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Superintendencia de Industria y Comercio
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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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Trámite : 011
Evento : 379
Actuación : 412

Referencia : Expediente No. 05.109.276
Asunto : Solicitud de patente para **ANTAGONISTAS DE INTERLEUKIN-6 Y COMPOSICIONES FARMACÉUTICAS QUE LOS CONTIENEN**
Solicitante : **CHUGAI SEIYAKU KABUSHIKI KAISHA**
Fecha de Solicitud : 26 de octubre de 2005

I. RECURSO VÍA GUBERNATIVA

Yo, **Luz Clemencia de Páez**, en mi condición de apoderada de la sociedad solicitante de la patente de la referencia, **CHUGAI SEIYAKU KABUSHIKI KAISHA**, por el presente escrito atentamente manifiesto a usted que debidamente notificada de la Resolución No. **19019** del 6 de abril de 2011, interpongo en su contra el recurso de **REPOSICIÓN**, para que sea revocada y en consecuencia se conceda el privilegio de patente de invención para "**ANTAGONISTAS DE INTERLEUKIN-6 Y COMPOSICIONES FARMACÉUTICAS QUE LOS CONTIENEN**".



II. OPORTUNIDAD DE LA IMPUGNACION

La Resolución No. **19019** del 6 de abril de 2011, fue notificada mediante edicto No. **7144** desfijado el 2 de mayo de dos mil once (2011), por lo que el término para interponer el recurso de reposición vence el 9 de mayo de 2011. En consecuencia, me encuentro dentro de la oportunidad legal.

III. LA DECISION RECURRIDA

3.1 Mediante la Resolución objeto de la presente impugnación, el Superintendente de Industria y Comercio decidió:

3.1.1 Negar el privilegio de patente para la invención **"ANTAGONISTAS DE INTERLEUKIN-6 Y COMPOSICIONES FARMACÉUTICAS QUE LOS CONTIENEN"**.

3.2 En la parte motiva de la providencia, se mencionaron los siguientes fundamentos:

3.2.1 "Así las cosas, la reivindicación 1 resulta obvia para el experto en la materia, quien enfrentado al problema de obtener composiciones farmacéuticas para el tratamiento de enfermedades relacionadas con interleukin IL-6, incluiría un anticuerpo monoclonal tal como PM-1, con el fin de declinar la cantidad de IL-6 que se revela en el documento D3, logrando de esta forma las composiciones farmacéuticas objeto de esta solicitud".

3.3 En cuanto a los argumentos presentados por mi representada en favor del nivel inventivo de la presente solicitud, ese Despacho mencionó lo siguiente:

3.3.1 "El artículo mencionado por la solicitante se refiere a las propiedades de la interleuquina IL-6, reportadas hacia el año 1991; sin embargo, en estudios posteriores como los que se reportaron en el documento D4, se enseña que las citoquinas IL-6 se encuentran entre las responsables de las manifestaciones de artritis reumatoide

El documento referenciado no evidencia que al emplear un anticuerpo anti-IL-6R tal como PM-1 y metotrexato, sea sinérgico y que además no se presenten expresiones negativas con el uso dichas composiciones, que anulen o disminuyen el efecto sobre las citocinas IL-6R".

3.3.2 "El experto en la materia, de acuerdo con sus conocimientos en farmacología, utilizaría compuestos que inhiben la interleuquina IL-6 con un antagonista tal como PM-1 y



un agente anti-reumático o inmunosupresor como metotrexato para el tratamiento de artritis reumatoide y los incluiría en una única composición farmacéutica. El documento D3 ya enseñaba las composiciones de PM-1, las cuales se incluirían en composiciones que tienen la combinación de anticuerpos monoclonales con metotrexato (inmunosupresor) en el tratamiento de la artritis reumatoide, como lo sugiere el documento D4. (D3 Pág 72), y D2 Fl. 185, D1-columna 23, líneas 1-5). En la solicitud no se aprecia un efecto sinérgico para las composiciones PM-1, que evidencia que supera las demás composiciones ya conocidas en este campo tecnológico para el tratamiento de artritis reumatoide”.

3.3.3 “Sobre el particular es oportuno precisar que, la disminución de las citoquinas IL-6 por parte del metotrexato ya se encontraba divulgado en el documento D3 (Pág. 73), puesto que es de conocimiento para el experto en la materia, su efecto como anti-reumático; además, los resultados presentados para la composición MRA y metotrexato (MTX) de acuerdo con los cuales esta combinación es levemente sinérgica, no son evidencia de que las composiciones que contienen PM-1 y MTX sean también sinérgicas y que las logren superar”.

3.3.4 “Las enseñanzas del estado de la técnica llevarían al experto en la materia, a combinar dos ingredientes activos que disminuyen la interleuquina IL-6, para el tratamiento de la artritis reumatoide, de forma tal que se encuentren en una única composición farmacéutica”.

IV. OBJETO DEL RECURSO

De acuerdo con los argumentos que expongo a continuación, solicito atentamente a su Despacho lo siguiente:



4.1. Revocar la Resolución No. **19019** del 6 de abril de 2011.

4.2. Conceder el privilegio de patente de invención para **“ANTAGONISTAS DE INTERLEUKIN-6 Y COMPOSICIONES FARMACÉUTICAS QUE LOS CONTIENEN”**.

V. FUNDAMENTOS DEL RECURSO

Respetuosamente me permito disentir de lo expuesto por ese Despacho en la Resolución objeto del presente recurso, ya que la presente solicitud posee nivel inventivo a la luz de los documentos "Methotrexate diminishes phlogiston production and responses" (D3) y US2001/0001663 (D4).

La anterior afirmación tiene justificación jurídica y técnica por las siguientes razones:

En primer lugar, deseamos hacer referencia al documento "Combination Therapy in Rheumatoid Arthritis – No Benefit of Addition of Hydroxychloroquine to Patients with a Suboptimal Response to Intramuscular Gold Therapy" presentado junto con el memorial de respuesta al Oficio No. 12873, radicado el 5 de abril de 2010, en donde se discute la terapia de combinación en el tratamiento de artritis reumatoide, y en los Resultados y en el Resumen se describe que "el papel de la terapia de combinación es controversial".

Algunas autoridades actualmente abogan el uso rutinario de la terapia de combinación a pesar de la insuficiencia de la evidencia para apoyar su método. Ha habido varios ensayos abiertos, no controlados, pero muy pocos ensayos controlados para evaluar la eficacia y seguridad de esta estrategia de tratamiento. La terapia de combinación de oro e hidroxiclороquinona (HCQ) es ampliamente prescrita, pero falta evidencia de este método. "(Véase, 645 página, columna izquierda, línea 3 de dicho documento J. Rheumatol. 1993, 20, 645-647); "Otros estudios controlados de combinación también han dado resultados negativos" "(Página 648, columna de la derecha, línea 21, de dicho documento J. Rheumatol. 1993, 20, 645-647); "Williams, et al. no pudieron demostrar ningún beneficio de la combinación de metotrexato y auranofina sobre la monoterapia. Antes de la utilización de la terapia de combinación en la Artritis Reumatoide, se vuelve más generalizado, además se necesita con urgencia ensayos controlados bien contruidos" (Página 648, columna derecha, de dicho documento J. Rheumatol. 1993, 20, 645-647).

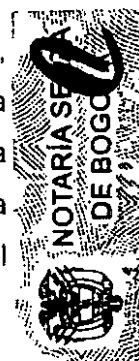


Como se puede apreciar de las descripciones mencionadas anteriormente no es claro si la terapia de combinación es de hecho efectiva o no, y es claro que una terapia de combinación de MTX (metotrexato) y auranofina, que es un agente anti-artritis reumatoide, no presenta efectos terapéuticos sobre un único uso, es decir la monoterapia.

Adicionalmente, cabe aclarar que no sólo no se proporciona ventaja mediante el uso de una combinación, sino también el uso de la combinación puede reducir un efecto proporcionado por la monoterapia, como puede verse a partir de la descripción "se ha realizado un estudio de doble ciego de HCQ vs penicilamina vs penicilamina/HCQ combinado, con el resultado sorprende de la eficacia reducida de la terapia de combinación en comparación con solo penicilamina" (Veáse página 648, columna derecha, línea 23, del documento J. Rheumatol. 1993, 20, 645-647) .

Así, como se puede observar de lo mencionado anteriormente, es bien sabido en el estado del arte, que es muy difícil hallar una combinación que proporcione un efecto terapéutico mejorado, y **por lo tanto, una persona medianamente versada en la materia no puede esperar que una combinación de dos agentes para tratar artritis reumatoide pueda proporcionar un efecto sinérgico.**

Adicionalmente, deseamos allegar a este memorial el documento, Genovese et al., Arthritis & Rheumatism, 50(5), 2004, p.1412-1419, el cual muestra un nivel técnico en la presentación de la actual solicitud y fue publicada justo después de la presentación de la actual solicitud. En dicho documento se describe que no hay diferencia en el efecto para el tratamiento de la artritis reumatoide entre un uso combinado del agente anti-IFN- α y el agente anti-IL-6, y el uso único del mismo, y que el uso combinado no es recomendable.



De esta manera, como se puede observar de lo anterior, es muy difícil encontrar una combinación que exhiba un efecto sinérgico, y que el uso combinado necesariamente proporciona un efecto sinérgico.

Por otra parte, deseamos llamar la atención del Examinador en cuanto a que de acuerdo con lo mostrado en los Ejemplos de la presente invención, se ha demostrado bien que un

efecto terapéutico proporcionado por un uso combinado de un anticuerpo del receptor anti-IL-6 y metotrexato (MTX), es mayor que el efecto terapéutico proporcionado por el uso único del mismo. Por ejemplo, en la Tabla 3, el ACR70 en un uso único de metotrexato (MTX) es del 16.3%, y el ACR70 en uso único de 8 mg/kg de MRA (anticuerpo del receptor anti-IL-6) es de 15.7%. Por otra parte, el ACR70 en un uso combinado de 8 mg/kg de MRA + MTX es 36.7%, es decir mayor a la suma de las monoterapias.

Bajo el entendimiento general que se tiene a partir de lo revelado en el estado del arte y en la presente invención, con respecto a que un uso combinado de dos diferentes medicamentos no proporciona necesariamente un efecto aditivo, que un efecto terapéutico proporcionado por un uso combinado puede no ser diferente del efecto terapéutico proporcionado por un único uso del mismo, y que hay un caso en donde un efecto terapéutico proporcionado por el uso combinado es menor que un efecto terapéutico proporcionado por un único uso, debido a la interferencia de sus efectos, cabe recalcar que **es muy difícil encontrar una combinación de dos medicamentos que proporcione un efecto sinérgico.**

Aún más, deseamos poner de presente que, considerando especialmente el efecto in vivo que incluye el efecto aditivo y el efecto sinérgico, en donde un efecto terapéutico es un resultado de varias reacciones, una persona medianamente versada en la técnica no puede considerar que un efecto de uso combinado de dos medicamentos de los cuales los mecanismos de acción son diferentes uno del otro, es sinérgico, o incluso aditivo. Por ejemplo, en relación con un efecto aditivo, en el supuesto de utilizar en combinación dos de cada medicamento que exhiben un 50% de efecto terapéutico, una persona medianamente versada en la técnica no puede considerar un uso combinado de los mismos que proporcione el 100% de efecto terapéutico que es un efecto aditivo.



De acuerdo con la presente invención, como se describió anteriormente, un uso combinado de (1) metotrexato (MTX), que proporciona 16.3% en un uso único y (2) ACR70 que proporciona el 15.7%, proporciona 36.7% (efecto sinérgico), que es mayor de 32%, correspondiente al efecto aditivo (=16.3% + 15.7%).

En consecuencia, contrario a lo afirmado por el Examinador en la Resolución 19019, el anterior efecto sinérgico de la presente invención no se puede esperar de las enseñanzas encontradas en el estado de la técnica.

Como prueba adicional para el efecto sinérgico de la presente invención, deseamos allegar a este memorial el documento R.N. Maini et al., Arthritis & Rheumatism, 54 (9), 2006, p. 2817-2329, que se basa en el presente invención. En la página 2822, columna izquierda de dicho documento, se describe que con respecto al DAS (Disease Activity Score), el índice de remisión del MTX en monoterapia es del 8%, y que el de la monoterapia de 8 mg /kg de MRA es del 17%, mientras que un índice de remisión de uso combinado de 8 mg/kg MRA + MTX es de 34%, que es superior al 25% (=8% + 17%) del efecto aditivo. De esta manera, deseamos reiterar, que lo anterior es muestra del efecto sinérgico de la presente invención.

De otro lado, con respecto a las enseñanzas encontradas en D3, deseamos aclarar que dicha anterioridad en el párrafo 71, se refiere a "una reducción en los niveles del factor reumatoide en pacientes con Artritis Reumatoide tratados con bajas dosis de metotrexato. La reducción en los niveles del factor reumatoide IgM están en paralelo con una reducción en los niveles de suero de IL-6". Sin embargo, D3 no describe en lo absoluto ni sugiere que la inhibición de IL-6 pueda ser útil para el tratamiento de artritis reumatoide. Como hemos argumentado previamente, no hay relación entre el nivel de citocina en sangre o un sitio de una enfermedad y que si es útil o no la inhibición de las citocinas para el tratamiento de las enfermedades. En algunas ocasiones, la inhibición de las citocinas empeora la enfermedad.



Teniendo en cuenta lo anterior, una persona versada en la materia no encontraría motivación alguna para utilizar una combinación de un anticuerpo del receptor anti-IL-6 y MTX para el tratamiento de la artritis reumatoide. Además, no hay ninguna base en absoluto para esperar que una combinación de un anticuerpo del receptor anti-IL-6 y MTX para tratar la artritis reumatoide, sea útil.

Así las cosas, deseamos se tenga en cuenta adicionalmente que D3, en el párrafo 75, se refiere a "la concentración en suero de IL-6 declina después de la terapia con metotrexato en asociación con la mejora de los parámetros clínicos en tres estudios, pero no cambió en los demás ". Esta descripción significa que como cuestión básica, no es muy claro si el nivel de IL-6 se reduce por MTX, y por lo tanto, la disminución del nivel IL-6 por MTX no es conocida comúnmente en el estado de la técnica.

Así entonces, podemos concluir que la Resolución acá impugnada fue expedida con violación del artículo 18 de la Decisión 486 debido a que no era posible derivar de manera obvia la presente invención a partir de los documentos más cercanos del estado del arte encontrados por su Despacho (D3 y D4).

VI. PETICIÓN

Por todo lo anterior, solicito a ese Despacho lo siguiente:

- 6.1. Que al considerar las razones precedentes se revoque la decisión contenida en la Resolución No. **19019** del 6 de abril de 2011, en cuando resuelve negar el privilegio de patente de invención.
- 6.2. Que se otorgue el privilegio de patente de la solicitud para: **ANTAGONISTAS DE INTERLEUKIN-6 Y COMPOSICIONES FARMACÉUTICAS QUE LOS CONTIENEN**".



VII. ANEXOS

- Copia del artículo: Genovese *et al.*, Arthritis & Rheumatism, 50(5), 2004, p.1412-1419
- Copia del artículo: R.N. Mainini *et al.*, Arthritis & Rheumatism, 54(9), 2006, p.2817-2329

VIII. NOMBRE Y DIRECCION

El nombre y la dirección de la sociedad recurrente es el siguiente:

CHUGAI SEIYAKU KABUSHIKI KAISHA

5-1, Ukima 5-chome,
Kita-ku, Tokyo 115-8543
Japan

IX. NOTIFICACIONES

Atentamente manifiesto a usted que recibiré notificaciones en la Secretaria de su Despacho o en mi oficina de abogada situada en la Carrera 4ª. No. 72-35, piso 4º, de esta ciudad de Bogotá, D.C.

Señor Superintendente,

Luz Clemencia Suarez
LUZ CLEMENCIA DE PAEZ
CC. 35.456.444 de Usaquén
T.P.A. No. 23.555



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No. M-2011-192291\DVF

COMO NOTARIO SEXTO DEL CIRCULO DE SANTAFE DE BOGOTA, HAGO CONSTAR QUE LA(S) FIRMA(S) DE

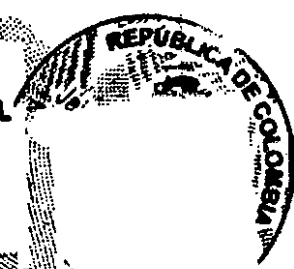
Luz clemencia Suarez de paez

COINCIDE(N) CON LA FIRMA REGISTRADA POR EL (ELLOS) EN ESTA NOTARIA SANTAFE DE BOGOTA, **09 MAY 2011**



9

NOTARIA SEXTA DE BOGOTÁ
RECONOCIMIENTO Y PRESENTACION PERSONAL
Bogotá, D.C. **09 MAY 2011**
Ante el
NOTARIO SEXTO (E) DEL CIRCULO DE
BOGOTÁ D.C.
Compareció (eron) *Juan Clemenencia*
Guerra de paz
Guerra de paz
C. *f-35-456-444*
f-23555
Y declaró(aron) que la(s) firma(s) que aparece(n)
en el presente documento es(son) la(s) suya(s) y
que el contenido del mismo es cierto.
En constancia se firma esta diligencia.
Juan Clemenencia Guerra
TP # 23555-456



Combination Therapy With Etanercept and Anakinra in the Treatment of Patients With Rheumatoid Arthritis Who Have Been Treated Unsuccessfully With Methotrexate

Mark C. Genovese,¹ Stanley Cohen,² Larry Moreland,³ Deborah Lium,⁴ Sean Robbins,⁴ Richard Newmark,⁴ and Pirow Bekker,⁴ for the 20000223 Study Group

Objective. To determine the potential for additive or synergistic effects of combination therapy with the selective anti-tumor necrosis factor α agent etanercept and the anti-interleukin-1 agent anakinra.

Methods. Two hundred forty-four patients in whom rheumatoid arthritis (RA) was active despite methotrexate therapy were treated with subcutaneous etanercept only (25 mg twice weekly), full-dosage etanercept (25 mg twice weekly) plus anakinra (100 mg/day), or half-dosage etanercept (25 mg once weekly) plus anakinra (100 mg/day) for 6 months in a double-blind study at 41 centers in the US. Patients had never previously received anticytokine therapy. Patient response was measured with the American College of Rheumatology (ACR) core set criteria, a health-related quality-of-life questionnaire, and the Disease Activity Score. Safety was assessed by the number of adverse events and clinical laboratory values. Plasma concentrations of both agents and antibody formation against both agents were also assessed.

Results. Combination therapy with etanercept plus anakinra provided no treatment benefit over etanercept alone, regardless of the regimen, but was associ-

ated with an increased safety risk. Thirty-one percent of the patients treated with full-dosage etanercept plus anakinra achieved an ACR 50% response, compared with 41% of the patients treated with etanercept only. This result was not statistically significant ($P = 0.914$). The incidence of serious infections (0% for etanercept alone, 3.7–7.4% for combination therapy), injection-site reactions, and neutropenia was increased with combination therapy. Combination therapy had no effect on the pharmacokinetics or immunogenicity of either agent.

Conclusion. Combination therapy with etanercept and anakinra provides no added benefit and an increased risk compared with etanercept alone and is not recommended for the treatment of patients with RA.

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease characterized by inflammation that often leads to the progressive destruction of articular structures and significant disability. The etiology of RA remains unclear, but it is thought to be mediated in part by antigen-driven T cells and macrophages that produce interleukin-1 (IL-1) and tumor necrosis factor α (TNF α), 2 cytokines involved in the inflammatory cascade (1–3). Specific blockade of these individual cytokines has recently been shown in large, place-controlled trials to be safe and effective in the treatment of RA (4–9).

Although selective anticytokine therapy has improved patient outcomes, it does not necessarily produce disease remission. Thus, the more aggressive treatment option of combining anticytokine agents has been explored in animal studies. Combination treatment with anakinra, a recombinant IL-1 receptor antagonist (IL-1Ra) (Kineret; Amgen, Thousand Oaks, CA), and polyethylene glycol-conjugated soluble TNF receptor type I

Supported by Amgen, Inc., Thousand Oaks, California.

¹Mark C. Genovese, MD: Stanford University, Palo Alto, California; ²Stanley Cohen, MD: St. Paul Medical Center, Dallas, Texas; ³Larry Moreland, MD: University of Alabama at Birmingham; ⁴Deborah Lium, PhD, Sean Robbins, MS, Richard Newmark, PhD, Pirow Bekker MD, PhD: Amgen, Thousand Oaks, California. Members of the 20000223 Study Group are shown in Appendix A.

Address correspondence to Mark C. Genovese, MD, Stanford University, 1000 Welch Road, Suite 203, Palo Alto, CA 94304. E-mail: genovese@stanford.edu. Address reprint requests to Pirow Bekker, MD, PhD, Amgen, One Amgen Center Drive, 38-2-B, Thousand Oaks, CA, 91320.

Drs. Genovese, Cohen, and Moreland have served as consultants for Amgen, Inc.

Submitted for publication September 3, 2002; accepted in revised form January 29, 2003.

resulted in synergistic improvement in the symptoms of adjuvant-induced and collagen-induced arthritis in rats relative to the improvement observed with either agent alone (10,11). Additive or synergistic improvement was seen with a variety of dose combinations of each agent, but synergistic improvement was particularly evident when suboptimal doses of each agent were given in combination.

The present study was designed to test the hypothesis that combination therapy with the anti-TNF agent etanercept, a soluble TNF α receptor (Enbrel; Amgen), and the anti-IL-1 agent anakinra at their approved dosages would safely provide superior efficacy relative to etanercept alone in patients with RA. Secondly, the study examined the possibility that anakinra given with etanercept at a dosage of 25 mg once weekly (half the approved weekly dose) would still provide superior efficacy compared with full-dosage etanercept alone.

PATIENTS AND METHODS

Patients. This study enrolled patients who were at least 18 years old and had a >6-month history of RA, as diagnosed by the American College of Rheumatology (ACR) classification criteria (12). Patients had at least 6 swollen joints and 9 tender/painful joints and at least 2 of the following: morning stiffness lasting at least 45 minutes, a serum C-reactive protein (CRP) level of at least 1.5 mg/dl, or an erythrocyte sedimentation rate (ESR) of at least 28 mm/hour. Patients had received methotrexate (MTX) for at least 16 weeks, with the dosage stable at 10–25 mg/week for at least 8 weeks. All patients gave informed consent, and the study protocol was approved by the institutional review boards for each study site.

Patients were not eligible to enroll in the study if they had received any disease-modifying antirheumatic drug other than MTX within the past 4 weeks, had ever been treated with anakinra or any protein-based TNF α inhibitor (e.g., etanercept, infliximab), had received any intraarticular or systemic corticosteroid injections within the past 4 weeks, or had a recent history of significant infection or other important concurrent illness.

Study design and treatment. Patients were randomly assigned in a 1:1:1 ratio to receive 25 mg of etanercept twice weekly plus anakinra placebo once daily, 25 mg of etanercept once weekly plus 100 mg of anakinra daily, or 25 mg of etanercept twice weekly plus 100 mg of anakinra once daily (hereafter referred to as etanercept only, half-dosage etanercept plus anakinra, and full-dosage etanercept plus anakinra, respectively). Both etanercept and anakinra were administered subcutaneously for 24 weeks. In order to blind patients to the treatment assignment, additional sham injections of etanercept were administered as necessary, so that all patients received twice weekly injections of etanercept/sham and once daily injections of anakinra or matched placebo. Patients continued to receive stable doses of MTX and other medications (e.g., corticosteroids) throughout the study. After screening, patients were evaluated at baseline (day 1) and at weeks 2, 4, 8, 12, 16,

20, and 24, with followup evaluation 4 weeks after completion or at the time of early discontinuation.

Endogenous human IL-1Ra was isolated, purified, and produced by recombinant DNA technology using *Escherichia coli* fermentation. The resulting product, anakinra, is identical to the naturally occurring nonglycosylated form of human IL-1Ra except for the addition of an N-terminal methionine residue. Anakinra was provided by Amgen in single-use vials as a liquid containing 1 ml of 100 mg/ml anakinra. The formulation consisted of sodium citrate, sodium chloride, disodium EDTA, and polysorbate 80. The placebo formulation was the same, but without anakinra. Both solutions were pH 6.5.

Etanercept is a soluble TNF receptor fusion protein produced by recombinant DNA technology in a Chinese hamster ovary mammalian cell expression system. Etanercept was supplied by Amgen in 25-mg single-use vials containing etanercept lyophilized powder, mannitol, sucrose, and tromethamine. After reconstitution with bacteriostatic water, the solution had a mean ($\pm$ SD) pH of 7.4 ± 0.3 .

Efficacy assessment. At every study visit, patients were assessed for the components of the ACR core set of disease activity measures (13), the modified Disease Activity Score (DAS) (14), the European League Against Rheumatism (EULAR) response (14), and the duration of morning stiffness. A health-related quality-of-life evaluation with the Short Form 36 (SF-36) healthy survey (15) was performed at baseline and at weeks 4, 12, and 24 (or at the time of early termination).

The primary end point was the proportion of patients achieving an ACR 50% (ACR50) response (16) at week 24. Secondary efficacy end points included the ACR20 and ACR70 response rates at week 24, the ACR response at week 12, the sustained ACR20 response (response for at least 4 monthly measurements, not necessarily consecutive, with 1 occurring at month 6), a good or moderate EULAR response at week 24, improvement in the ACR core criteria components, duration of morning stiffness, the DAS, and the SF-36 score.

Patients were considered ACR50 responders if they had at least a 50% reduction in the number of tender and swollen joints and in 3 of the following 5 measures: patient's assessment of disease activity by visual analog scale (VAS), physician's assessment of disease activity by VAS, patient's assessment of pain by VAS, the disability score as measured by the Health Assessment Questionnaire (17), and acute-phase reactants (CRP or ESR). The joint counts (66 joints evaluated for swelling, and 68 joints evaluated for tenderness/pain) were assessed by the same qualified independent assessors at each study center throughout the study. To preserve blinding of the study, injection sites were covered with clothing during the joint counts to insure that the assessors would not be influenced by injection-site reactions.

Safety and pharmacokinetic assessment. Safety assessment data that were collected at every study visit were the number of adverse events/infectious events and the clinical laboratory values. An adverse event was defined as follows: "Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment" (18). A serious adverse event was defined as follows: "Any untoward medical occurrence that at

Table 1. Baseline characteristics of study patients*

Characteristic	Etanercept only (n = 80)	Half-dosage etanercept + anakinra (n = 81)	Full-dosage etanercept + anakinra (n = 81)
% female	82.5	71.6	77.8
% white race	86.3	77.8	75.3
Age, years	54.4 ± 13.6	53.8 ± 11.8	55.7 ± 13.0
% age ≥ 65 years	25.0	18.5	29.6
Weight, kg	75 ± 18	82 ± 21	80 ± 23
Duration of RA, years	9.7 ± 9.4	9.5 ± 10.3	10.6 ± 9.8
% rheumatoid factor positive	65.0	75.3	71.6
% receiving corticosteroids	48.8	54.3	44.4
NSAID use, %	96.3	95.1	96.3
MTX dosage, mg/week	16.1 ± 4.5	16.2 ± 4.2	15.7 ± 5.0
No. of tender/painful joints	31.0 ± 14.2	31.0 ± 15.4	35.9 ± 14.9
No. of swollen joints	21.4 ± 9.4	19.8 ± 9.6	23.4 ± 12.0
HAQ score	1.5 ± 0.6	1.5 ± 0.6	1.6 ± 0.6
Serum CRP, mg/dl	2.0 ± 2.2	2.4 ± 3.5	2.0 ± 2.5
ESR, mm/hour	44.6 ± 21.51	49.2 ± 22.81	49.9 ± 23.94
Duration of morning stiffness, minutes	145.3 ± 102.3	154.4 ± 162.1	159.5 ± 134.0
SF-36 score			
Physical component	28.7 ± 9.7	28.8 ± 7.9	29.1 ± 7.9
Mental component	46.9 ± 12.3	47.9 ± 10.9	44.5 ± 11.9

* Except where indicated otherwise, values are the mean ± SD. Etanercept only = etanercept 25 mg twice weekly; half-dosage etanercept + anakinra = etanercept 25 mg once weekly plus anakinra 100 mg daily; full-dosage etanercept + anakinra = etanercept 25 mg twice weekly plus anakinra 100 mg daily; RA = rheumatoid arthritis; NSAID = nonsteroidal antiinflammatory drug; MTX = methotrexate; HAQ = Health Assessment Questionnaire; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; SF-36 = Short Form 36.

any dose results in death, is life threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly/birth defect” (18).

Blood samples were collected for measurement of plasma anakinra and etanercept concentrations (at baseline and weeks 4, 12, and 24 [or at the time of early termination]) and anti-anakinra and anti-etanercept antibodies (at baseline and weeks 12 and 24 [or at the time of early termination]). Plasma anakinra concentrations were assessed by antibody-capture enzyme-linked immunoassay (ELISA), and plasma etanercept concentrations were assessed by a solid-phase sandwich ELISA kit. Antibody samples testing positive in a screening biosensor immunoassay (Biacore, Uppsala, Sweden) were retested for neutralizing antibodies, using a bioassay.

Statistical analysis. Results were analyzed using a modified intent-to-treat method that included all randomized patients who received at least 1 dose each of anakinra/placebo and etanercept/sham. Patients with missing ACR scores at a particular visit were considered nonresponders at that visit.

The primary comparison was the full-dosage etanercept plus anakinra group against the etanercept-only group. The analysis was 1-tailed for the primary comparison and 2-tailed for the secondary comparisons. Odds ratios (ORs) and their confidence intervals (CIs) were calculated for comparisons between groups. Binary efficacy end points were analyzed using a logistic regression model. Continuous end points were analyzed over time using a repeated-measures mixed model.

Adverse events were tabulated for comparison across treatment groups, and summary statistics were calculated for laboratory values.

RESULTS

Characteristics of the study patients. The baseline demographics and disease characteristics of the patients are shown in Table 1. Most of the patients were women with long-standing and very active disease. Characteristics were balanced across treatment groups. Com-

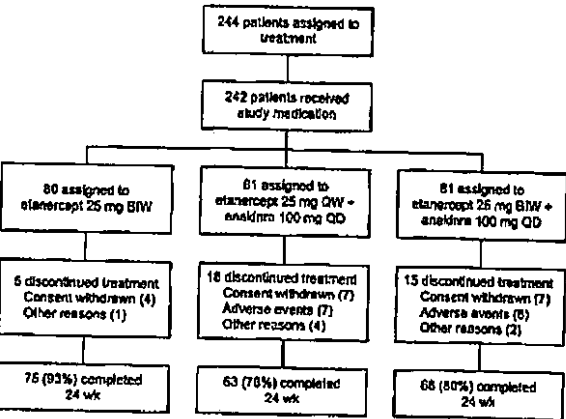


Figure 1. Disposition of patients. Other reasons for premature withdrawal included protocol violations and patients being lost to followup. BIW = twice weekly; QW = once weekly; QD = once daily.

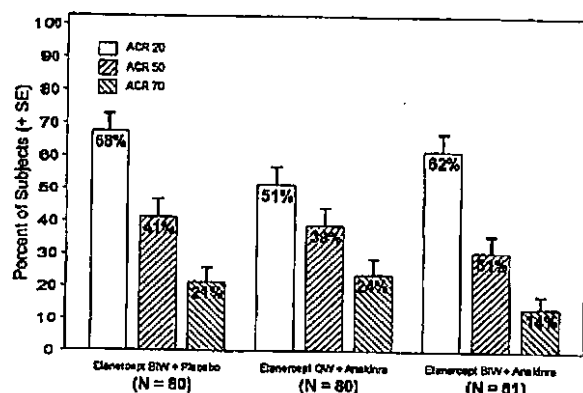


Figure 2. Percent of patients achieving an American College of Rheumatology 20% (ACR20), ACR50, or ACR70 response at week 24. BIW = twice weekly; QW = once weekly.

pletion rates ranged from 78% to 93%, with the highest rate in the etanercept-only group (Figure 1). Significantly fewer patients in this group withdrew as a result of adverse events compared with the combination-therapy groups.

Efficacy results. Patients in all treatment groups showed improvement from baseline at week 24 (Figure 2). Therapy in the etanercept-only group resulted in an ACR50 of 41%, compared with 31% in the full-dosage etanercept plus anakinra group ($P = 0.914$, by 1-tailed t -test). The OR for achieving an ACR50 response in the full-dosage etanercept plus anakinra group relative to that in the etanercept-only group was 0.64 (90% CI 0.37–1.09). The OR for achieving an ACR50 response in the etanercept-only group relative to that in the half-

dosage etanercept plus anakinra group was 1.11 (95% CI 0.59–2.09). For the comparison of patients in the full-dosage (twice weekly) combination-therapy group relative to those in the low-dosage (once weekly) combination therapy group, the OR was 0.71 (95% CI 0.37–1.35).

Because the dropout rate was higher in the groups receiving combination therapy, it was important to determine whether this influenced the efficacy results. Sensitivity analyses, including a completers analysis (all patients who completed the study) and a last observation carried forward analysis, yielded results similar to those observed in the modified intent-to-treat analysis (data not shown), indicating that differential dropout rates did not influence the outcome. In addition, results were unaffected when they were adjusted for baseline covariates (data not shown).

Evaluation of the ACR20 and ACR70 response rates confirmed that combination therapy was not superior to etanercept alone. The only comparison yielding a statistically significant difference between treatments indicated that at week 24, the ACR20 response of patients treated with etanercept alone was superior to that of patients treated with etanercept once weekly plus anakinra (OR 1.98, 95% CI 1.05–3.78; $P = 0.037$).

Between 43% and 54% of patients in each treatment group achieved a sustained ACR20 response during the study, and most patients achieved a EULAR response at week 24 (79% of patients in etanercept-only group, 73% of those in the full-dosage etanercept plus anakinra group, and 66% of patients in the half-dosage etanercept plus anakinra group received a good or moderate rating). At week 24, the mean percent reduction from baseline in the DAS was 39% in the

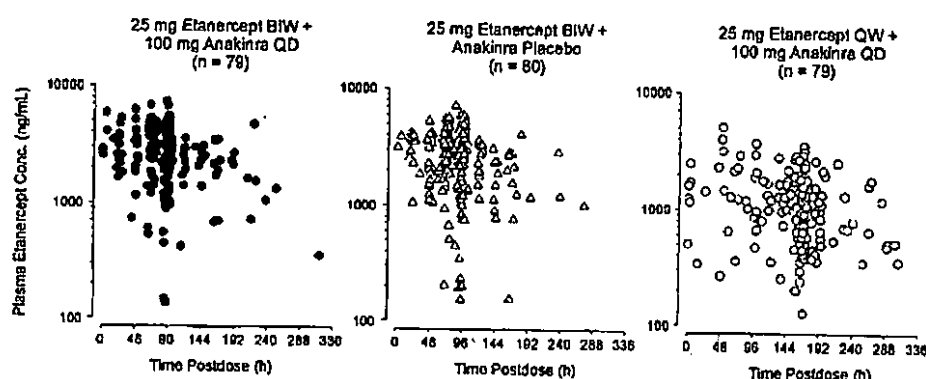


Figure 3. Individual plasma etanercept concentrations (Conc). Because the results at each week indicated that the pharmacokinetic steady state of etanercept was reached by week 4, the data for all visits (weeks 4, 12, and 24) were pooled for analysis. BIW = twice weekly; QW = once weekly; QD = once daily; h = hours.

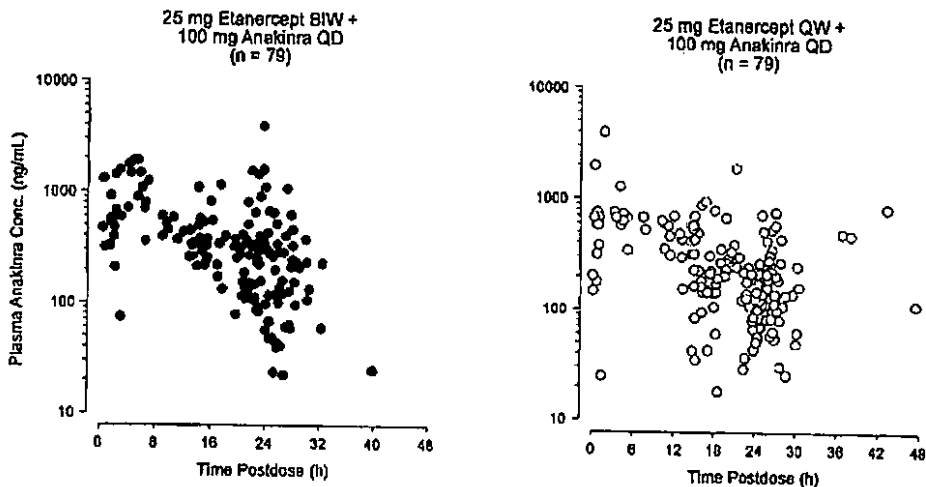


Figure 4. Individual plasma anakinra concentrations. Because the results at each week indicated that the pharmacokinetic steady state of anakinra was reached by week 4, the data for all visits (weeks 4, 12, and 24) were pooled for analysis. See Figure 3 for definitions.

etanercept-only group, 40% in the half-dosage etanercept plus anakinra group, and 41% in the full-dosage etanercept plus anakinra group. Radiographs were not obtained in this study.

Pharmacokinetics. Plasma concentrations for weeks 4, 12, and 24 indicated that the pharmacokinetic steady state was reached by week 4 for both etanercept and anakinra, and thus the data for all visits were pooled for analysis. The pharmacokinetics of each agent appeared unaffected by the concomitant administration of the other (Figures 3 and 4). Plasma concentrations of anakinra and etanercept were similar to those observed

in previous studies of anakinra or etanercept alone (Genovese MC, et al: unpublished observations).

Safety. The proportion of patients reporting adverse events was similar for each treatment group (90–95%) (Table 2). However, combination therapy with anakinra and etanercept was associated with a higher overall incidence of serious adverse events, events causing patient withdrawal from study, and injection-site reactions.

Serious adverse events were generally individual occurrences, with no single category of event accounting for the increase observed with combination therapy

Table 2. Incidence of adverse events during treatment*

Event	Etanercept only (n = 80)	Half-dosage etanercept + anakinra (n = 81)	Full-dosage etanercept + anakinra (n = 81)
Any adverse event	72 (90.0)	77 (95.1)	76 (93.8)
Most common adverse events			
Injection-site reaction	32 (40.0)	55 (67.9)	57 (70.4)
Upper respiratory infection	16 (20.0)	9 (11.1)	11 (13.6)
Any adverse event causing withdrawal	0 (0)	7 (8.6)	6 (7.4)
Any serious adverse event	2 (2.5)	4 (4.9)	12 (14.8)
Any infection	32 (40.0)	30 (37.0)	38 (46.9)
Infection resulting in antibiotic administration or hospitalization	0 (0.0)	3 (3.7)	6 (7.4)
Infection leading to withdrawal	0 (0.0)	2 (2.5)	2 (2.5)
Serious infection	0 (0)	3 (3.7)	6 (7.4)
Serious pneumonia	0 (0)	1 (1.2)	2 (2.5)
Serious cellulitis	0 (0)	1 (1.2)	2 (2.5)

* Values are the number (%) of patients. See Table 1 for definitions.

Table 3. Serious adverse events that occurred during the study*

Event	Etanercept only (n = 80)	Half-dosage etanercept + anakinra (n = 81)	Full-dosage etanercept + anakinra (n = 81)
Cellulitis	0 (0)	1 (1.2)	2 (2.5)
Pneumonia	0 (0)	1 (1.2)	1 (1.2)
Pneumonia and pulmonary fibrosis leading to respiratory insufficiency	0 (0)	0 (0)	1 (1.2)
Gastroenteritis	0 (0)	0 (0)	1 (1.2)
Herpes zoster	0 (0)	0 (0)	1 (1.2)
Lymphoma, malignant	0 (0)	0 (0)	1 (1.2)
Neuralgia	0 (0)	0 (0)	1 (1.2)
Back pain	0 (0)	0 (0)	1 (1.2)
Chest pain, cardiac	0 (0)	0 (0)	1 (1.2)
Chest pain, noncardiac	0 (0)	0 (0)	1 (1.2)
Pyelonephritis	0 (0)	0 (0)	1 (1.2)
Transient ischemic attack	0 (0)	0 (0)	1 (1.2)
Arrhythmia, atrial	1 (1.3)	0 (0)	0 (0)
Dyspnea	0 (0)	1 (1.2)	0 (0)
Gastric ulcer, hemorrhage	0 (0)	1 (1.2)	0 (0)
Personality disorder	1 (1.3)	0 (0)	0 (0)
Pneumonitis	0 (0)	1 (1.2)	0 (0)

* Values are the number (%) of patients. See Table 1 for definitions

(Table 3). However, infections accounted for serious events in 9 of 16 patients receiving combination therapy. The reported serious infections were as follows: pneumonia and cellulitis (3 patients each), herpes zoster (1 patient), pneumonitis (1 patient), and pyelonephritis (1 patient). One 70-year-old patient with pulmonary fibrosis was diagnosed as having pneumonia and died of pulmonary insufficiency. Serious infections occurred an average of 2 months after exposure to combination treatment (range 1 week to 5 months) in patients whose mean age was 60 years (range 42–77 years). No cases of tuberculosis or opportunistic infections were reported.

The incidence of injection-site reactions was more than 50% higher with combination therapy than with etanercept alone. These reactions were transient, rarely severe (a combined incidence of 2% in the combination-therapy groups), and typically involved no clinical sequelae. However, they were the most common cause of adverse event-related withdrawal during the study for patients receiving combination therapy, accounting for 5 of 13 adverse event-related withdrawals. Injection-site reactions were less likely to occur after the first month of therapy.

The mean neutrophil counts decreased to a similar extent in all 3 groups within the first 2 weeks of initiating treatment but remained stable thereafter. Two patients in the full-dosage etanercept plus anakinra group experienced neutropenia (neutrophil count $<1.0 \times 10^9/\text{liter}$) during the study. Both patients completed the study, and no clinical events were associated

with the neutropenia. No subjects had changes in the neutrophil count that appeared to be associated with their serious infectious episodes. None of the subjects who experienced serious infectious episodes experienced neutropenia. No other clinically significant trends in the laboratory results were apparent. Three patients receiving anakinra and no patients receiving etanercept had evidence of potentially neutralizing anti-anakinra and anti-etanercept antibodies, respectively. The presence of antibodies had no apparent effect on efficacy or safety.

DISCUSSION

The clinical hypothesis for this study, that combination treatment with selective anticytokine therapies (etanercept and anakinra) would safely provide superior improvement in the signs and symptoms of RA compared with etanercept alone, proved false. Analyses of the primary efficacy end point, the ACR50 response at week 24, showed no significant differences between groups regardless of the combination regimen used (etanercept twice weekly or once weekly). In fact, patients who received etanercept only in this study had the highest ACR responses, similar to those seen in previous studies of etanercept (4,8), and encountered the fewest safety problems.

Results of preclinical experiments suggested that simultaneous blockade of IL-1 and TNF α would be more effective than either approach alone in inhibiting

progression of RA, with a combination of suboptimal doses of each anticytokine agent providing a synergistic effect. The reasons for the discrepancy between the preclinical and clinical results are unclear, but several theories present themselves. First, there may have been a negative interaction between the compounds. This theory seems unlikely, because the pharmacokinetic results show that plasma concentrations for both agents remained unchanged relative to observations for each agent independently. Also, anakinra is eliminated renally (19–21), while etanercept is eliminated by the Kupffer cells of the liver (Genovese MC, et al: unpublished observations). It is also unlikely that anakinra and etanercept would bind and block the effect of each other.

Second, anti-anakinra antibodies could be responsible for the lack of benefit of combination therapy. This too is unlikely, because a low percentage of patients had potentially neutralizing anti-anakinra antibodies, and the antibody results from this study were consistent with the antibody data from other anakinra studies in which efficacy was demonstrated. Third, anti-TNF therapy could have down-regulated IL-1 expression, rendering any impact of anti-IL-1 therapy negligible. This remains a possibility, although it would not explain the inconsistency between the clinical and preclinical results. Fourth, the degree of overlap and interplay between IL-1 and TNF may leave little room for improvement beyond the efficacy attainable with an effective TNF inhibitor. This is an intriguing possibility that requires further study.

The safety results for this study showed that combination anticytokine therapy was associated with a higher incidence of serious infections than was observed with the use of etanercept alone. These results are comparable with what was previously reported in a small open-label study of the combination of etanercept and anakinra (22). Many patients in this study were receiving a combination of 4 potentially immunosuppressive agents, including corticosteroids, MTX, anakinra, and etanercept, all of which could have contributed to this finding. Combination therapy was also more commonly associated with neutropenia, which is probably not surprising because decreases in the neutrophil count have been associated with both etanercept and anakinra (Genovese MC, et al: unpublished observations). It is notable, however, that in this study neutropenia was not associated with the risk of serious infection.

These results suggest that use of combination treatment with anakinra and etanercept is not justified in patients with RA who are naive to biologic therapy.

However, the possibility that combination anticytokine treatment could benefit certain patients cannot be excluded based on this relatively small study. The response to combination therapy might be different in patients with partial or inadequate responses to prior anticytokine treatment, for example, although the safety concerns raised by this study would remain. Furthermore, these results do not preclude the possibility of successful combination therapy with future agents selectively blocking other pathways.

Overall, the results from this study provide no evidence of an additional treatment benefit of combination therapy with etanercept plus anakinra in patients with active RA despite the use of MTX. In fact, the combination posed an increased risk of serious infection and neutropenia. Treatment with etanercept or anakinra alone, or either agent in combination with MTX, has been demonstrated to be effective and safe in previous studies, and the findings from this study do not affect the profiles of the individual compounds. The findings do raise important questions about the role of each of these cytokines in the pathophysiology of RA and other inflammatory diseases. The development of anticytokine therapies has epitomized the translation of research from bench to bedside, and the results of this study highlight the need to translate these results back to the bench to better understand the interrelationship of these cytokines in human disease and to explain the incongruity of the preclinical and clinical findings.

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APPENDIX A: MEMBERS OF THE 20000223 STUDY GROUP

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Double-Blind Randomized Controlled Clinical Trial of the Interleukin-6 Receptor Antagonist, Tocilizumab, in European Patients With Rheumatoid Arthritis Who Had an Incomplete Response to Methotrexate

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for the CHARISMA Study Group

Objective. To establish the safety and efficacy of repeat infusions of tocilizumab (previously known as MRA), a humanized anti-interleukin-6 (IL-6) receptor antibody, alone and in combination with methotrexate (MTX), for the treatment of rheumatoid arthritis (RA).

Methods. The study group comprised 359 patients with active RA in whom the response to MTX was

inadequate. During a stabilization period, these patients received their current dose of MTX for at least 4 weeks. Following stabilization, they were randomized to 1 of 7 treatment arms, as follows: tocilizumab at doses of 2 mg/kg, 4 mg/kg, or 8 mg/kg either as monotherapy or in combination with MTX, or MTX plus placebo.

Results. A 20% response (improvement) according to the American College of Rheumatology criteria (ACR20 response) was achieved by 61% and 63% of patients receiving 4 mg/kg and 8 mg/kg of tocilizumab as monotherapy, respectively, and by 63% and 74% of patients receiving those doses of tocilizumab plus MTX, respectively, compared with 41% of patients receiving placebo plus MTX. Statistically significant ACR50 and ACR70 responses were observed in patients receiving combination therapy with either 4 mg/kg or 8 mg/kg of tocilizumab plus MTX ($P < 0.05$). A dose-related reduction in the Disease Activity Score in 28 joints was observed from week 4 onward, in all patients except those receiving monotherapy with 2 mg/kg of tocilizumab. In the majority of patients who received 8 mg/kg of tocilizumab, the C-reactive protein level/erythrocyte sedimentation rate normalized, while placebo plus MTX had little effect on these laboratory parameters. Tocilizumab was mostly well tolerated, with a safety profile similar to that of other biologic and immunosuppressive therapies. Alanine transaminase and aspartate transaminase levels followed a sawtooth pattern (rising and falling between infusions). There were moderate but reversible increases in the nonfasting total cholesterol and triglyceride levels and reversible reductions in the high-density lipoprotein cholesterol and neutrophil levels. There were 2 cases of sepsis, both of which occurred

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in patients who were receiving combination therapy with 8 mg/kg of tocilizumab plus MTX.

Conclusion. These results indicate that targeted blockade of IL-6 signaling is a highly efficacious and promising means of decreasing disease activity in RA.

Interleukin-6 (IL-6) is a proinflammatory cytokine that is abundantly expressed in patients with rheumatoid arthritis (RA) and is detectable in the joints and circulation of such patients during active phases of the disease (1-4). IL-6 binds to its soluble and membrane-bound receptors, and their interaction with gp130 transduces intracellular signals that mediate gene activation and a wide range of biologic activities (5). Preclinical and human studies have demonstrated IL-6 activities relevant to RA. These include the differentiation of B cells into immunoglobulin-secreting plasma cells, activation of T cells, induction of acute-phase proteins by hepatocytes, and production of platelets (6-9). IL-6 induces the recruitment of chemokines and leukocytes (10) and has been shown to induce proliferation of synovial fibroblasts (11). In addition, IL-6 has a profound effect on bone and can induce osteoclast differentiation and activation in vitro (12). IL-6 appears also to be involved in damage to cartilage, by decreasing aggrecan protein and type II collagen production by chondrocytes (13). The inflammatory destructive effects of IL-6 have been validated, with data in animals suggesting an important role of IL-6 in the induction and maintenance of chronic synovial inflammation (14-16). IL-6 gene-knockout mice have been demonstrated to be protected from arthritis (17). Because of the key role played by IL-6 in several phenomena typical of RA, IL-6 is a candidate target for therapeutic inhibition as a novel approach to the treatment of RA.

Monoclonal antibodies (infliximab and adalimumab) and a soluble tumor necrosis factor (TNF) receptor fusion protein (etanercept), which inhibit another proinflammatory cytokine TNF, have become established as effective options for the treatment of RA, especially in patients who experience an inadequate response to disease-modifying antirheumatic drugs (DMARDs), including methotrexate (MTX) (18). The efficacy of anti-TNF therapy is increased by concomitant treatment with MTX (19,20). Nonetheless, 20-40% of patients have an inadequate response to such treatment, and there is an unmet need for new therapeutic interventions with new modes of action for the treatment of RA.

Tocilizumab (previously known as MRA) is one of the molecules in development that offers a new

mechanism of action. Tocilizumab, a humanized anti-human IL-6 receptor antibody of the IgG1 subclass, was developed collaboratively by Osaka University and Chugai Pharmaceutical Company Ltd (Japan). Tocilizumab is humanized by the grafting of complementarity-determining regions of a mouse anti-human IL-6 receptor monoclonal antibody onto human IgG1, using recombinant DNA technology. Tocilizumab has been shown to compete for both the membrane-bound and soluble forms of human IL-6 receptor, thus inhibiting the binding of IL-6 to its receptors and its proinflammatory activity. Short-term clinical trials of tocilizumab in adult-onset RA and systemic-onset juvenile idiopathic arthritis have demonstrated acceptable safety and significant efficacy (21-24).

The objective of this multicenter randomized clinical trial (the Chugai Humanized Anti-Human Recombinant Interleukin-6 Monoclonal Antibody [CHARISMA] study) was to establish the safety and efficacy of repeat infusions of tocilizumab, alone and in combination with MTX, in patients with established RA in whom the response to MTX was inadequate and who had continuing disease activity. Tocilizumab was used either as monotherapy (by discontinuation of MTX) or concomitantly with MTX therapy and compared with placebo infusions in patients who continued to receive a fixed dose of MTX during this 20-week study.

PATIENTS AND METHODS

Role of the funding source. Chugai Pharma Europe (a member of the Roche Group) provided funding for the trial and was responsible for supply of the study medication (active and placebo), for data processing and management, statistical analysis, and consulting via a clinical research organization (Covance), for ongoing medical safety reviews, and for reporting of serious adverse events. Dr. Maini was responsible for the trial design and manuscript preparation. Both Chugai and Roche Pharmaceuticals checked and approved the submitted manuscript.

Patient and treatment protocol. In this randomized, double-blind, controlled trial, the study group comprised 359 patients fulfilling the American College of Rheumatology (ACR; formerly, the American Rheumatism Association) revised criteria for a diagnosis of RA (25); all patients had active disease, had experienced an inadequate response to MTX, and had been stabilized on their current dose of MTX for at least 4 weeks before randomization. Three groups of patients received intravenous infusions of tocilizumab (2 mg/kg, 4 mg/kg, and 8 mg/kg, respectively) every 4 weeks, plus MTX placebo once weekly. Another 3 groups of patients received intravenous infusions of tocilizumab (2 mg/kg, 4 mg/kg, and 8 mg/kg, respectively) every 4 weeks, plus MTX once weekly. A control group received placebo infusions every 4 weeks and MTX once weekly. Thus, all patients received a total of 4 infusions of tocilizumab or tocilizumab placebo once every 4 weeks, to-

gether with 10–25 mg of MTX or MTX placebo each week. End point efficacy evaluations were performed at week 16; followup evaluations for safety were performed at week 20, after which patients were no longer monitored. A total of 359 patients from 57 rheumatology centers in Europe were randomized to participate in this study. Members of the CHARISMA Study Group and the European countries involved are shown in Appendix A.

Randomization was performed centrally. When a patient was eligible for randomization into the study, an interactive voice response system was used to allocate treatment, by determining inclusion into the group that would minimize any imbalances between MTX dose level at baseline and between patients from a center. All patients and investigators were blinded to the study treatments.

Tocilizumab or placebo (an aqueous solution of sucrose and polysorbate 80, resembling the active treatment) was diluted in normal saline and administered by intravenous infusion over 1 hour. Pre-prepared capsules containing 10-mg, 12.5-mg, 15-mg, 17.5-mg, 20-mg, 22.5-mg, and 25-mg doses of MTX were blinded by over-capsulation using a lactose filler; the matching placebo capsules contained only filler.

The MTX dose remained unchanged for at least 4 weeks prior to baseline and throughout the study. Patients at all centers were supplied (separately, from the same source) 5 mg of folic acid weekly, for the duration of the study. Patients randomly allocated to receive tocilizumab monotherapy were switched to MTX placebo on day 0.

Inclusion criteria were a diagnosis of RA according to the ACR revised criteria (25), disease duration of at least 6 months, active disease (defined as ≥ 6 tender joints and ≥ 6 swollen joints, based on a 28-joint count), an erythrocyte sedimentation rate (ESR) ≥ 28 mm/hour, and/or a C-reactive protein (CRP) level ≥ 1.0 mg/dl. Patients must also have shown an inadequate response to MTX or a disease flare while receiving MTX (at a dosage of 10–25 mg weekly) during a minimum of 6 months of therapy. An inadequate response was defined as the presence of active disease, as described above, despite MTX therapy. If patients required concomitant treatment with nonsteroidal antiinflammatory drugs and/or oral steroids, the dose must have been stable for at least 4 weeks prior to study entry and during the course of the study (for steroids, ≤ 10 mg prednisolone or equivalent).

Exclusion criteria included leukopenia (white blood cell count $< 4.0 \times 10^9$ /liter, absolute neutrophil count $< 2.0 \times 10^9$ /liter) and/or thrombocytopenia (platelet count $< 150 \times 10^9$ /liter), any hepatic dysfunction as shown by aspartate transaminase (AST) and alanine transaminase (ALT) levels > 1.5 -fold the upper limit of normal or significant renal impairment (serum creatinine level > 1.5 -fold the upper limit of normal). Patients who received DMARDs (excluding MTX) within 4 weeks prior to the start of the study were excluded, as were patients who received anti-TNF agents within 12 weeks or leflunomide within 6 months of infusion of study medication.

Ethical approval was obtained from the appropriate authorities in each country, and each patient gave informed consent. The first patient was enrolled on May 14, 2001, and the final patient exited the study on September 3, 2002.

Efficacy assessments. The primary clinical end point was the percentage of patients who achieved improvement of 20% according to the ACR criteria (ACR20 response) at week

16; secondary end points included the ACR50 and ACR70 responses (26). For the analysis of efficacy (i.e., the ACR20 response at 16 weeks), all patients who withdrew before the week 16 assessment because of lack of efficacy were included in the analysis as nonresponders, as per protocol. For patients who withdrew for other reasons, the last recorded assessment of the ACR20 response was carried forward.

Other secondary end points included percent and absolute change from baseline in individual disease activity measures (swollen joint count, tender joint count, physician's global assessment of disease activity, patient's global assessment of disease activity, patient's pain score, ESR, CRP level, and Health Assessment Questionnaire [HAQ] score), as well as changes in the duration of early morning stiffness, and the Disease Activity Score in 28 joints (DAS28) (27). In this study, the DAS28 was calculated using the ESR, according to a previously described formula (28). Remission was defined according to the European League Against Rheumatism (EULAR) definition of a DAS28 score < 2.6 (29).

Safety assessments. Safety assessments included physical examinations, pre- and postdose electrocardiograms, and laboratory analyses of hematology, serum biochemistry, coagulation, immunologic parameters, and urine. Adverse events were recorded throughout the study. A treatment-emergent adverse event was defined as any adverse event that occurred after commencement of allocated treatment or an adverse event that occurred prior to the allocated treatment but worsened in severity after commencement of the allocated treatment. A treatment-related adverse event was defined as any adverse event whose relationship to the study drug was described as possible, probable, or definite, as documented in the case report forms.

Statistical analysis. The primary efficacy end point was the ACR20 response at week 16, using an intent-to-treat full-analysis set. All patients who were allocated to receive study medication, had received at least 1 dose, and had postrandomization efficacy data available were included in the full-analysis set. All patients who withdrew because of lack of efficacy before the week 16 assessment were included in the analysis as nonresponders. For patients who withdrew because of other reasons, the last recorded assessment of the ACR20 response was carried forward. Differences between treatment groups in the proportion of responders were assessed using logistic regression. A model was fitted for the treatment groups to which patients were assigned in a center and the stratification of weekly doses of MTX. Analyses similar to the primary efficacy analysis were performed for the secondary efficacy end points.

Treatment groups were formally compared at the week 4, week 8, week 12, week 16, and week 20 assessments, separately; changes from baseline to each assessment were analyzed using analysis of covariance, with an effect for treatment, center, and the respective baseline value fitted as a covariate. The *P* value for the overall test of difference between treatment groups from this model was determined. If results of the overall test of difference between treatment groups from this model were statistically significant, the linear and quadratic components of the dose-response relationship were assessed. *P* values for linear, quadratic, and average dose effect were determined for the groups receiving monotherapy and the groups receiving combination therapy.

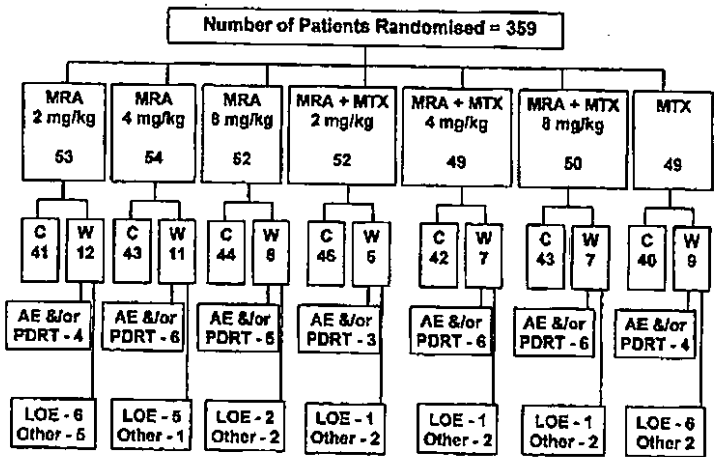


Figure 1. Diagram showing randomization, numbers of patients who completed or withdrew from the trial, and reasons for withdrawal. More than 1 reason for withdrawal could be given. MRA = tocilizumab; MTX = methotrexate; C = completed; W = withdrawn; AE = adverse event; PDRT = possible drug-related toxicity; LOE = lack of efficacy; Other = withdrew consent, used prohibited medication, or experienced intercurrent illness.

Table 1. Characteristics of patients at baseline*

Characteristic	MRA monotherapy			Combination therapy, MRA plus MTX			MTX (n 49)	All groups (n 359)
	2 mg/kg (n 53)	4 mg/kg (n 54)	8 mg/kg (n 52)	2 mg/kg (n 52)	4 mg/kg (n 49)	8 mg/kg (n 50)		
Age, years	52.2	49.3	50.1	49.2	50.2	50.1	50.9	50.3
No. of men/no. of women	9/44	13/41	14/38	7/45	12/37	11/39	11/38	77/282
Duration of RA, months	9.19	9.79	9.21	9.33	7.82	10.62	11.24	-
Tender joint count (28 joints)	15	15	15	15	13	15	16	15
Swollen joint count (28 joints)	11	11	12	11	11	11	12	11
ESR, mm/hour	39†	41	39‡	45§	40	39¶	43	41
C-reactive protein, mg/liter	26†	19	22‡	28§	31	24¶	32	27
DAS28	6.48†	6.55	6.43‡	6.58§	6.34	6.47¶	6.75	-
Rheumatoid factor positive, no.	44	39	43	46	38	40	47	-
MTX dose*								
Low	20	20	19	19	17	18	17	-
Medium	25	25	24	25	24	24	24	-
High	8	9	9	8	8	8	8	-
Patient's assessment of pain	58.9†	62.2	58.5‡	59.7§	60.2	57.0¶	63.8	-
Duration of MTX therapy, mean ± SD months	33.0 ± 25.5	34.1 ± 26.5	40.0 ± 30.5	38.8 ± 27.1	29.0 ± 17.7	40.4 ± 32.7	33.3 ± 27.8	35.5 ± 27.3
Patient's global assessment of disease activity	62.0†	63.6	60.7‡	62.3§	60.9	62.1¶	68.8	-
Physician's global assessment of disease activity	61.3†	62.1	59.5‡	59.2§	59.5	61.3¶	66.0	-
Duration of early morning stiffness, minutes	90.0†	110.0	120.0†	110.0§	90.0	120.0¶	90.0	-

* Except where indicated otherwise, values are the mean. MRA = tocilizumab; MTX = methotrexate; RA = rheumatoid arthritis; ESR = erythrocyte sedimentation rate; DAS28 = Disease Activity Score in 28 joints; low 10 mg or 12.5 mg weekly; medium 15 mg or 17.5 mg weekly; high = 20 mg, 22.5 mg, or 25 mg weekly.
† n = 52 patients.
‡ n = 51 patients.
§ n = 50 patients.
¶ n = 49 patients.

RESULTS

Baseline characteristics and patient flow. Three hundred fifty-nine patients were randomly allocated to the 7 treatment groups. Patient flow through the trial and randomization to each treatment arm are shown in Figure 1. Approximately equal numbers of patients were randomly allocated to each arm of the study. During the trial, 60 patients withdrew (34 patients withdrew due to adverse events and/or possible drug-related toxicity). Of the 359 patients randomized to receive study medication, all were included in the safety population, and 354 patients were included in the full-analysis set (5 patients were excluded from the full-analysis set because of a protocol violation). The baseline demographics of the treatment groups were similar, and no statistically significant differences were observed (Table 1).

All patients had previously been treated with DMARDs, the most common of which was MTX. The mean duration of prior MTX treatment varied across the groups, with the longest duration recorded in the group receiving 8 mg/kg of tocilizumab plus MTX (40 months), and the shortest duration recorded in the group receiving 4 mg/kg of tocilizumab plus MTX (29 months). Most patients had previously been treated with 1–3 different DMARDs, although several patients had previously received more than 5 different DMARDs. Cyclophosphamides had been taken by 1.4% of patients, leflunomide by 6.4%, sulfasalazine by 53.5%, azathioprine by 6.7%, cyclosporine by 20.3%, etanercept by 2.8%, auranofin by 2.8%, chloroquine by 23.4%, gold by 3.1%, hydroxychloroquine by 12.0%, penicillamine by 7.2%, and sodium aurothiomalate by 21.2% of patients. Fifty patients had received TNF inhibitors (infliximab or etanercept) prior to study enrollment. A high proportion of patients in each treatment group was serum rheumatoid factor positive (overall mean 82.7%), and the proportion was highest in the group receiving placebo plus MTX (95.9%).

Primary efficacy end point. The primary end point of the study, an ACR20 response at week 16, was achieved by 61% and 63% of the patients receiving monotherapy with 4 mg/kg and 8 mg/kg of tocilizumab, respectively, compared with 41% of patients receiving placebo plus MTX ($P < 0.05$). The responses of the group receiving tocilizumab at a dose of 2 mg/kg were not significantly different from those of the group assigned to receive placebo plus MTX (Figure 2). The ACR20 responses among patients receiving combination therapy with tocilizumab (2 mg/kg, 4 mg/kg, and 8

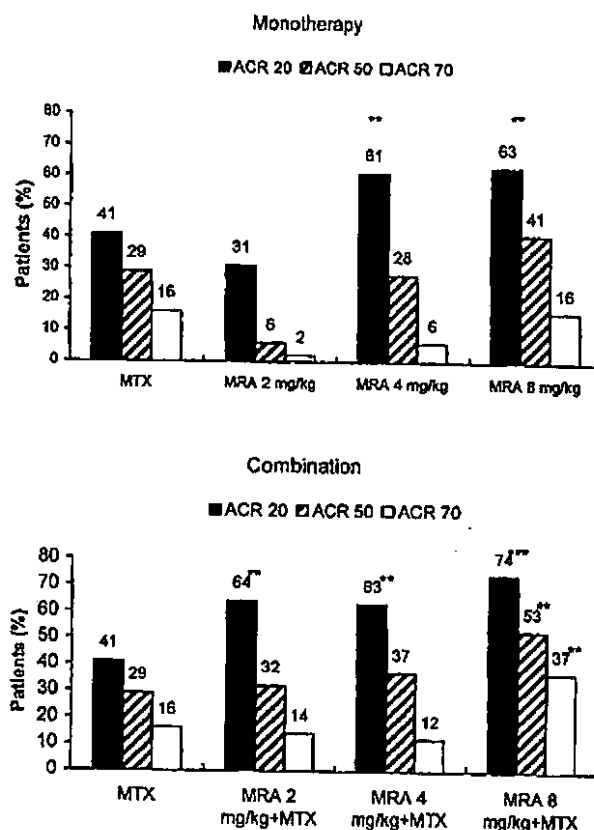


Figure 2. Efficacy end points. American College of Rheumatology 20% (ACR20), ACR50, and ACR70 response rates at week 16 in the groups of patients receiving methotrexate (MTX) plus placebo, those receiving MRA (tocilizumab) monotherapy, and those receiving combination therapy with MRA plus MTX. ** $P < 0.05$; *** $P = 0.001$ versus MTX.

mg/kg) plus MTX exceeded those among patients receiving placebo plus MTX (64%, 63%, and 74%, respectively, versus 41%; $P < 0.05$, $P < 0.05$, and $P < 0.001$, respectively) (Figure 2).

Secondary efficacy end points. The ACR50 and ACR70 response rates at week 16 were measured, as shown in Figure 2. Only combination therapy with 8 mg/kg of tocilizumab plus MTX was significantly better than placebo plus MTX in terms of both the ACR50 and the ACR70 ($P < 0.05$). There was a similar pattern of change in DAS28 scores, showing a clear dose response with tocilizumab and significant differences from week 4 onward, except in the group receiving monotherapy with 2 mg/kg of tocilizumab. The maximum reduction in the DAS28 was achieved with the 8-mg/kg doses. Among patients assigned to the 8-mg/kg dose of tocilizumab in

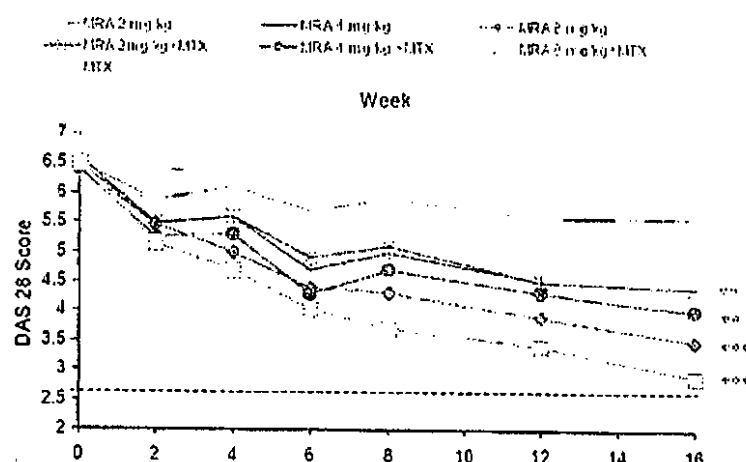


Figure 3. Mean change from baseline in the Disease Activity Score in 28 joints (DAS28). From week 4 onward, the groups receiving 8 mg/kg of MRA (tocilizumab), with or without methotrexate (MTX), showed a statistically significant decrease in the DAS28 compared with the group receiving MTX plus placebo. At weeks 4, 8, and 12, the groups receiving 4 mg/kg of MRA, with or without MTX, and the group receiving 2 mg/kg of MRA plus MTX demonstrated a statistically significant decrease in the DAS28 compared with the group receiving MTX plus placebo. At week 16, this decrease was maintained in the group receiving 4 mg/kg of MRA plus MTX. The broken horizontal line shows the score (2.6) below which disease is considered to be in remission. ** = $P < 0.05$ versus MTX; *** = $P < 0.001$ versus MTX.

combination with MTX, the mean DAS28 at 16 weeks was 2.9. All groups receiving tocilizumab, alone or in combination with MTX (except for the group assigned to monotherapy with 2 mg/kg of tocilizumab), showed a greater decrease in the DAS28 by week 16 than did the group receiving placebo plus MTX (for combination therapy with 8 mg/kg of tocilizumab and monotherapy with 8 mg/kg of tocilizumab, $P < 0.001$; for combination therapy with 4 mg/kg of tocilizumab, $P < 0.05$ versus placebo plus MTX) (Figure 3).

A further analysis was performed to calculate the percentage of patients who achieved remission according to the DAS, in the group receiving 8 mg/kg of tocilizumab plus MTX, the group receiving 8 mg/kg of tocilizumab as monotherapy, and the group receiving placebo plus MTX. The analysis showed that the rate of remission was 34% among those assigned to 8 mg/kg of tocilizumab plus MTX, 17% among those receiving 8 mg/kg of tocilizumab as monotherapy, and 8% among those receiving placebo plus MTX. Among patients responding to tocilizumab treatment, a response was observed by week 4, and this was maintained during the course of the study. The slope of the graph suggested that a peak response had not been reached by the end of

the study, and that further improvement could be attained.

Each of the individual clinical components of the ACR response assessment (swollen joint count, tender joint count, physician's global assessment of disease activity, patient's global assessment of disease activity, patient's pain score, and HAQ score), as well as the duration of morning stiffness, showed an improvement at doses of tocilizumab ≥ 4 mg/kg. However, comparison with the placebo plus MTX control group at week 16 consistently showed that monotherapy with 2 mg/kg of tocilizumab performed less well than did placebo plus MTX. For the swollen joint counts, the mean reduction observed among patients receiving 8 mg/kg of tocilizumab, alone and in combination with MTX, was statistically significant compared with the reduction in the group assigned to placebo plus MTX ($P = 0.01$ and $P < 0.001$, respectively). Additionally, the mean reduction in the tender joint count in the group receiving combination therapy with 8 mg/kg of tocilizumab plus MTX was statistically significantly superior compared with the mean reduction in the group assigned to receive placebo plus MTX ($P = 0.009$).

The mean CRP level and the mean ESR showed

a marked decrease over time in all of the patients receiving tocilizumab except those assigned to 2 mg/kg as monotherapy (both the ESR and the CRP level) and those receiving 4 mg/kg as monotherapy (the CRP level only), while placebo plus MTX had little effect on these laboratory parameters (Figure 4). Especially in the groups receiving 4 mg/kg of tocilizumab, as monotherapy or combination therapy, there was a sawtooth pattern, with a decrease in these parameters 2 weeks after dosing (maximum observed biologic effect) and an increase prior to the next infusion. The 8-mg/kg dose of tocilizumab produced a more sustained decrease. The mean CRP level normalized after the first infusions of 8 mg/kg of tocilizumab (as both monotherapy and combination therapy) and 4 mg/kg of tocilizumab as combination therapy, while the mean ESR normalized after the third infusions of tocilizumab at a dose of 8 mg/kg (as both monotherapy and combination therapy). The comparison between 8 mg/kg of tocilizumab, as monotherapy or combination therapy, and placebo plus MTX showed the most significant differences ($P \leq 0.001$) at week 16. The mean ESR and the mean CRP level returned toward baseline values within 4–8 weeks after the last infusion of tocilizumab.

Other direct and indirect laboratory markers of inflammation that returned toward baseline levels after tocilizumab treatment included serum ferritin, serum amyloid A, C4 complement, fibrinogen, neutrophils, platelets, and hemoglobin.

Safety. In general, tocilizumab was well tolerated in this trial (see Table 2). Approximately half of the patients experienced adverse events after treatment, the majority of which were mild or moderate in intensity, and only one-fourth of which were judged to be treatment-related. The most frequent treatment-emergent adverse events were infections (mixed viral and bacterial), musculoskeletal disorders (all of which were compatible with RA), gastrointestinal disorders (a variety of minor disorders, the incidence of which was higher among those receiving tocilizumab compared with those receiving placebo plus MTX), skin rash, pruritus, and erythema.

There was no clear pattern of occurrence of adverse events by dose group, except for serious adverse events (Table 2). Thirty patients experienced 35 treatment-emergent serious adverse events. The incidence of treatment-emergent serious adverse events was highest in the group receiving 2 mg/kg of tocilizumab as monotherapy and lowest in the group receiving 4 mg/kg of tocilizumab plus MTX (Table 2). The most common

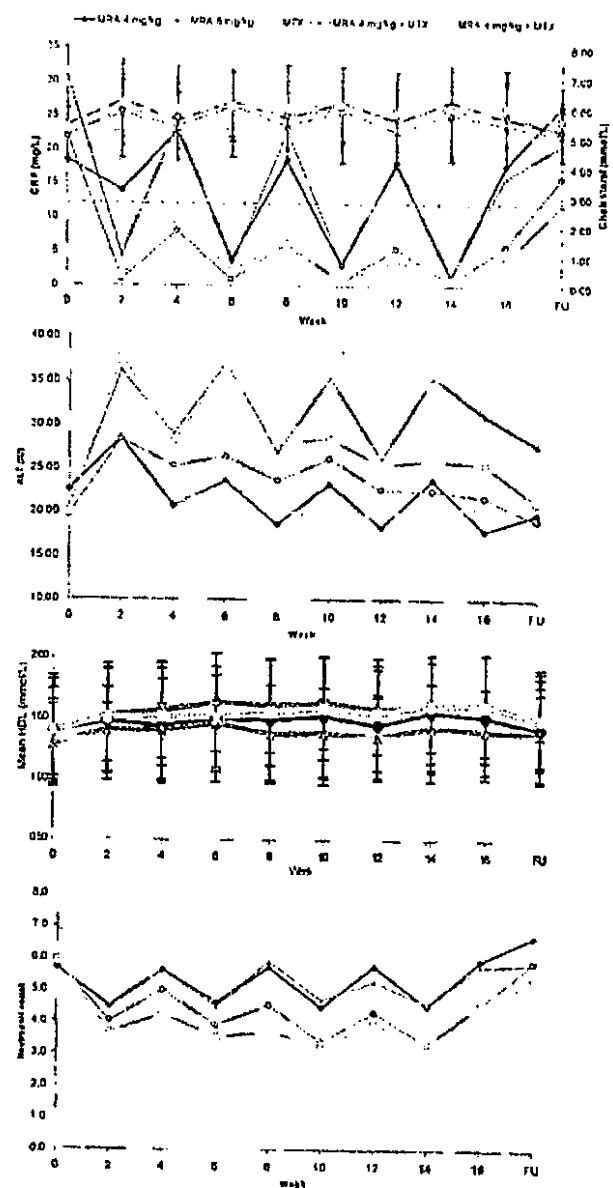


Figure 4. Effects of different doses of MRA (tocilizumab) on the C-reactive protein (CRP) level, the alanine transaminase (ALT) level, the high-density lipoprotein (HDL) cholesterol level, and the neutrophil count. Mean CRP values (solid lines in top panel) demonstrated a maximum observed biologic effect after dosing with MRA, correlating with changes in the nonfasting total cholesterol level (broken lines in top panel). Mean ALT values during MRA therapy followed a sawtooth pattern, rising and falling between infusions. Moderate, reversible changes in the nonfasting HDL cholesterol level occurred during treatment with MRA. A dose-dependent, reversible decrease in the neutrophil count occurred following dosing with MRA. FU = followup.

Table 2. Summary of adverse events (AEs)*

	MRA monotherapy			Combination therapy, MRA plus MTX			
	2 mg/kg (n = 53)	4 mg/kg (n = 54)	8 mg/kg (n = 52)	2 mg/kg (n = 52)	4 mg/kg (n = 49)	8 mg/kg (n = 50)	MTX (n = 49)
Patients with ≥ 1 treatment-emergent AE	30	27	31	30	19	27	23
Patients with ≥ 1 treatment-related AE	13	14	15	12	9	14	9
Patients who discontinued due to an AE	4	5	5	3	6	6	4
Patients with ≥ 1 serious treatment-emergent AE	8	5	3	4	1	7	2
Patients with ≥ 1 serious treatment-related AE	5	2	1	2	0	4	0
Serious treatment-emergent infections†	4	0	0	0	0	3	0
Serious treatment-emergent anaphylactic reaction/shock‡	3	1	0	0	0	0	0

* MRA = tocilizumab; MTX = methotrexate.

† In the 2-mg/kg monotherapy group, infections included a limb abscess/osteomyelitis not otherwise specified (NOS), a pleural infection NOS, and a lower respiratory tract infection NOS. In the 8-mg/kg combination therapy group, infections included infective arthritis NOS and 2 cases of sepsis NOS.

‡ In the 2-mg/kg monotherapy group, an additional patient experienced a hypersensitivity reaction. In the 2-mg/kg combination therapy group, 1 patient had a nonserious anaphylactic reaction. In the 8-mg/kg combination therapy group, 1 patient experienced a hypersensitivity reaction NOS.

treatment-emergent serious adverse events were aggravated RA (2 cases in the group receiving low-dose combination therapy and 1 case in the group receiving placebo plus MTX), 4 infections in 3 patients in the 2-mg/kg monotherapy group (1 patient with a limb abscess and osteomyelitis and 2 patients with respiratory infections), 3 infections in the 8-mg/kg plus MTX group (including 1 patient with infective arthritis and 2 with sepsis not otherwise specified), and anaphylactic shock/reaction and hypersensitivity (4 cases in the 2-mg/kg monotherapy group and 1 case in the 4-mg/kg monotherapy group). Of the 2 patients for whom sepsis was reported as a serious adverse event, septicemia developed in 1 patient following a *Staphylococcus aureus*-infected skin wound; in the other patient, septicemia was diagnosed, but there were no signs of focal infection and no organisms were isolated on blood culture, and pyelitis was suspected. That patient had a history of streptococcal septicemia due to a dental abscess (10 months previously).

Additionally, 2 cases of nonserious anaphylactic reactions and hypersensitivity were reported (1 in a patient receiving monotherapy with 4 mg/kg of tocilizumab, and 1 in a patient receiving combination therapy with 2 mg/kg of tocilizumab). Overall, anti-tocilizumab antibodies developed in 25 patients, all of whom were in the groups receiving either 2 mg/kg or 4 mg/kg of tocilizumab as monotherapy; anti-tocilizumab antibodies developed in none of the patients receiving treatment with 8 mg/kg of tocilizumab, as either monotherapy or combination therapy. No cases of reactivation of tuber-

culosis were seen, and no opportunistic infections were observed in this study.

Liver function test changes. During the dosing period of this trial, the mean ALT level and, to a lesser extent, the mean AST level increased in all patients receiving tocilizumab, following a sawtooth pattern (rise and fall) between infusions (Figure 4). This increase was tocilizumab-related but was accentuated in patients receiving tocilizumab in combination with MTX (>3 -fold the upper limit of normal in 2% of the patients receiving combination therapy). All of the mean values returned to near-baseline values within 8 weeks of the final infusion. The mean ALT level increased in a dose-related manner in the groups receiving tocilizumab monotherapy; the maximum increase from baseline at week 2 (45%) was observed in the group receiving 8 mg/kg of tocilizumab. Larger dose-related increases in the ALT level occurred in the combination-therapy groups; the maximum increase from baseline at week 2 (88%) was observed in the group receiving combination therapy with 8 mg/kg of tocilizumab plus MTX. No increase in the mean ALT level was observed in the group receiving placebo plus MTX. A similar pattern of changes in the mean AST level was seen, but these changes were of lesser magnitude.

Certain patients showed marked but reversible increases in the level of transaminases, which in some cases led to discontinuation of the study treatment. Five patients who had ALT levels >100 IU/liter were withdrawn from the study; all of these patients were in the groups receiving tocilizumab plus MTX. In 4 patients,

the ALT level returned to a level within the normal range within 4 weeks of withdrawal. Overall, 127 patients treated with tocilizumab experienced an ALT level greater than the upper limit of normal during the study; 35 of these patients had mildly abnormal ALT values at baseline, compared with 12 patients who were treated with MTX alone. In 112 tocilizumab-treated patients, the ALT level returned to near-baseline values by the time of the followup visit. Eighteen patients had ALT values >100 IU/liter during the study, and all of these patients had been treated with tocilizumab.

Mean total bilirubin values increased gradually during the study period in tocilizumab-treated patients, but this increase was not exacerbated by the addition of MTX. Among the groups receiving tocilizumab monotherapy, the maximum increase from baseline in mean total bilirubin levels was 83% at week 16; this was observed in the group receiving 8 mg/kg of tocilizumab. Among patients receiving combination therapy, the maximum increase in bilirubin levels (59%) was observed at week 14 in the group receiving 4 mg/kg of tocilizumab plus MTX. Again, values had largely returned to normal by the followup visit, and the group receiving placebo plus MTX was unaffected. Twenty-one patients had bilirubin levels >21 μ moles/liter during the study, but these patients had high total bilirubin levels when they entered the study (mean 17 μ moles/liter, which is the upper end of the normal range) and demonstrated predominantly unconjugated hyperbilirubinemia. In 86% of this subgroup, total bilirubin levels returned to near-baseline values by the time of followup. No relationship between elevated ALT levels and elevated bilirubin levels was observed. No individual patient experienced significant elevations in both the ALT level and the bilirubin level simultaneously.

Lipid changes. During the study period, moderate but reversible increases in the mean levels of non-fasting total cholesterol, high-density lipoprotein cholesterol, and triglycerides were seen in the groups assigned to receive treatment with tocilizumab. The levels increased initially and then stabilized and did not continue to increase during the treatment period (Figure 4). Importantly, the mean atherogenic index remained largely unchanged despite some rise and fall between infusions in the higher-dose groups. In both of the groups receiving 8 mg/kg of tocilizumab, the atherogenic index was reduced to below its initial level by the 20-week followup visit. Again, certain individuals experienced abnormally high lipid levels, but in the majority of these patients the levels were high at study entry and showed a relatively small increase during treatment.

Neutrophils. There was a general dose-dependent reduction in the neutrophil count following treatment with tocilizumab, but the neutrophil count recovered when tocilizumab was withdrawn (Figure 4). Forty patients experienced neutropenia during treatment with tocilizumab. Reductions in the neutrophil counts of 47% and 43%, respectively, were seen at week 14 in patients receiving 8 mg/kg of tocilizumab as monotherapy and in those receiving 8 mg/kg of tocilizumab plus MTX. Of the 13 patients who experienced grade 2 neutropenia (between 1,000 and 1,500 neutrophils/mm³) or grade 3 neutropenia (between 1,000 and 500/mm³) during the dosing period excluding baseline, all had received higher doses of tocilizumab, with or without MTX. The addition of MTX did not seem to exacerbate the effect, based on the relative incidence of neutropenia in the groups receiving tocilizumab as monotherapy or combination therapy. Infections were not associated with reduced neutrophil counts. The lowest neutrophil count observed was 0.88×10^9 /liter at week 8 (in patients receiving 8 mg/kg of tocilizumab), and no clear pattern in the occurrence of neutropenia was observed across study visits. Some patients experienced neutropenia after the first infusion of tocilizumab, and some patients experienced neutropenia after later infusions, and there was no evidence that continued dosing worsened the effect. The neutrophil count returned to the normal range by the followup visit in all except 1 patient, in whom the count was abnormally low at baseline.

The number of patients with low neutrophil counts was related to the dose of tocilizumab administered (0, 5, and 14 patients in the 2-mg/kg, 4-mg/kg, and 8-mg/kg tocilizumab groups, respectively). The proportion of patients who experienced infections was no different between those with low neutrophil counts (7 of 40 patients) and those with normal neutrophil counts (62 of 359 patients).

DISCUSSION

Previously, a monoclonal antibody to the IL-6 molecule was shown to induce improvements in patients with RA. However, the effects were short-lived, and neutralizing antibodies developed against this murine antibody (30). Clinical studies in Japan, in which tocilizumab was used as monotherapy (4 mg/kg or 8 mg/kg) in adults who had previously received DMARDs (including MTX at a dosage less than 8 mg/week) (22,23), and a dose-ranging study in Europe in which patients received a single dose of either 0.1 mg/kg, 1 mg/kg, 5 mg/kg, or 10

mg/kg of tocilizumab (21) have provided proof of concept that IL-6 blockade by tocilizumab is efficacious in the treatment of RA. Based on these data and those from our study, it may be concluded that IL-6 is an important driver of inflammation in RA. The blockade by tocilizumab of the membrane-bound as well as the soluble IL-6 receptor thus appears to be a direct and manipulable method for achieving the target of reducing the pathologic effects of excess IL-6.

The results of this study clearly show that infusions of tocilizumab every 4 weeks, with or without background MTX therapy, can produce marked and dose-related improvement in RA disease activity as measured by ACR20, ACR50, and ACR70 responses and clinically meaningful changes in the DAS28. The study also showed that the 4-mg/kg and 8-mg/kg doses of tocilizumab were associated with high percentages of ACR50 and ACR70 responses after only 4 infusions (the difference between patients receiving placebo plus MTX and those receiving 8 mg/kg of tocilizumab in combination with MTX was statistically significant) (Figure 2). Assessment of efficacy over time showed continued improvement in all dose groups. This improvement was seen up to the end of the 16-week trial, and there was an indication that maximum efficacy may not have been achieved in this short-term study.

The mean DAS28 among patients receiving combination therapy with 8 mg/kg of tocilizumab plus MTX approached the EULAR criteria for remission (DAS28 <2.6). The primary and secondary efficacy end points for tocilizumab therapy at the highest dose demonstrate that the efficacy of this therapy is in the same range as that of the currently available anti-TNF therapies (18). Thirty-seven percent of the patients in this high-dose group also achieved an ACR70 response, and 34% achieved EULAR-defined remission based on the DAS28.

One feature of note in this study is that a relatively high proportion of patients in the group receiving placebo plus MTX also continued to respond. The ACR20 response rate of 41% in this study contrasts with a rate of 11% among placebo-treated patients in the Japanese study (23). Because the Japanese study compared a placebo infusion with treatment that commenced after a DMARD washout period, and patients in our study received a placebo infusion during continuation of MTX therapy, the high response rate associated with placebo likely indicates that our patients were not MTX nonresponders, but rather that they had not yet fully responded to MTX at the time of trial entry. In the current study, the response to placebo plus MTX was

high, and a better control group might have been produced if the period of time during which controls received stable doses of MTX prior to trial entry had been longer than 4 weeks. The study design required that background MTX be withdrawn abruptly at the start of the study in the groups receiving tocilizumab monotherapy, thus placing these groups at a relative disadvantage in terms of efficacy in a population of incomplete MTX responders. This may have contributed to the negative comparison between the group receiving 2 mg/kg of tocilizumab and those receiving placebo plus MTX and to the apparent superiority of combination therapy over monotherapy at each dose level. However, the unexpectedly high rate of ACR20 responses in the group of patients who received MTX alone has no effect on the numbers of patients who achieved ACR50 or ACR70 responses while receiving tocilizumab plus MTX.

The durability of the response to tocilizumab was not investigated in this study; however, data from a phase II study performed in Japan demonstrated that with continued treatment, efficacy (as measured by ACR20, ACR50, and ACR70 responses) was maintained at least until week 72 (22). These data were obtained from an open-label extension study in which patients received tocilizumab at a dosage of 8 mg/kg every 4 weeks for an unrestricted period of time (22).

The most important safety signals in this study were related to changes in the results of liver function tests and serum lipid levels. A dose-related increase in the mean serum transaminase level was seen during tocilizumab treatment. This change appeared to be drug-related, due to the sawtooth pattern of rise and fall between infusions, most of which were observed after the first infusion of tocilizumab. More importantly, certain individuals experienced clinically significant increases in the transaminase level. The addition of MTX appeared to accentuate this effect. Mean bilirubin levels showed a gradual elevation over the study period. It is clear that the pattern of change for the mean bilirubin level was not the same as that for the mean transaminase level, and larger individual rises in the bilirubin level were not observed in the same patients as those experiencing rises in the levels of transaminases or lipids. Also, the total bilirubin elevations were mild (<3-fold the upper limit of normal). In a retrospective analysis of 8 patients with high levels of bilirubin, the increase appeared to be primarily in the unconjugated bilirubin fraction and possibly associated with underlying Gilbert's syndrome. Hemolysis may possibly have contributed to the bilirubin level, but there are no data sup-

porting this hypothesis, which needs to be investigated in the future.

There was no evidence of frank clinical hepatitis or predictors of the occurrence of hepatitis in any of the patients who had elevated levels of ALT and bilirubin. Based on a review of individual patients at each time point, there was no temporal correlation between ALT, total bilirubin, and cholesterol and clinical symptoms consistent with hepatitis. No patient had any 2 of the 3 laboratory abnormalities at the same time. In the Japanese extension study, bilirubin levels did reach a plateau in patients treated for up to 72 weeks.

Treatment with tocilizumab also resulted in an increase in the total cholesterol level as well as an increase in the level of triglycerides and HDL cholesterol (in this study, low-density lipoprotein cholesterol was not measured). At the same time, the mean atherogenic index was unchanged. As with bilirubin, changes in the cholesterol level were not temporally related to changes in the ALT level; however, there was a temporal correlation between total cholesterol levels and the CRP levels (CRP levels were dramatically reduced). The degree of elevation of the cholesterol level may be related to the degree of suppression of inflammation (31). It has also been observed that use of the anti-TNF monoclonal antibody infliximab to treat RA leads to an elevated cholesterol level (32). Increases in plasma lipid levels and/or CRP levels are associated with an increased risk of cardiovascular events (33,34), and a major cause of death in patients with RA is heart disease (35). The significance of the increases in lipid levels and the decreases in CRP levels observed in this study is unclear (especially because the lipid samples were nonfasting), and further studies are clearly indicated to investigate the clinical significance of these effects on the morbidity and mortality associated with ischemic heart disease. In addition, the mechanism of this lipid level elevation following DMARD treatment in general and tocilizumab in particular is unclear and should be further evaluated.

Favorable effects of tocilizumab were also demonstrated by other direct and indirect laboratory indicators of inflammatory disease (levels of serum ferritin, serum amyloid A, complement C4, fibrinogen, rheumatoid factor, and serum albumin, the neutrophil count, the platelet count, and the hemoglobin concentration), suggesting that suppression of inflammation by tocilizumab is not associated with only a few of the markers of inflammation.

A small number of patients in whom the neutrophil count was normal at the start of the study (unlike the majority of the study population) experienced neu-

tropenia when treated with tocilizumab at a dose of 8 mg/kg. An increased incidence of infections, including life-threatening conditions, has been observed with other monoclonal antibodies targeting the components of the immune system. In this study, serious infections were seen in the group receiving combination therapy with 8 mg/kg of tocilizumab plus MTX, as might be expected with the combination of 2 potentially immunosuppressant drugs, although serious infections were also seen in the group receiving monotherapy with 2 mg/kg of tocilizumab. As the most clinically effective dose, 8 mg/kg of tocilizumab may be considered the optimal therapeutic dose. Although the evidence clearly is not conclusive, IL-6 is known to demarginate neutrophils (36), and inhibition of IL-6 function could be expected to marginate neutrophils, thus showing a decrease in the number of circulating neutrophils. Larger studies in which the period of exposure to the study drug is longer will be required to accurately assess the risk of serious infection associated with tocilizumab treatment.

Anaphylaxis and anaphylactoid reactions are also expected and serious adverse events associated with treatment with humanized monoclonal antibodies. In the case of tocilizumab, as with infliximab, such reactions occurred only at low doses and in the absence of MTX, against a background of "insufficient" immunosuppression, or high-dose antigen-induced immunologic tolerance may have occurred to prevent formation of anti-tocilizumab antibodies (19). Among patients in whom anti-tocilizumab antibodies developed, the number was highest in the groups receiving monotherapy, which suggests that MTX provides some protection against anti-tocilizumab antibody production. This trend is also seen when MTX is given in combination with infliximab (19).

The highest responses to tocilizumab monotherapy have been achieved using the 4-mg/kg (minimally efficacious) and 8-mg/kg (optimally efficacious) doses; therefore, those doses are proposed for use in future clinical studies. Monthly administration of 8 mg/kg of tocilizumab is proposed as the highest dosage, because in this study both efficacy and safety appeared to be dose-related, and increasing the dose beyond 8 mg/kg may increase the possibility that adverse events will occur. Combination therapy with tocilizumab plus MTX demonstrated superior efficacy compared with tocilizumab monotherapy.

In conclusion, the results of this study indicate that targeted blockade of IL-6 is a highly efficacious and promising means of decreasing disease activity in patients with RA, and that tocilizumab treatment (as

monotherapy or in combination with MTX) is well tolerated in the majority of cases, having a safety pattern consistent with that of other biologic and immunosuppressive therapies. Large-scale phase III studies are currently ongoing in an attempt to validate the encouraging hypothesis generated in this and other studies, and to investigate the potentially beneficial radiographic outcomes related to treatment with tocilizumab in patients with RA.

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APPENDIX A: THE CHARISMA STUDY GROUP

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