclamación de los mismos por medio de generalidades y/o resultados esperados y/o posibilidades inespecíficas y/o por su relación con otras moléculas y/o por su actividad o efecto esperado y/o por transformaciones de los mismos y/o según condiciones totalmente conocidas en el estado de la técnica, todas éstas que no permiten establecer las propiedades nuevas e inventivas de lo reclamado (p.e. cláusula 5, 8, 9, 27, 44);
- 2) se repite materia idéntica en cláusulas únicas ya incluidas en reivindicaciones anteriores, existiendo una duplicidad de contenido pues las cláusulas posteriores no aportan detalle de la invención, ni especifican su alcance (p.e. cláusulas 18 y 19, cláusulas 20 y 21, cláusulas 13, 24, 25 y 26);
- 3) se reclama material sin sustento descriptivo suficiente, existiendo una inconsistencia con el capítulo descriptivo (p.e. cláusula 1, 8, 32, 36) ;
- 4) en cláusulas dependientes se incluye materia más amplia que la reclamada en la reivindicación independiente, ampliando la materia inicial y estableciendo falsas dependencias entre las mismas (p.e. cláusulas 10, 12, 20).

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

5. **Determinación del Estado de la Técnica**

La fecha que se ha tenido en cuenta para determinar el Estado de la Técnica es:

Fecha de presentación de la solicitud prioritaria: 06/06/2001.

Teniendo en cuenta la fecha indicada se realizó la búsqueda en el estado de la técnica de documentos relacionados con la solicitud encontrándose los siguientes documentos más relevantes:

Nº	Documento	Título	Fecha de publicación
D1	WO9825971	"Monovalent antibody fragments"	18/07/1998
D2	WO92011383	"Recombinant antibodies specific for TNFα"	09/07/1999

6. **Análisis de patentabilidad (según requisito) (artículo 14 D486)**

6.1. Nivel inventivo (artículo 18 D486)

La solicitud en estudio reclama una molécula de anticuerpo quimérico humanizado que tiene especificidad para TNFα humano que comprende un CDRH1 de secuencia SEQ No.1, un CDRH2 de secuencia SEQ No. 2 ó 7 o un CDHR3 con secuencia SEQ No. 3 para la cadena pesada y un CDRL1 de secuencia SEQ No.4, un CDRL2 de secuencia SEQ No. 5 o un CDHL3 con secuencia SEQ No. 6 para la cadena ligera, compuestos que lo contienen, un anticuerpo híbrido, fragmentos Fab, el ADN codificante, vectores de clonación, la célula huésped transformada con el vector, su proceso de producción, una composición terapéutica o de diagnóstico y un polipeptido (reivindicación 1 y dependientes, 51, 57, 59).

Adicionalmente se reclama otros anticuerpos quiméricos humanizados que contienen

vector, su proceso de producción, una composición terapéutica o de diagnóstico y un polipeptido (reivindicación 1 y dependientes, 51, 57, 59).
Adicionalmente se reclama otros anticuerpos quiméricos humanizados que contienen otras secuencias.

Considerando: 1) que en la solicitud en estudio no se evidencia un efecto técnico sorprendente del desarrollo reclamado; 2) que el documento WO9825971 (D1) divulga el anticuerpo recombinante murino hTNF40 y fragmentos Fab del mismo, conjugados y compuestos que los contienen (página 13 y ejemplo 2), su método de producción por medio técnicas de variación molecular, transformación de vectores y cultivo de células transformadas con el vector, método además usual para este tipo de desarrollos (ver p.e. página 5, párrafo 10; página 10, párrafo 15 y el documento en general); 3) que D1 es el estado de la técnica más cercano y que la única diferencia que se presenta se refiere a la introducción de secuencias específicas en los anticuerpos anti TNF α (por lo demás se divulga iguales procedimientos y productos (compuestos, conjugados, vectores, células huésped)) y, 4) que el concepto de desarrollar anticuerpos quiméricos humanizados en el laboratorio, entre los que se encuentran anticuerpos anti-TNF α , y diseñar y alternar secuencias específicas para mejorar su afinidad a la molécula objetivo (ver WO92011383, D2 (todo el documento)) era ampliamente conocido en el estado de la técnica, la invención presentada solo puede reconocerse como una alternativa a los anticuerpos presentes en el estado de la técnica al momento de su reclamación.

De esta manera, según el estado de la técnica, 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.

7. Conclusión:

Los requisitos de patentabilidad de la presente solicitud se encuentran afectados así:

N°	Documento N°	Reivindicaciones afectadas	Requisito que afecta
D1	WO9825971	1-59	Nivel Inventivo
D2	WO92011383	1-59	Nivel Inventivo

8. Respuesta a los argumentos del solicitante:

En relación con los argumentos del solicitante sobre la afectación de la materia reclamada con los documentos anteriormente citados, como ya se menciona en el aparte de nivel inventivo, el diseño y construcción de anticuerpos con secuencias específicas para mejorar la afinidad de los mismos se constituye como una práctica cotidiana para este tipo de desarrollos desde hace ya varias décadas, por esto no se encuentra plausible que este pueda ser el núcleo de la invención, máxime sin existir sustento experimental del desarrollo de un anticuerpo con la conformación reclamada (cláusula 1), ni evidencia de un efecto técnico sorprendente probado asociado a ésta, y por ende una solución positiva del problema técnico planteado. En cuanto a la afinidad aumentada de anticuerpos anti-TNF α , referida por el solicitante, ésta también ha sido divulgada con anterioridad (ver D1 p.e.) en relación con diferentes conformaciones moleculares de los mismos, por lo que esto no puede considerarse como el efecto técnico sorprendente y por lo mismo como un salto cualitativo en el estado de la técnica; y respecto a los documentos de fases clínicas

452

452

Adicionalmente, en referencia al documento WO92011383 (D2) se considera que los fragmentos de anticuerpos son derivaciones de los mismos y por tanto proceden de las mismas técnicas y pueden entenderse como el mismo concepto, muestra de esto se encuentra en que tanto la solicitud en estudio como el mismo documento (D2) divulgan y reclaman estos dos elementos relacionalmente.

Por último, tal como se observa en este concepto, se considera que no se ha presentado subsanación total de los requerimientos realizados en el concepto técnico No. 8514.

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

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 N° 10 de 2001.

ALIX CARMENZA CÉSPEDES DE VERGEL
Jefe de División de Nuevas Creaciones

El anterior requerimiento fue notificado por fijación en lista, de fecha
18 NOV 2007

CONCEPTO TÉCNICO No. 2164	EXAMINADOR TÉCNICO Código No.202081
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452