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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 05-109276- -00012-0000

Fecha: 2010-04-05 17:06:29 Dep: 2020 DIR.NUEVASCR  
Tra: 11 PATENTEIYII Lve: 379 FASENACIONALII  
Act: 444 RESPUESTAREQUE Folios: 15

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14  
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Señora Directora  
**DIRECCIÓN DE NUEVAS CREACIONES**  
Superintendencia de Industria y Comercio  
E. S. D.

Trámite : 011  
Evento : 379  
Actuación : 444

Referencia : Expediente No. 05109276  
Asunto : Solicitud de patente para: **ANTAGONISTAS DE  
INTERLEUKIN-6 Y COMPOSICIONES FARMACÉUTICAS  
QUE LOS CONTIENEN**  
Solicitante : CHUGAI SEIYAKU KABUSHIKI KAISHA  
Clase : A61K 039/395, A61K 039/505, A61P 037/000  
Fecha solicitud: 26 de octubre de 2005

1. Yo, Luz Clemencia DE PÁEZ, en mi condición de apoderada de la sociedad solicitante de la patente de la referencia, de acuerdo con la sustitución del poder a mí otorgada por el doctor Emilio Ferrero, me refiero al oficio No. **12873** notificado por fijación en lista del 20 de noviembre de 2009.

2. Mediante memorial radicado el día 16 de febrero de 2010 solicité plazo de treinta (30) días para atender el oficio No. **12873**.

3. Mediante oficio No. **12873** 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:

### 3.1 De la solicitud de patente

#### **3.1.1 Descripción**

Señala el examinador que no se encontraron sustentadas las reivindicaciones de las composiciones farmacéuticas de la solicitud, mediante ejemplos que describan la forma de realizar el invento.

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## 3.1.2 Reivindicaciones

El Examinador considera que la reivindicación 1 se presenta de manera amplia y general al referirse a un anticuerpo contra el receptor anti-interleukin-6.

## 3.2 Análisis de patentabilidad

### 3.2.1 Nivel inventivo

Se ha citado nuevamente el documento D1: US 5,210,075 considerando que éste y un nuevo documento D3: B.N Cronstein et al. "Methotrexate Diminishes Phlogiston Production and Responses" afectan el nivel inventivo de la presente solicitud.

Según el examinador, el problema objetivo de la solicitud el cual es obtener composiciones farmacéuticas para el tratamiento de enfermedades relacionadas con interleukina-6, queda resuelto comparativamente con lo previamente divulgado en el estado de la técnica, pues sería solucionado fácilmente por el experto en la materia con base en las composiciones divulgadas en D1 a la luz de D3, utilizando un antagonista de interleukina-6 y un inmunosupresor tal como metotrexato. De acuerdo con esto, el Examinador afirma que el efecto que provee la presente solicitud no es inesperado.

4. Con el fin de subsanar las objeciones expuestas, se desean presentar las siguientes modificaciones y argumentos:

### 4.1 Modificación de una solicitud en trámite

De acuerdo con el artículo 34 de la Decisión 486 de la Comisión de la Comunidad Andina, donde se establece que la modificación de una solicitud es posible en cualquier momento del trámite mientras no implique una ampliación de la solicitud inicial, me permito presentar un nuevo pliego reivindicatorio conformado por 2 reivindicaciones.

De esta manera, siguiendo las observaciones del Examinador, se ha modificado la reivindicación 1 para indicar que el anticuerpo contra el receptor anti-interleukina-6 (IL-6) es un anticuerpo PM-1.

La nueva reivindicación 2, señala entonces que dicho anticuerpo es un anticuerpo quimérico, humanizado o de tipo humano.

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Las anteriores modificaciones tienen por objeto definir la invención de manera más clara y concisa. Adicionalmente, las nuevas reivindicaciones se encuentran debidamente sustentadas en la descripción, ya que tal como se mencionó en respuesta al oficio No. 12203 la descripción contiene un ejemplo que da a conocer los efectos de la composición farmacéutica que contiene metotrexato y MRA (el cual es un anticuerpo PM-1), sobre un número determinado de pacientes y adicionalmente, presenta 4 ejemplos de referencia para la preparación del receptor soluble IL-6 y del anticuerpo IL-6.

Es de relevancia poner de presente que una persona medianamente versada en la materia sería capaz de obtener la composición farmacéutica con base en sus conocimientos y en la información contenida en la descripción. Ver por ejemplo página 32, último párrafo de la descripción donde se indica:

"Los compuestos farmacéuticos de la invención pueden ser aquellos que contienen los vehículos farmacéuticamente aceptables y aditivos dependiendo de las formas de administración. Ejemplos de tales vehículos y aditivos incluyen agua, solventes orgánicos farmacéuticamente aceptables, colágeno, alcohol polivinil, polivinilpirrolidona, polímero de carboxivinil, carboximetilcelulosa sódica, poliacrilato de sodio, arginato de sodio, dextran soluble en agua, almidón de carboximetil sódico, pectina, metil celulosa, etil celulosa, goma xantana, goma arábica, caseína, gelatina, azarosa, diglicerina, propilenglicol, polietileno glicol, petrolatum, parafina, alcohol estearil, ácido esteárico, albúmina de suero humano (HSA), manitol, sorbitol, lactosa, surfactantes adecuados como aditivos farmacéuticos y los similares. Los aditivos usados son seleccionados apropiadamente de los de arriba o en combinación dependiendo de si formulación, pero no están limitados a ellos.

De este modo, consideramos que la composición farmacéutica reivindicada se encuentra debidamente sustentada en la descripción y que las objeciones formuladas con respecto a la falta de soporte y concisión se ven superadas. Solicitamos atentamente tener en cuenta este nuevo pliego reivindicatorio que cumple con las disposiciones de la Decisión 486.

#### **4.2 Argumentos a favor del nivel inventivo**

Señala el Artículo 18 de la Decisión 486 que una invención se considerará inventiva si la misma no hubiese resultado obvia a partir

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del arte previo, para una persona del oficio normalmente versada en la materia. Esto a su vez implica que el Examinador a cargo de esta evaluación debe colocarse en el momento exacto en el que el inventor buscaba resolver el problema técnico contemplado en la solicitud y para el cual poseía la literatura y el conocimiento disponibles en dicho momento.

Siguiendo entonces los lineamientos del Manual Andino de Patentes y de la Jurisprudencia aplicable al análisis del nivel inventivo de una solicitud de patente, se considera que una anterioridad o una combinación de dos anterioridades puede afectar dicho requerimiento de patentabilidad, si una persona medianamente versada en la materia encuentra enseñanzas o sugerencias claras que le indiquen cómo llegar a la invención reivindicada. Adicionalmente se considera que una mejora sustancial, un efecto inesperado o una ventaja con respecto al arte previo es un indicio de nivel inventivo, por cuanto aportan un salto tecnológico en la regla técnica.

En el presente caso y así como se indicó en respuesta al oficio No. 12203, D1 se refiere de manera general a los inmunosupresores, pero no indica en ningún caso el metotrexato.

Por su parte, D3 **no** describe ni sugiere una combinación de un anticuerpo contra el receptor anti-IL-6 y metotrexato en absoluto y mucho menos el efecto ventajoso conseguido con la presente invención, es decir, el efecto sinérgico que provee dicha combinación.

Antes de la fecha de prioridad de la presente solicitud, debido al mecanismo complicado y a la causa de la artritis reumatoide, no se tenía presente que la combinación de dos agentes terapéuticos para tratar la artritis reumatoide generaba necesariamente un efecto sinérgico. Por el contrario, se conocía un caso en el que el efecto de una combinación era menor en comparación con la administración individual de los componentes, dado que el efecto de un agente era negado por el otro.

Para ilustrar mejor estas afirmaciones se presenta junto con el presente memorial copia del Artículo: Porter et al. "Combination Therapy in Rheumatoid Arthritis - No Benefit of Addition of Hydroxychloroquine to Patients with a Suboptimal Response to

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Intramuscular Gold Therapy", J. Rheumatol., 1993, 20, p. 645-649 en el cual se estudió una combinación para el tratamiento de artritis reumatoide y tanto en los resultados como en el resumen se describe que "no existe diferencia en el resultado en términos de la eficacia y la toxicidad". Esta descripción reporta que la combinación no resultó en un efecto sinérgico.

Adicionalmente, el mismo artículo describe que no fue posible demostrar ningún beneficio a partir de la combinación de metotrexato con auranofin y que el estudio de HCQ vs penicilamina vs penicilamina/HCQ reveló un resultado sorpresivo de eficacia reducida, comparado con la eficacia de la penicilamina administrada de manera individual.

Esto, sumado al conocimiento que una persona medianamente versada en la materia tiene, permite determinar que la combinación de dos agentes terapéuticos no genera necesariamente un efecto sinérgico, dado que existen interacciones de dichos agentes que no pueden ser predichas y por lo tanto, nos apartamos de la opinión del Examinador cuando afirma que el efecto inesperado aportado por la presente solicitud no resulta inesperado. Bajo ninguna circunstancia consideramos que la simple combinación de dos agentes terapéuticos resulte **necesariamente** en un efecto sinérgico, porque en dicho caso, todos los medicamentos que se encuentran en el mercado resultarían de la simple combinación de agentes que ya son conocidos. Si bien el efecto sinérgico es un efecto técnico buscado, por cuanto aporta un beneficio adicional, no es una regla en el campo de la química farmacéutica y no resulta de una simple combinación.

Por lo tanto, consideramos que ni el documento D1 ni el documento D2 pueden afectar el nivel inventivo de la presente solicitud, puesto que ninguno de éstos revela la combinación revelada y mucho menos la posibilidad de un efecto sinérgico.

De acuerdo con lo anterior se tiene que:

- Ni D1 ni D2 ni su combinación revelan la composición farmacéutica reivindicada o el posible efecto sinérgico;
- La composición reivindicada ofrece un efecto sinérgico, es decir, una ventaja o mejora con respecto al arte previo; y

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- El efecto sinérgico no es un resultado esperado.

Consecuentemente, teniendo en cuenta que D1 y D2 no son anterioridades válidas capaces de afectar el nivel inventivo de la presente solicitud, consideramos que la objeción formulada a este respecto se debe tener por superada.

Solicitamos atentamente tener en cuenta los argumentos que se presentan y que demuestran que la presente solicitud tiene nivel inventivo.

5. 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.

### 6. ANEXOS

- Nuevo pliego reivindicatorio conformado por 2 reivindicaciones.
- Formulario de modificaciones
- Copia del artículo: Porter et al. "Combination Therapy in Rheumatoid Arthritis - No Benefit of Addition of Hydroxychloroquine to Patients with a Suboptimal Response to Intramuscular Gold Therapy", J. Rheumatol., 1993, 20, p. 645-649
- Sustitución de poder otorgado por el Doctor Emilio FERRERO a mi favor, y en consecuencia solicito me sea reconocida personería para proseguir con el trámite de este asunto a favor de la sociedad solicitante.

Señora Directora,

*Luz Clemencia de Páez*  
**LUZ CLEMENCIA DE PÁEZ**

C.C. 35.456.344 de Usaquén

T.P.A. No. 23.555

COLO-14419-841-002-5  
JMS\JMS\ID-168375\WPC14419  
No. M-2010-168375\JMS

Espacio reservado para el adhesivo de radicación

**FORMULARIO UNICO DE CORRECCIONES Y MODIFICACIONES**

1

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

2

**SOLICITANTE****Nombre:** CHUGAI SEIYAKU KABUSHIKI  
KAISHASociedad constituida y existente bajo las leyes de  
Japón.**Dirección:** 5-1 Ukima 5-chome,  
Kita-ku,  
Tokio 1158543,  
Japón**Domicilio:** Tokio,  
Japón**Teléfono:** 546 09 70 **Fax:** 249 40 70**E-mail:** [clientes@cavelier.com](mailto:clientes@cavelier.com)**IDENTIFICACION**C.C. ☐ NIT ☐  
C.E. ☐ Otro ☐**Número:** \_\_\_\_\_

3

**REPRESENTANTE  
O APODERADO****Nombre:** LUZ CLEMENCIA DE PÁEZ**Dirección:** Carrera 4ª. No. 72-35**Teléfono:** 347 36 11**Fax:** 211 86 50**E-mail:** [clientes@cavelier.com](mailto:clientes@cavelier.com)**Domicilio:** Bogotá, Colombia.**IDENTIFICACIÓN**C.C. ☒ NIT ☐  
C.E. ☐ Otro ☐**Cual** \_\_\_\_\_**Número** 35.456.344 T. P. 23.555

4

**Número de expediente:** 05.109.276

5

**Descripción de la corrección o modificación solicitada:**

Presento nuevo capítulo reivindicatorio compuesto por 2 reivindicaciones con el fin de superar las objeciones hechas por Su Despacho a la presente solicitud de patente y pido respetuosamente que el examen de patentabilidad sea realizado sobre estas nuevas reivindicaciones presentadas.

6

**Comprobante de pago No.** \_\_\_\_\_**Fecha:** \_\_\_\_\_

7

**Anexos**

- ☐ Comprobante de pago de la tasa por \$114.000, por concepto de modificaciones.
- ☐ Poderes, si fuere el caso
- ☐ Documento que acredita la existencia y representación legal de las partes, cuando sean personas jurídicas

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NOMBRE: LUZ CLEMENCIA DE PÁEZ

FIRMA: *Luz Clemencia de Páez*

C.C. 35.456.344 de Usaquén T.P.A. 23.555

JMS

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Señores

**SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO**

E. S. D.

Poderdante : **CHUGAI SEIYAKU KABUSHIKI KAISHA**

Poder conferido el : 16/09/2004

Yo, EMILIO FERRERO, obrando como apoderado principal del poderdante arriba mencionado, mediante el presente escrito sustituyo el poder a mi otorgado a la doctora LUZ CLEMENCIA DE PAEZ, identificada con cédula de ciudadanía No. 35.456.344 y Tarjeta Profesional N°23.555, con todas las facultades que me fueron conferidas.

Hago la anterior sustitución conforme al artículo 66 del Código de Procedimiento Civil.

Señora Jefe,



EMILIO FERRERO

T.P.A. N° 31.929

C.C. N° 438.019 de Usaquén

Acepto:



LUZ CLEMENCIA DE PAEZ

T.P.A. N° 23.555

C.C. No. 35.456.344 de Usaquén



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## REIVINDICACIONES

1. Una composición farmacéutica que comprende un antagonista de interleukin-6 y un inmunosupresor, caracterizada porque el antagonista de interleukin-6 es un anticuerpo receptor anti-interleukin-6 (anti-IL-6R), el cual es un anticuerpo PM-1 y el inmunosupresor es metotrexato (MTX).
2. La composición farmacéutica de acuerdo con una de la reivindicación 1, caracterizada porque dicho anticuerpo anti-IL-6R, el cual es un anticuerpo PM-1, es un anticuerpo quimérico, un anticuerpo humanizado o un anticuerpo de tipo humano.

JMS\JMS\ID-168417\WPC14419

- El Ac. carad. eson. composición leq. debe definirse por su estructura, ~~es~~ cat. ligera y pesada o los 6 CDR's.  
- No tipo de hibridoma

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# Combination Therapy in Rheumatoid Arthritis — No Benefit of Addition of Hydroxychloroquine to Patients with a Suboptimal Response to Intramuscular Gold Therapy

DUNCAN R. PORTER, HILARY A. CAPELL, and JOHN HUNTER

**ABSTRACT.** *Objective.* To determine if there is any advantage in adding hydroxychloroquine to intramuscular gold therapy in patients with rheumatoid arthritis (RA) with a suboptimal response to gold after 6 months of treatment.

*Methods.* Prospective double blind placebo controlled study at the Centre for Rheumatic Diseases, Glasgow Royal Infirmary and Gartnavel General Hospital, Glasgow. Patients — 440 patients with RA began intramuscular gold therapy. One hundred forty-two patients with a suboptimal response at 6 months were randomized to receive additional treatment with hydroxychloroquine (400 mg/day) or placebo, and followed for a further 6 months. Outcome measures were erythrocyte sedimentation rate, C-reactive protein, Ritchie articular index, grip strength, visual analog pain score, duration of morning stiffness, health assessment questionnaire, and rate of side effects.

*Results.* There was no difference in outcome in terms of efficacy or toxicity.

*Conclusion.* There is no justification for using a combination of intramuscular gold and hydroxychloroquine in patients with RA with a partial response to gold. (*J Rheumatol* 1993;20:645-9)

**Key Indexing Terms:**

GOLD

COMBINATION THERAPY

HYDROXYCHLOROQUINE  
RHEUMATOID ARTHRITIS

Disease modifying antirheumatic drug (DMARD) therapy in rheumatoid arthritis (RA) is of limited efficacy in some patients, and the role of combination therapy is controversial<sup>1</sup>. Some authorities currently advocate the routine use of combination therapy despite a paucity of evidence to support this approach<sup>2</sup>. There have been several open, uncontrolled trials<sup>3,4</sup>, but very few controlled trials<sup>5-8</sup> to assess the efficacy and safety of this treatment strategy. Gold and hydroxychloroquine (HCQ) combination therapy is widely prescribed — 45% of Canadian rheumatologists have used this combination<sup>9</sup> — but evidence for this approach is lacking.

The protocol for trials in combination DMARD therapy is important<sup>1</sup>. Some patients make an excellent response in the short term to DMARD therapy, and the risk of additional toxicity from a 2nd DMARD from the outset is not justifiable in these patients. One alternative approach is to assess the effect of a 2nd DMARD in patients with a suboptimal response to another. We have conducted a double blind controlled trial of the addition of either HCQ, or placebo to

patients with a suboptimal response to gold therapy after 6 months. This combination of drugs was chosen since in general their side effect profiles differ, thus facilitating management decisions should adverse events supervene.

## MATERIALS AND METHODS

Four hundred forty patients with RA (revised ARA criteria<sup>10</sup>) were deemed to require DMARD therapy on the basis of active synovitis, or prolonged morning stiffness, with or without a raised erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP). IM gold therapy was given at a dose of 50 mg/week until a response was achieved, when the frequency of injections was reduced. One hundred forty patients stopped IM gold therapy before 6 months, the majority because of the side effects (Figure 1). After 6 months of treatment, the patients still receiving treatment were assessed by a clinical interview and examination, and the response to gold therapy was assessed, according to the criteria shown in Table 1A. Patients who had a good response to therapy were not considered for combination therapy (110 patients). One hundred ninety patients with a suboptimal response were identified. Forty-eight of these patients refused to consent to the trial, mainly because they felt much better than at the time of starting gold and did not wish to risk an additional drug, or were not offered enrollment (protocol violations); the remaining 142 patients gave informed consent, and were randomized to receive either HCQ (400 mg/day) or placebo (2 matching tablets) in addition to their gold therapy. The dose of gold therapy was altered at the discretion of the attending rheumatologist. Clinical and laboratory variables of disease activity were measured at the start of gold therapy (Gold 0), at randomization (Combined 0), and after 6 months of combined therapy (Combined 6) by a single, experienced nurse metrologist. The metrologist, physicians and patients were all blinded to the additional treatment received.

The measures of disease activity used were Ritchie articular index (AI), duration of morning stiffness (MS), grip strength, health assessment questionnaire (HAQ score), visual analog pain score (VAS), erythrocyte sedimentation rate (ESR).

From the Centre for Rheumatic Diseases, Glasgow Royal Infirmary, and Gartnavel General Hospital, Glasgow, Scotland.

D.R. Porter, MRCP, Rheumatology Registrar, Centre for Rheumatic Diseases; H.A. Capell, FRCP, Consultant Rheumatologist, Centre for Rheumatic Diseases; J. Hunter, FRCP, Consultant Rheumatologist, Gartnavel General Hospital.

Address reprint requests to Dr. D. Porter, Centre for Rheumatic Diseases, Glasgow Royal Infirmary, Glasgow G4 0SF, Scotland.  
Submitted March 31, 1992 revision accepted September 11, 1992.

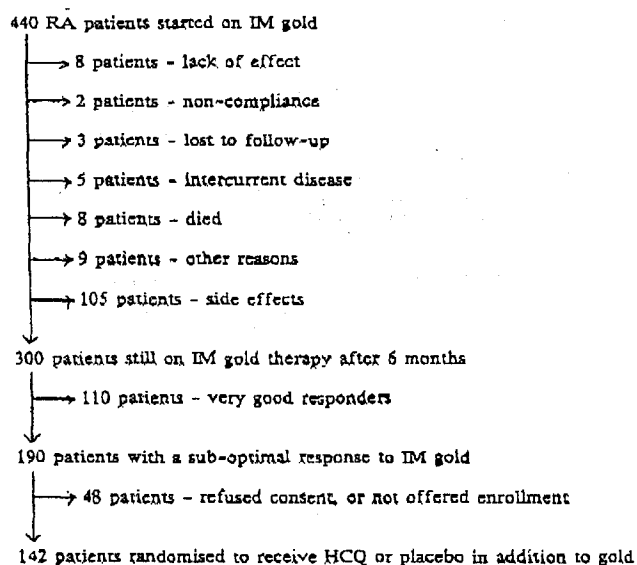


Fig. 1. Treatment details of study patients with RA.

tation rate (ESR) and C-reactive protein (CRP). Adverse effects were documented, and therapy discontinued if intolerable or potentially serious side effects arose. Patients were assessed at Combined 0 and Combined 6 by an ophthalmologist who was also blinded to the patient's treatment.

No patient received systemic corticosteroids or other DMARD at any time during gold therapy or in the 3 months preceding the start of gold therapy.

**Statistical analysis.** For between groups comparisons we used Mann-Whitney analysis, and paired Wilcoxon matched pairs signed rank tests were used for within groups analyses. The group of good responders was compared to the suboptimal responders at Comb 0 to assess whether the entry criteria were successful in selecting a group of patients with a suboptimal response. Only patients continuing with their assigned therapy were included in the analysis at Comb 6, and intention to treat analysis was not performed.

## RESULTS

Analysis of clinical laboratory variables at the time of allocation to HCQ/placebo (Combined 0) confirmed that the entry criteria employed had succeeded in separating those patients with a good response from those with a suboptimal response. The good responders had a median ESR and CRP within the normal range (17 mm/h and < 10 mg/l, respec-

Table 1A. Criteria used for assessing response to gold therapy after 6 months

| Good Response                                                                                                    | Suboptimal Response                                                 |
|------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------|
| ESR < 30 mm/h AND                                                                                                | ESR > 30 mm/h OR                                                    |
| CRP < 20 mg/l AND                                                                                                | CRP > 20 mg/l OR                                                    |
| Articular index < 8 AND                                                                                          | Articular index > 8 OR                                              |
| am stiffness < 15 min                                                                                            | am stiffness > 15 min                                               |
| OR                                                                                                               | AND                                                                 |
| Patient and physician agreed that response was very good, such that neither would contemplate additional therapy | Patient and physician agreed that further improvement was possible. |

Table 1B. Clinical and laboratory features in good and suboptimal responders at outset and after 6 months of gold (medians and ranges)

|                | Too Good<br>n = 110 |               | Suboptimal<br>n = 142 |              |
|----------------|---------------------|---------------|-----------------------|--------------|
|                | 0                   | 6 mths        | 0                     | 6 mths       |
| ESR (mm/h)     | 54<br>0-150         | 17<br>0-55    | 60<br>2-150           | 39<br>1-150  |
| CRP (mg/l)     | 36<br>10-193        | 10<br>3-56    | 45<br>10-256          | 18<br>6-168  |
| AI             | 11<br>0-39          | 3<br>0-33     | 14<br>0-42            | 8<br>0-27    |
| Grip L (mm Hg) | 80<br>36-204        | 106<br>36-300 | 70<br>36-205          | 76<br>36-220 |
| Grip R (mm Hg) | 76<br>38-300        | 100<br>40-300 | 70<br>36-290          | 76<br>40-300 |
| MS (min)       | 90<br>0-720         | 5<br>0-720    | 120<br>0-720          | 60<br>0-720  |
| HAQ            | 1.75                | 1.75          | 2.12                  | 2.0          |
| Pain (VAS)     | 18<br>4-28          | 9<br>0-30     | 18<br>4-30            | 15<br>0-30   |

Wilcoxon within groups — all values for both groups  $p < 0.0001$ .

Mann Whitney between groups at time 0 — AI, morning stiffness and HAQ higher in group subsequently randomized  $p < 0.01$ , other variables NS. Mann Whitney between groups at 6 months — all variables in suboptimal group significantly worse than too good group  $p < 0.0001$ .

tively), and all variables were highly significantly lower in this group when compared with the patients who were randomized ( $p < 0.0001$ , Table 1B). Not all of the good responders had normal values of all variables, but only 14% had ESR > 30, 13% had CRP > 20, 8% had articular index > 8, and 23% had morning stiffness > 15 min. The degree of response to im gold was not determined by the total dose of gold received, since the good responders had received no more injections than the suboptimal responders (median cumulative dose after 6 months = 960 mg in all 3 groups, i.e., those too good for additional treatment ( $n = 110$ ), those allocated to gold + HCQ ( $n = 72$ ), and those allocated to gold + placebo ( $n = 70$ )).

Before gold therapy started, those patients destined to be randomized to the Gold/HCQ group tended to have more active disease than those assigned to placebo. There was a trend for all the measures of disease activity to be higher in the Gold/HCQ group, although this only reached statistical significance in grip strength of the left hand and HAQ score. However, after 6 months of gold therapy, at randomization, (Comb 0) the Gold/HCQ and Gold/placebo groups were well matched for age (median age Gold/HCQ = 58 vs 60 years Gold/placebo) and disease duration (median disease duration Gold/HCQ = 6 vs 5 years Gold/placebo). Disease activity in the 2 groups was also similar, except for significantly higher AI in the Gold/HCQ group (median 10.5 vs 7,  $p < 0.05$  [Table 2]). After 6 months of combined treatment there were no differences between the groups for any variable (Table 2), nor in the mean change in any variable (data not shown). Within group analysis showed that between

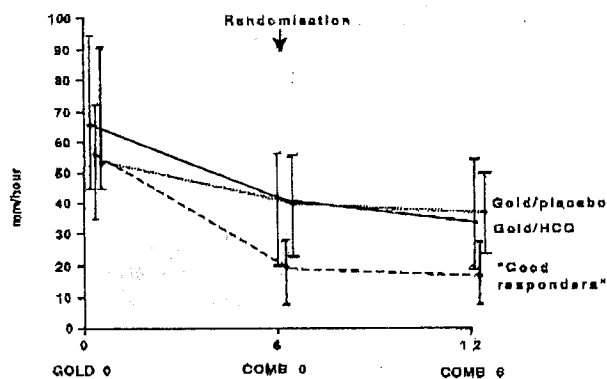
278  
220

Fig. 2A. Median (interquartile ranges) ESR at Gold 0, Comb 0 and Comb 6.

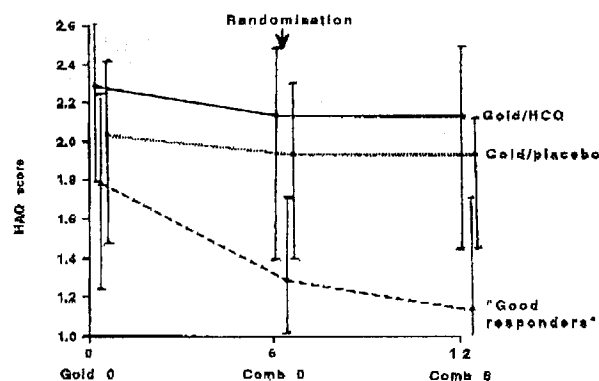


Fig. 2B. Median (interquartile ranges) HAQ score at Gold 0, Comb 0 and Comb 6.

Combined 0 and Combined 6, the Gold/HQC group improved significantly in AI ( $p < 0.005$ ), left hand grip strength ( $p < 0.05$ ) and morning stiffness ( $p < 0.02$ ); in the Gold/placebo group morning stiffness significantly improved ( $p < 0.05$ ). Over the 6 months of combined therapy, the Gold/HQC group improved more in 3 variables (ESR, AI, grip strength), and the Gold/placebo group improved more in 3 (CRP, morning stiffness, pain score) (Table 2). Both groups received very similar cumulative doses of gold by one year (median cumulative dose Gold/HQC = 1450 mg vs 1510 mg Gold/placebo).

The reasons for stopping therapy are shown in Table 3. If both gold and HCQ (or placebo) were stopped at the same time, it was not possible to be confident which drug was responsible for the side effect, and the cause is listed under both drugs. Of the Gold/HQC group 12.5% and of the gold/placebo group 16% stopped gold therapy; 17% of the Gold/HQC group stopped HCQ, and 21% of the Gold/placebo group stopped taking placebo (for side effects in 9%).

## DISCUSSION

One of the features to emerge from our study was that evaluation of combination therapy in this way is by no means straightforward. It proved difficult to define a suboptimal response in a global fashion but the results gave unequivocal evidence that the groups with which we were dealing had different responses to the initial 6 months of gold treatment. Although the patients randomized to gold + HCQ or gold + placebo were selected on the basis of a suboptimal

Table 2. Median (interquartile range) of all variables

| Month           |          | Gold 0         | Comb 0<br>(Gold 6) | Comb 6        |
|-----------------|----------|----------------|--------------------|---------------|
| Age             | Gold/HQC | 58 (48,65)     |                    |               |
| (years)         | Gold/Pbo | 60 (52,65)     |                    |               |
| Disease dur.    | Gold/HQC | 6 (2,12)       |                    |               |
| (years)         | Gold/Pbo | 5 (3,11)       |                    |               |
| ESR             | Gold/HQC | 64 (45,94)     | 39 (18,54)         | 32 (18,55)    |
| (mm/h)          | Gold/Pbo | 52 (44,90)     | 38 (23,53)         | 35 (24,50)    |
| CRP             | Gold/HQC | 49 (26,76)     | 16 (10,37)         | 18 (10,35)    |
| (mg/l)          | Gold/Pbo | 45 (24,78)     | 20 (12,36)         | 16 (10,27)    |
| Grip strength   | Gold/HQC | 66 (48,88)     | 76 (60,100)        | 80 (60,102)   |
| (mm Hg) R       | Gold/Pbo | 70 (58,90)     | 80 (60,100)        | 80 (70,106)   |
| Grip strength   | Gold/HQC | 64 (50,80)*    | 71 (56,110)        | 80 (58,106)†  |
| (mm Hg) — L     | Gold/Pbo | 76 (60,92)     | 78 (64,100)        | 81 (68,100)   |
| Articular index | Gold/HQC | 17 (11,23)     | 11 (5,15)*         | 6 (3,12)†     |
|                 | Gold/Pbo | 12 (8,19)      | 7 (3,12)           | 6 (3,10)      |
| am Stiffness    | Gold/HQC | 180 (60,300)   | 60 (30,150)        | 60 (15,120)†  |
| (min)           | Gold/Pbo | 120 (60,240)   | 60 (15,120)        | 30 (10,60)†   |
| HAQ score       | Gold/HQC | 2.3 (1.8,2.6)* | 2.1 (1.4,2.5)      | 2.1 (1.5,2.5) |
|                 | Gold/Pbo | 2.0 (1.5,2.4)  | 1.9 (1.4,2.3)      | 1.9 (1.4,2.1) |
| Pain score      | Gold/HQC | 19 (15,24)     | 15 (8,20)          | 15 (9,20)     |
|                 | Gold/Pbo | 18 (15,22)     | 15 (10,18)         | 14 (9,18)     |

\* =  $p < 0.05$  Mann-Whitney Gold/HQC vs Gold/placebo.

† =  $p < 0.05$  paired Wilcoxon Comb 0 vs Comb 6.

Gold/HQC — Comb 0,  $n = 72$ ; Comb 6,  $n = 57$ .

Gold/placebo — Comb 0,  $n = 70$ ; Comb 6,  $n = 55$ .

Table 3. Reasons for discontinuing therapy during combination therapy

|                     | Gold/HQC Group<br>n = 72 |     | Gold/Placebo Group<br>n = 70 |         |
|---------------------|--------------------------|-----|------------------------------|---------|
|                     | Gold                     | HQC | Gold                         | Placebo |
| Lack/loss of effect | 1                        | 0   | 1                            | 3       |
| Rash                | 2                        | 1   | 2                            | 2       |
| Mouth ulcers        | 1                        | 0   | 1                            | 0       |
| Thrombocytopenia    | 1                        | 1   | 0                            | 0       |
| Proteinuria         | 4                        | 0   | 5                            | 0       |
| Diarrhea            | 0                        | 1   | 0                            | 0       |
| Ophthalmological*   | 0                        | 6   | 0                            | 1       |
| Headache            | 0                        | 2   | 0                            | 2       |
| Nausea              | 0                        | 0   | 0                            | 3       |
| Other**             | 0                        | 1   | 2                            | 4       |

\* 4 patients did not begin HCQ at the advice of the ophthalmologist because of preexisting senile macular degeneration (n = 1), diabetic maculopathy (n = 1), undiagnosed maculopathy (n = 1), and poor visual acuity (n = 1); 3 patients stopped their own therapy within a month of starting, because of blurred vision (n = 2) and painful eyes (n = 1).

\*\* noncompliance, lost to followup.

response to gold therapy, there had been a highly significant improvement in almost all the variables of disease activity after 6 months of gold. There was very little further improvement over the next 6 months, with or without additional HCQ therapy, and it seems that combined therapy does not confer any significant additional benefit over gold therapy alone during this period. The patients initially considered too good for combination therapy were still much better than the other groups at the end of the 6 month combination period. There was, however, no detectable increased toxicity associated with combined therapy.

At the outset of gold therapy the 2 groups subsequently assigned to combination were not as closely matched as they were at randomization, but it is not clear what relevance this has. It could be argued that the Gold/HQC had more severe disease, compensating for an additional benefit of HCQ; alternatively, it could be argued that if the Gold/HQC group started with more severe disease, but had comparable disease activity at randomization, these patients must have had a better response to gold. This might be expected to continue over the next 6 months of gold, producing a bias in favor of the combined group.

The only other double blind placebo controlled trial of gold plus HCQ therapy concluded that there may be a 20-25% superiority in efficacy of the combination over gold alone, at the expense of 80% more toxicity, but the trial lacked the statistical power to confirm this<sup>5</sup>. The trial used a different protocol, in which the patients were randomized to gold plus HCQ or placebo at the outset of the gold therapy. As the authors acknowledge, the difficulty with this approach is that large numbers of patients stop gold therapy because of side effects in the first 6 months of therapy; and others have such an excellent response that additional DMARD therapy is not

needed. The result is 2-fold: first, the trial has a high dropout rate, and secondly some patients are unnecessarily exposed to the side effects of a 2nd drug.

In our study the response of the Gold/HQC and Gold/placebo groups were so similar that it is very unlikely that there had been a clinically significant Type II error. Standard estimates for determining the sample size when the trial was being designed were attempted despite little data in the literature about the expected response to im gold over the 2nd 6 months of treatment on which to base such estimates. Our statistician calculated that to show a difference of 20% between the 2 treatment groups with a power of 80% we would need 86 patients to complete the combination assessment period (43 in each group). We became aware during this study of the considerable difficulties of defining a sub-optimal response in a way which satisfied patient, physician and statistician. For that reason we continued recruiting beyond the stage when 86 patients might be expected to complete the study in order to ensure that a Type II error was avoided.

Other controlled studies of combination therapy have also yielded negative results.

A double blind study of HCQ vs penicillamine vs combined penicillamine/HQC has been performed, with the surprising result of reduced efficacy of combination therapy compared to penicillamine alone<sup>6</sup>; the trial used small numbers and the findings have not been confirmed in a one year study of 72 patients with early RA<sup>7</sup>. It seems that there is no benefit in using this combination. Williams, *et al*<sup>8</sup> were unable to demonstrate any benefit from the combination of methotrexate and auranofin over either drug used alone.

Before the use of combination therapy in RA becomes more widespread, further well constructed controlled trials are urgently needed. Proponents of combination therapy must be careful to perform a thorough examination of the benefit/risk ratio, and the temptation to use unproven treatment regimens on the basis of anecdotal report or uncontrolled studies should be strongly resisted.

## ACKNOWLEDGMENT

Sterling Winthrop kindly provided hydroxychloroquine and placebo. We are grateful to our metrologist Mrs. Anne Thomson, our computing assistant Mrs. D. McKnight, and to Professor R. Sturrock for allowing us to study his patients. Our thanks are also due to Miss A. Tierney who typed the manuscript and to all medical and nursing staff who helped in the care of this large group of patients.

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US 20010001663A1

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231(19) **United States**(12) **Patent Application Publication**  
**Kishimoto et al.**(10) **Pub. No.: US 2001/0001663 A1**(43) **Pub. Date: May 24, 2001**(54) **CHRONIC RHEUMATOID ARTHRITIS  
THERAPY CONTAINING IL-6 ANTAGONIST  
AS EFFECTIVE COMPONENT**(76) **Inventors: Tadamitsu Kishimoto, Osaka (JP);  
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**Washington, DC 20007-5109 (US)**(21) **Appl. No.: 09/756,125**(22) **Filed: Jan. 9, 2001****Related U.S. Application Data**(60) **Division of application No. 09/233,474, filed on Jan.  
20, 1999, which is a division of application No.  
08/817,084, filed on Apr. 7, 1997, now Pat. No.**

5,888,510, which is a continuation of application No. PCT/JP95/01144, filed on Jun. 7, 1995 and which is a continuation-in-part of application No. 08/971,997, filed on Feb. 21, 1997, now abandoned, which is a continuation of application No. 08/268,520, filed on Jun. 30, 1994, now abandoned.

(30) **Foreign Application Priority Data**

Oct. 7, 1994 (JP) ..... 6-244035

**Publication Classification**(51) **Int. Cl.<sup>7</sup> ..... A61K 39/395**  
(52) **U.S. Cl. .... 424/141.1**(57) **ABSTRACT**

There is provided a synovial cell growth inhibitor, or a pharmaceutical composition for treatment of chronic rheumatoid arthritis based on the synovial cell growth inhibitor.

The pharmaceutical composition for treatment of chronic rheumatoid arthritis or synovial cell growth inhibitor contains an IL-6 antagonist, such as IL-6 antibody or IL-6R antibody, as an effective component.

exist regarding the relationship between IL-6 and chronic rheumatoid arthritis, and the relationship is as yet unclear.

[0013] Recently, Wendling et al. have reported that administration of anti-IL-6 antibodies to chronic rheumatoid arthritis patients temporarily alleviates the clinical and biological symptoms, while also increasing IL-6 levels in the serum (*J. Rheumatol.* 20:259-262, 1993).

[0014] These reports provide no data at all about whether IL-6 accelerates growth of chronic rheumatoid arthritis synovial cells or has an inhibitory effect, and thus it is still unknown whether or not IL-6 has a direct effect on synovial cells of chronic rheumatoid arthritis patients.

#### DISCLOSURE OF THE INVENTION

[0015] Anti-inflammatory steroidal agents such as corticosteroids have been used as rheumatoid arthritis

#### BRIEF DESCRIPTION OF THE DRAWINGS

[0016] FIG. 1 is a graph showing  $^3\text{H}$ -thymidine uptake into synovial cells in the presence of either IL-6 or sIL-6R alone and in the presence of both IL-6 and sIL-6R.

[0017] FIG. 2 is a graph showing the effect of IL-6 antibody or IL-6R antibody on  $^3\text{H}$ -thymidine uptake into synovial cells in the presence of both IL-1 $\beta$  and sIL-6R.

[0018] FIG. 3 is a graph showing the effect of IL-6 antibody or IL-6R antibody on  $^3\text{H}$ -thymidine uptake into synovial cells in the presence of both IL-6 and sIL-6R.

[0019] FIG. 4 is a graph showing the suppressive effect of IL-6R antibody on the onset of mouse collagen-induced arthritis models.

[0020] FIG. 5 is a graph showing serum anti-collagen antibody levels in arthritic mice.

[0021] FIG. 6 is a photograph of histopathological examination of hind paw joint of a collagen-arthritis mouse. (a) is a photograph from a mouse in an IL-6 receptor antibody-administered group, and (b) is from a mouse in a control antibody-administered group. In the IL-6 receptor antibody-administered group, invasion of granulation tissue into the cartilage and bone (chronic proliferative synovitis) was clearly suppressed.

#### DETAILED DESCRIPTION OF THE INVENTION

[0022] A pharmaceutical composition for treatment of a chronic rheumatoid arthritis according to the invention is a drug which when administered to chronic rheumatoid arthritis patients suppresses growth of synovial cells in joints and has an alleviating and therapeutic effect on the symptoms.

[0023] The IL-6 antagonist used according to the invention may be derived from any source so long as it is a substance which blocks IL-6 signal transfer and inhibits IL-6 biological activity. IL-6 antagonists include IL-6 antibody, IL-6R antibody, gp130 antibody, modified IL-6, antisense IL-6R and partial peptides of IL-6 or IL-6R.

[0024] An antibody used as an antagonist according to the invention, such as IL-6 antibody, IL-6R antibody or gp130 antibody, may be of any derivation or type (monoclonal, polyclonal), but monoclonal antibodies derived from mam-

malian animals are especially preferred. These antibodies bind to IL-6, IL-6R or gp130 to inhibit binding between IL-6 and IL-6R or IL-6R and gp130 and thus block IL-6 signal transduction, inhibiting IL-6 biological activity.

[0025] The animal species for the monoclonal antibody-producing cells is not particularly limited so long as it is a mammal, and human antibodies or antibodies derived from a mammal other than human may be used. Monoclonal antibodies derived from a mammal other than human are preferably monoclonal antibodies derived from rabbits or rodents because they are easier to prepare. There is no particular restriction on the rodents, but preferred examples are mice, rats and hamsters.

[0026] Examples of such antibodies which are IL-6 antibodies include MH166 (Matsuda et al., *Eur. J. Immunol.* 18:951-956, 1988) and SK2 antibody (Sato et al., *Journal for the 21st General Meeting of the Japan Immunology Association*, 21:116, 1991). Examples of IL-6R antibodies include PM-1 antibody (Hirata et al., *J. Immunol.* 143:2900-2906, 1989), AUK12-20 antibody, AUK64-7 antibody and AUK146-15 antibody (Intl. Unexamined Patent Application No. WO92-19759). An example of gp130 antibody is AM64 antibody (Japanese Unexamined Patent Publication No. 3-219894).

[0027] Among these, PM-1 antibody is preferred.

[0028] Monoclonal antibodies may be prepared in the following manner which is based on a known technique. That is, IL-6, IL-6R or gp130 is used as the sensitizing antigen for immunization according to a conventional immunizing method, and the resulting immunocytes are then fused with known parent cells by a conventional cell fusion method and monoclonal antibody-producing cells are screened by a conventional screening method to prepare the antibodies.

[0029] More specifically, the monoclonal antibodies may be prepared in the following manner. For example, if the sensitizing antigen is human IL-6, the antibodies are obtained using the gene sequence for human IL-6 disclosed by Hirano et al., *Nature*, 324:73, 1986. The human IL-6 gene sequence is inserted into a publicly expression vector system and used to transform suitable host cells, after which the desired IL-6 protein is purified from the host cells or from the culture supernatant and the purified IL-6 protein is then used as the sensitizing antigen.

[0030] In the case of human IL-6R, the IL-6R protein may be obtained by the same method as for human IL-6 described above, using the gene sequence disclosed in European Patent Application No. EP325474. Two types of IL-6R exist, one expressed on the cell membrane and a soluble form (sIL-6R) which is separated from the cell membrane. sIL-6R consists mainly of the extracellular domain of IL-6R which is attached to the cell membrane, and it differs from the membrane-bound IL-6R in that it lacks the transmembrane domain or the transmembrane domain and the intracellular domain.

[0031] In the case of human gp130, the gp130 protein may be obtained by the same method as for human IL-6 described above, using the gene sequence disclosed in European Patent Application No. EP411946.

[0032] The mammalian animals immunized with the sensitizing antigen are not particularly restricted, but they are

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with 0.005 ml of the  $^{35}\text{S}$ -labelled IL-6R solution prepared earlier. The immunoprecipitated substance was analyzed by SDS-PAGE, and the hybridoma culture supernatants reacting with IL-6R were examined. As a result, a reaction-positive hybridoma clone PM-1 was established. The IL-6R antibody PM-1 produced by hybridoma PM-1 has an IgG1K subtype.

[0075] The inhibiting activity of the antibody produced by hybridoma PM-1 against binding of IL-6 to human IL-6R was investigated using the human myeloma cell line U266. Human recombinant IL-6 was prepared with *E. coli* (Hirano et al., Immunol. Lett., 17:41, 1988) and  $^{125}\text{I}$ -labelled with Bolton-Hunter reagent (New England Nuclear, Boston, Mass.) (Taga et al., J. Exp. Med. 166:967, 1987).

[0076]  $4 \times 10^5$  U266 cells were cultured at room temperature in the presence of a 100-fold excess of non-labelled IL-6 for one hour, together with 70% (v/v) of hybridoma PM-1 culture supernatant and 14000 cpm of  $^{125}\text{I}$ -labelled IL-6. A 70  $\mu\text{l}$  sample was overlaid onto 300  $\mu\text{l}$  of FCS placed in a 400  $\mu\text{l}$  microfuge polyethylene tube, and after centrifugation the radioactivity on the cells was measured.

[0077] As a result it was demonstrated that the antibodies produced by hybridoma PM-1 inhibited binding of IL-6 to IL-6R.

#### Reference Example 4. Preparation of mouse IL-6 receptor antibody

[0078] Monoclonal antibodies against mouse IL-6 receptor were prepared by the method described in Japanese Patent Application No. 6-134617.

[0079] Following the method of Saito et al. (J. Immunol., 147, 168-173, 1993), CHO cells producing mouse soluble IL-6 receptor were cultured in IMDM medium containing 10% FCS, and the mouse soluble IL-6 receptor was purified from the culture supernatant using the mouse soluble IL-6 receptor antibody RS12 (see *ibid.* Saito et al.) and an affinity column immobilizing Affigel 10 gel (Biorad).

[0080] A 50  $\mu\text{g}$  of the obtained mouse soluble IL-6 receptor was mixed with Freund's complete adjuvant and intraperitoneally injected into wistar rats (Nihon Charles River Co.). Booster immunizations were given with Freund's incomplete adjuvant after 2 weeks. On the 45th day the rats were butchered, and about  $2 \times 10^8$  splenic cells thereof were used for cell fusion with  $1 \times 10^7$  mouse P3U1 myeloma cells by a conventional method utilizing 50% PEG1500 (Berlinger Mannheim), after which the hybridomas were screened with HAT medium.

[0081] After adding the hybridoma culture supernatants to an immunoplate coated with rabbit anti-rat IgG antibody (Cappel Co.), mouse soluble IL-6 receptor was reacted therewith and the hybridomas producing antibodies against mouse soluble IL-6 receptor were screened by the ELISA method using rabbit anti-mouse IL-6 receptor antibody and alkali phosphatase-labelled sheep anti-rabbit IgG. The hybridoma clones in which antibody production was confirmed were subjected to subscreening twice to obtain a single hybridoma clone. This clone was named MR16-1.

[0082] The neutralizing activity of the antibody produced by this hybridoma against mouse IL-6 signal transduction was investigated by incorporation of  $^3\text{H}$ -thymidine using

MH60.BSF2 cells (Matsuda et al., J. Immunol. 18, 951-956, 1988). MH60.BSF2 cells were added to a 96-well plate to  $1 \times 10^5$  cells/200  $\mu\text{l}$ /well, and then mouse IL-6 (10 pg/ml) and MR16-1 antibody or RS12 antibody were added to 12.3-1000 ng/ml prior to culturing at  $37^\circ\text{C}$ , in 5%  $\text{CO}_2$  for 44 hours, after which  $^3\text{H}$ -thymidine (1  $\mu\text{Ci}$ /well) was added and the uptake after 4 hours was measured. As a result, MR16-1 antibody was found to inhibit uptake of  $^3\text{H}$ -thymidine by MH60.BSF2 cells.

#### Experiment 1. Establishment of chronic rheumatoid arthritis-derived synovial cell line

[0083] (1) Preparation of synovial cells

[0084] Synovial tissue was obtained during surgical operation on the joint of a chronic rheumatoid arthritis patient. The synovial tissue was minced with scissors and then subjected to enzymatic dissociation by incubation for one hour at  $37^\circ\text{C}$  with 5 mg/ml of TYPE I collagenase (product of Sigma Chemical Co.) and 0.15 mg/ml of bovine pancreatic DNase (product of Sigma Chemical Co.) in IMDM (Iscoe's modified Dulbecco's medium), and passed through a mesh to obtain single cells. These obtained cells were then cultured overnight in a culture flask using IMDM containing 5% FCS, after which the non-adherent cells were removed to obtain the synovial cells. The synovial cells were passaged 3 to 6 times and used for the following experiment.

[0085] (2) IL-6 production by synovial cells

[0086] The synovial cells obtained as described above were suspended in IMDM culture medium containing 5% FCS (product of Hyclone Laboratories Inc.), 10 U/ml of penicillin G and 100  $\mu\text{g}$ /ml streptomycin to an amount of  $3 \times 10^5$  cells/well, and were then cultured in 96-well microtiter plate (product of Falcon Co.), which human interleukin- $1\beta$  (IL- $1\beta$ ), human tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), human platelet-derived growth factor (PDGF)AB and human basic fibroblast growth factor (bFGF) were added to concentrations of 0.01 or 0.1, 0.1 or 1, 1 or 10 and 1 or 10 ng/ml, respectively, and upon culturing at  $37^\circ\text{C}$  for 72 hours the culture supernatants were collected.

[0087] A 100  $\mu\text{l}$  of anti-human IL-6 antibody MH166 (1  $\mu\text{g}$ /ml) was added to a 96-well ELISA plate (Immunoplate: product of Nunc Co.) and incubated at  $4^\circ\text{C}$  for 24 hours. Each well was subsequently washed with PBS containing 0.05% Tween 20, and blocked at  $4^\circ\text{C}$  overnight with PBS containing 1% BSA. The culture supernatants obtained previously were then diluted with PBS containing 1% BSA, added to the wells, and then incubated at room temperature for 2 hours. After washing with PBS containing 0.05% Tween20, 2.5  $\mu\text{g}$ /ml of rabbit polyclonal anti-human IL-6 antibody purified with a 100  $\mu\text{l}$  protein A column (product of Pharmacia) was added.

[0088] After incubating at room temperature for 2 hours, the rabbit polyclonal anti-IL-6 antibody binding to IL-6 in the culture supernatants was reacted with alkali phosphatase-bound anti-rabbit IgG antibody (product of Tago Co.). And then 1 mg/ml of Sigma104 alkali phosphatase substrate (product of Sigma Co.) was added according to the attached instructions and the absorbance at 405-600 nm was measured with an MPR A4 microplate reader (product of Tosoh Co.).

[0089] Calibration curves were prepared for the recombinant IL-6 during each assay for conversion of the absor-

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Current Opinion in Molecular Therapeutics (2003) 5(1):64-69  
© Current Drugs ISSN 1464-8431

*Chugai, the Japanese subsidiary of Roche, is developing a humanized anti-interleukin (IL)-6 receptor monoclonal antibody MRA for the potential treatment of multiple myeloma, rheumatoid arthritis, Crohn's disease and other IL-6-related disorders. MRA is currently undergoing phase II clinical trials for these indications.*

### Introduction

Interleukin (IL)-6 is a pleiotropic cytokine with a wide range of biological activities, including regulation of immune responses, support of hematopoiesis and generation of acute-phase reactions [475335]. Deregulation of IL-6 production has been implicated in the pathogenesis of a variety of diseases. Chronic inflammation of the joint in rheumatoid arthritis (RA) induces IL-6 production by synovial cells, macrophages and lymphocytes in the affected synovium. Overproduction of IL-6 appears to be involved in the pathogenesis of pannus formation, angiogenesis, infiltration of mononuclear cells and destruction of cartilage and bone [475335], [475553], [475555]. IL-6 is also present at very high levels in the serum and/or related tissue from patients with Crohn's disease (CD) [475336], Castleman's disease [475337], multiple myeloma (MM) [475338] and systemic lupus erythematosus (SLE) [475341]; it may, therefore, play a crucial role in the pathogenesis of these diseases. As a result, therapy involving blockade of IL-6 functions may constitute a new therapeutic strategy.

The functions of IL-6 are mediated through a receptor system comprising two cell-surface molecules, a signal transducer and a binding molecule (the IL-6 receptor, IL-6R). Blockade of IL-6 binding to its receptor seems to be specific and effectively inhibits IL-6 functions. Chugai is therefore developing MRA, a humanized anti-IL-6R monoclonal antibody for potential use in the treatment of IL-6-related disorders, including RA, CD and Castleman's disease [459399], [466916], [469932].

### Synthesis and SAR

PM-1, a mouse monoclonal antibody against human recombinant IL-6R, was generated from a mouse immunized with IL-6R partially purified from the human myeloma cell line U266 [475342]. In humans, the production of neutralizing antibodies to mouse antibodies can be clinically problematic, and cause the effects of administered antibodies to be transient [154800]. To prevent the induction of such antibodies against the anti-IL-6R antibody, the PM-1 antibody was reshaped to create MRA [154800].

**Originator** Chugai Pharmaceutical Co Ltd

**Status** Phase II Clinical

**Action** IL-6 antagonist, Immunosuppressant

**Indication** Arthritis, Autoimmune disease, Castleman's disease, Crohn's disease, Myeloproliferative disorder, Rheumatoid arthritis, Systemic lupus erythematosus

**Biotechnology** Humanized monoclonal antibody

**Synonyms** Anti-interleukin-6 receptor mAb, hPM-1, IL-6 receptor MAb

**Registry No:** 339528-87-9

Complementarity-determining regions of the mouse PM-1 light and heavy chain variable regions were grafted to human REI and NEW framework regions (FRs), respectively. Template DNA was prepared using *NcoI*-*BamHI* fragments containing DNA sequences coding for the reshaped human light and heavy chain variable regions, which have FRs from human REI and NEW, respectively. These were then subcloned into the *HindIII*-*BamHI* site of pUC19 vector using a *HindIII*-*NcoI* adaptor. Using appropriate mutagenic PCR primers and template DNA, several versions were constructed. After DNA sequencing, the *HindIII*-*BamHI* fragments coding for reshaped human PM-1 variable regions were excised from pUC19 vectors and inserted in the *HindIII*-*BamHI* sites in human elongation factor expression vectors. Plasmid DNAs were transfected into COS cells for production of MRA and this antibody was purified. The ability to bind to IL-6R and the inhibitory effect of IL-6 function of the original PM-1 were conserved in the MRA molecule [154800]. Thus, the administration of MRA induces hardly any anti-MRA antibodies and it can be repeatedly administered [154800].

### Pharmacology

*In vitro* and *in vivo* data have shown that MRA blocks IL-6 functions in the cynomolgus monkey. MRA inhibited two functional parameters *in vitro* in this animal; T-cell proliferation stimulated by phytohemagglutinin and human IL-6, and IgG production evoked by *Staphylococcus aureus* Cowan-1- and human IL-6-stimulated B-lymphocytes [472428]. MRA (5 mg/kg iv) completely inhibited IL-6-induced typical responses (such as elevation of blood platelet counts and serum C-reactive protein (CRP) levels) in cynomolgus monkeys [472418], [472427].

The *in vivo* effect of MRA on the development of collagen-induced arthritis was examined in cynomolgus monkeys. MRA (10 mg/kg iv) given once weekly for 13 weeks significantly inhibited arthritis symptoms. The elevation of serum CRP and fibrinogen levels was also inhibited, as was erythrocyte sedimentation rate (ESR). Furthermore, radiographic and histological examination showed that MRA treatment suppressed joint destruction [472418]. In severe combined immunodeficiency (SCID) mice in which human RA

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

2020  
Bogotá, D.C.,

Abogado  
**LUZ CLEMENCIA DE PÁEZ**  
Apoderado

ASUNTO                      Radicación      05-109276  
                                 Trámite          002  
                                 Evento          001  
                                 Actuación      430  
                                 Oficio          10483  
                                 Folios          10

Patente de Invención      ☒      Patente de Modelo de Utilidad      ☐  
Vía Nacional                      ☐  
Vía PCT (fase nacional)      ☒      Cap. I      ☐      Cap. II      ☒  
No. Sol. Internacional              PCT/JP2004/006211  
Fecha de Presentación Internal.      28 de Abril de 2004

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**

Descripción:                      Folios: 61 a 104 y 115 como se radicó inicialmente  
Reivindicaciones:              Folios 105 a 114 como se radicó inicialmente  
                                 Folios 145 a 148 modificadas  
                                 Folio: 201 modificadas  
                                 Folio: 225 modificadas  
Prioridad:                      Folios: 1  
                                 28 de Abril de 2003, GB, 0309619.5  
Respuesta del solicitante:      Folios 193 a 200; Folios 216 a 221

**2. Análisis de la Prioridad**

La fecha de prioridad del 28 de Abril de 2003 y su correspondiente documento, son aceptados para el estudio de patentabilidad.

**3. Objeto de la invención**

El objeto de la presente solicitud de patente de invención consiste en métodos de tratamiento de enfermedades relacionadas con interleukina 6 mediante la combinación de un antagonista de interleukina-6 (antagonista IL-6) con inmunosupresores.

El problema planteado en esta solicitud es "obtener composiciones farmacéuticas para el tratamiento de enfermedades relacionadas con interleukina IL-6".

Para solucionar este problema técnico la solicitud en estudio proporciona una composición farmacéutica de un antagonista de interleukina -6 (antagonista IL-6) con inmunosupresores.

**Clasificación Internacional de Patentes (IPC):** A61K 39/505, A61K 39/395, A61K31/519

#### 4. De la solicitud de Patente

##### Descripción (artículo 28 D. 486)

No se encontraron soportadas las reivindicaciones con la suficiente información para que el experto en la materia, pueda realizar las composiciones farmacéuticas que contienen un anticuerpo anti-IL-6R y metotrexato.

A lo largo del capítulo descriptivo se encuentra sólo soportada la administración simultánea o con un intervalo de tiempo (Ver Folio 64-66), del anticuerpo anti-IL-6R y un inmunosupresor tal como metotrexato mediante combinación terapéutica, es decir referida a métodos de tratamiento. No se evidencia la composición farmacéutica que contenga ambos fármacos.

##### Reivindicaciones (artículo 30 D 486)

La reivindicación 1 referente a las composiciones farmacéuticas de un anticuerpo anti-IL-6R y metotrexato, no se encuentran sustentadas en la descripción.

La reivindicación 2 referente a las composiciones farmacéuticas que contienen el anti-IL-6R, el cual es un anticuerpo quimérico, humanizado o un anticuerpo de tipo humano, no se encuentran sustentadas en la descripción.

#### 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: 28 de Abril de 2003

Considerando que la fecha de presentación de la primera solicitud de patente de la cual se reivindica prioridad es: 28/04/2003 se encontraron los siguientes documentos del estado de la técnica relacionados, que pueden afectar la patentabilidad del objeto de la presente solicitud:

| Nº | Documento       | Título                                                                                 | Fecha de publicación |
|----|-----------------|----------------------------------------------------------------------------------------|----------------------|
| D4 | US 2001/0001663 | Chronic rheumatoid arthritis therapy containing IL-6 antagonist as effective component | 24/05/2001           |
|    | Methotrexate    |                                                                                        |                      |

## 6. Análisis de patentabilidad (artículo 14 D486)

### Nivel inventivo (artículo 18 D486)

La reivindicación 1 consiste en:

Una composición farmacéutica que comprende un antagonista de interleukin-6 y un inmunosupresor, caracterizada porque el antagonista de interleukin-6 es un anticuerpo receptor anti-interleukin-6 (anti-IL-6R), el cual es un anticuerpo PM-1 y el inmunosupresor es metotrexato (MTX).

El estado de la técnica más cercano es **D4**, pues enseña las composiciones farmacéuticas para el tratamiento de la artritis crónica reumatoide mediante el empleo de un antagonista IL-6, tal como un anticuerpo IL-6 o IL-6R como componente efectivo (Ver resumen) y donde el anticuerpo de preferencia es el PM-1 (Ver página 2, 0026-0027; página 6, 0077)

Las composiciones objeto de esta solicitud se diferencian de las composiciones de **D4**, en que adicionalmente contienen metotrexato.

El hecho de incluir metotrexato en las composiciones que se daban a conocer en **D4**, causa que las composiciones presenten un efecto sinérgico en el tratamiento de artritis reumatoide.

El Problema Técnico Objetivo era obtener composiciones farmacéuticas para el tratamiento de enfermedades relacionadas con interleukina IL-6.

**D3** describe una manera de solucionar el problema técnico, pues enseña que las citoquinas IL-6, entre otros, se encuentra entre las responsables de las manifestaciones de artritis reumatoide. Los efectos de la terapia de metotrexato sobre la respuesta y la producción de citoquinas, ha sido ampliamente estudiada en modelos animales, pacientes y células aisladas. (Ver página 73)

**D3** además enseña que una variedad de proteínas antiinflamatorias; han sido medidas en el suero de pacientes de AR tratados con metotrexato. De estas la que más consistentemente ha disminuido en el suero es IL-6. Las concentraciones en suero de IL-6, declinaron después de la terapia con metotrexato.

**D3** divulga los resultados de emplear metotrexato en AR frente a las interleuquinas IL-6, donde se obtiene que son reducidos durante el proceso de inflamación y cuyo efecto técnico logrado es reducir el proceso de la inflamación en artritis reumatoidea. (AR)

La reivindicación es obvia, porque el Experto en la Materia enfrentado al problema de obtener composiciones farmacéuticas para el tratamiento de enfermedades relacionadas con interleukina IL-6, incluiría metotrexato con el fin de declinar la cantidad de IL-6 que se revela en **D3**, dentro de las composiciones que se enseñan en **D4**, logrando de esta forma las composiciones farmacéuticas objeto de esta solicitud. Por tanto, la reivindicación 1 no tiene Nivel Inventivo. La reivindicación dependiente 2 no menciona alguna característica que haga inventivas a las

## 7. Conclusión:

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

| N° | Documento N°                                                            | Reivindicaciones afectadas | Requisito que afecta |
|----|-------------------------------------------------------------------------|----------------------------|----------------------|
| D4 | US 2001/0001663                                                         | 1-2                        | Nivel Inventivo      |
| D3 | Methotrexate,<br>B.N Cronstein, J.R.<br>Bertino, 2000, páginas<br>71-75 | 1-2                        | Nivel Inventivo      |

## 8. Respuesta a los argumentos del solicitante:

El solicitante se refiere en el Folio 217: "Se ha modificado la reivindicación 1 para indicar que el anticuerpo contra el receptor anti-interleukina-6 (IL-6) es un anti cuerpo PM-1" y "dicho anticuerpo es un anticuerpo quimérico, humanizado o de tipo humano"

De acuerdo con lo anterior dicha modificación al capítulo reivindicatorio no amplía la materia objeto de invención inicialmente presentada; sin embargo las reivindicaciones continúan sin presentar sustento en la descripción.

El solicitante reitera en el Folio 218: "ya que como tal se mencionó en respuesta en el oficio No 12203 la descripción contiene un ejemplo que da a conocer los efectos de la composición farmacéutica que contiene metotrexato y MRA (el cual es un anticuerpo PM-1), sobre un número determinado de pacientes y adicionalmente presenta 4 ejemplos de referencia para la preparación del receptor soluble IL-6 y del anticuerpo IL-6"

Esta Oficina reitera nuevamente al solicitante que se evidencia sólo el resultado clínico de la combinación mediante la administración por un método de tratamiento (Ver Folios 64-66), pero no sustenta de forma tangible, la composición farmacéutica de un antagonista IL-6 con metotrexato, junto con sus elementos esenciales constitutivos de acuerdo con el campo de la tecnología farmacéutica. Es decir el experto medio en la materia no tiene la suficiente información soportada en la descripción para desarrollar una composición que contenga a l mismo tiempo un anticuerpo (biofármaco) con un fármaco de origen químico.

En cuanto a que presenta 4 ejemplos de referencia para la preparación del receptor soluble IL-6 y del anticuerpo IL-6; es claro, que ésta es la mejor forma de realizar la invención en cuanto a la obtención de los anticuerpos; más no de la composición farmacéutica final como tal, que contiene metotrexato.

El solicitante afirma que el anticuerpo MRA es un anticuerpo PM-1, sin embargo ambos presentan diferente configuración, lo cual los hace diferentes en actividad biológica (Ver "Technology evaluation: MRA, Chugai, anexo informativo). Los datos anexados por el solicitante de actividad biológica corresponden a MRA y no al anticuerpo PM-1 reivindicado; por lo cual esta oficina reitera que las composiciones con PM-1 y metotrexato no se encuentran sustentadas en la

composición farmacéutica con base en sus conocimientos y en la información contenida en la descripción. Ver por ejemplo página 32, último párrafo de la descripción donde se indica:"

De acuerdo con lo anteriormente descrito, dicha información sólo corresponde a la característica de los compuestos farmacéuticos los cuales pueden ser aquellos que contienen los vehículos farmacéuticamente aceptables, y nombra una cantidad amplia y general de excipientes, los cuales no soportan ni dan suficiencia de información para elaborar una formulación que contiene un anticuerpo como PM-1 (biofármaco) con un fármaco como metotrexato (síntesis química)

Por lo tanto el argumento del solicitante en cuanto a que la composición farmacéutica reivindicada se encuentra debidamente sustentada en la descripción no es aceptado por parte de esta oficina.

El solicitante afirma en el Folio 219, en cuanto a nivel inventivo: "y así como se indicó en respuesta al oficio No 12203, **D1** se refiere de manera general a los inmunosupresores, pero no se indica en ningún caso el metotrexato"

Esta Oficina de acuerdo con el concepto técnico No 02463, se informó al solicitante que ya era conocida, la combinación de un inmunosupresor y antagonista IL-6. Por lo tanto el documento **D1**, es relevante pues ya enseñaba la combinación de un antagonista IL-6 y un inmunosupresor y es evidente que para el experto en la materia, con el fin de obtener sinergismo, emplear otro tipo de antagonistas IL-6, como PM-1 y un inmunosupresor conocido como metotrexato en combinación y colocarlos en una composición farmacéutica.

El solicitante se refiere en el Folio 219: "**D3** no describe ni sugiere una combinación de un anticuerpo contra el receptor anti-IL-6 y metotrexato en absoluto y mucho menos el efecto sinérgico que provee dicha combinación"

El documento **D3** claramente enseña que el empleo de metotrexato en la personas con artritis reumatoidea, reduce la producción de interleuquinas IL-6, por lo tanto con el fin de potenciar el efecto de reducir la producción de IL-6 combinar el metotrexato con un antagonista del IL-6, como lo enseña **D1**. De acuerdo con lo anterior esta oficina encuentra que no es válido, dicho argumento.

El solicitante afirma en el Folio 219: "antes de la fecha de prioridad de la presente solicitud, debido al mecanismo complicado y a la causa de la artritis reumatoide, no se tenía presente que la combinación de dos agentes terapéuticos para tratar la artritis reumatoide generaba necesariamente un efecto sinérgico" y anexa el artículo "Combination Therapy in Rheumatoid Arthritis-No benefit of Addition of Hydroxychloroquine to Patients with suboptimal Response to Intramuscular Gold Therapy", J. Rheumatol, 1993, 20, p 645-649 y afirma "Esta descripción reporta que la combinación no resultó un efecto sinérgico"

El artículo anexado por el solicitante (Ver Folios 226 a 230) evidencia la sinergia de la administración de los dos compuestos, sin embargo no sustenta la presencia de los dos compuestos en las composiciones farmacéuticas objeto de esta solicitud.

genera necesariamente un efecto sinérgico, dado que existen interacciones de dichos agentes que no pueden ser predichas"

De acuerdo con lo anterior, el objeto de la solicitud presentada son las composiciones farmacéuticas y no las combinaciones para uso terapéutico, por lo tanto, no se sustenta un antagonista IL-6 tal como PM-1 y metotrexato, de forma tal que se encuentren de forma tangible en una forma farmacéutica con la aplicación de una tecnología farmacéutica y que además presente sinergismo. La solicitud sólo evidencia la administración de los compuestos mediante un método terapéutico que mediante la administración combinada (Ver Folios 61, 63-68)

El solicitante argumenta en el Folio 220: "De acuerdo con lo anterior se tiene que: Ni **D1** ni **D2** ni su combinación revelan la composición farmacéutica reivindicada o el posible efecto sinérgico; la composición reivindicada ofrece un efecto sinérgico, es decir un ventaja o mejora con respecto al arte previo; y el efecto sinérgico no es un resultado esperado"

**D1** y **D2** mediante su combinación, ofrecen la información necesaria para realizar las composiciones objeto de esta solicitud y que a la luz de dichos documentos, resultaría en un sinergismo. Se reitera al solicitante que las composiciones declaradas en las reivindicaciones no se encuentran sustentadas en la descripción.

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 CESPEDES DE VERGEL**  
Directora de Nuevas Creaciones (C)

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

**20 ABO. 2001**

CONCEPTO TÉCNICO No. 02435

EXAMINADOR TÉCNICO  
Código No. 202070