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CASE BASED REVIEW

Successful treatment of adult-onset Still's disease with tocilizumab monotherapy: two case reports and literature review

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Abstract Adult-onset Still's disease (AOSD) is a systemic inflammatory disease of unknown etiology. Recently, it has been reported that quite a few cases of refractory AOSD were successfully treated with tocilizumab (TCZ) and corticosteroids were withdrawn in some of these patients. We report two AOSD patients who were treated successfully with TCZ monotherapy; thus, avoiding corticosteroid treatment. Because both of the patients refused to take corticosteroids, we planned to treat them with 8 mg/kg of TCZ monotherapy at weeks 0, 2, 6 and subsequently every 4 weeks. The efficacy of TCZ was assessed by patients' clinical symptoms such as fever, arthralgia, skin eruptions, and laboratory markers such as serum levels of CRP, ferritin, and IL-6. We also reviewed 14 previous case reports including 30 cases who had been treated with TCZ for AOSD. Our patients responded rapidly and have been maintained in clinical remission without corticosteroid treatment. In the literature review, concomitant corticosteroid treatment described in 13 cases was successfully tapered in 7 and discontinued in 6 cases. TCZ monotherapy can be a candidate for the first-line therapy for some AOSD patients.

Keywords Adult-onset Still's disease · Interleukin-6 · Monotherapy · Tocilizumab

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Introduction

Adult-onset Still's disease (AOSD) is a systemic inflammatory disease of unknown etiology and is characterized by remittent fever, evanescent salmon pink rash, and polyarthralgia frequently accompanied by neutrophilic leukocytosis [1, 2]. AOSD treatment comprises non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and immunosuppressive drugs such as methotrexate (MTX) [3]. Corticosteroids, in particular, still provide mainstay AOSD therapy despite various adverse effects [3].

Recently, numerous studies have revealed that proinflammatory cytokines such as IL-1, IL-6, IL-18, tumor necrosis factor (TNF), and interferon-gamma are involved in AOSD's pathogenesis [4, 5]. In fact, AOSD patients have been successfully treated with anti-cytokine therapies such as with TNF-α blocking agents [6, 7], an IL-1 receptor antagonist (anakinra) [8, 9], and an anti-IL-6 receptor monoclonal antibody (tocilizumab, TCZ) [10–23]. Most of the cases are refractory to conventional therapies including high-dose corticosteroids and immunosuppressive drugs (cyclosporin and methotrexate, etc.) [11, 14, 16–18, 20, 22]. Among these case reports, TCZ seems to be highly

effective for treating patients refractory to TNF antagonists [16, 17, 20] and anakinra [11, 14, 18, 22].

Here, we describe two patients with AOSD who were treated with TCZ monotherapy without corticosteroids. Table 1 summarizes the patients' characteristics.

Case reports

Case 1

A 32-year-old woman was admitted to our hospital in January 2009 with fever, polyarthralgia, sore throat, and skin eruptions. Two years earlier, she experienced self-limiting high fever and cervical lymphadenopathy. Eight months prior to admission, she developed eruptions on her back, both arms and legs, which were improved by olopatadine hydrochloride and clobetasol propionate ointment treatment. Two weeks prior to admission, she developed a sore throat followed by skin eruptions, fever and polyarthralgia, which did not respond to cefcapene pivoxil hydrochloride hydrate and NSAIDs.

Laboratory test results on admission were as follows: leukocyte count 13,000/μl (neutrophils, 10,900/μl), hemoglobin 13.4 g/dl, platelet count 198,000/μl, erythrocyte sedimentation rate (ESR) 110 mm/hr, C-reactive protein (CRP) 33.8 mg/dl, aspartate aminotransferase (AST) 29 IU/l, alanine aminotransferase (ALT) 22 IU/l, lactate dehydrogenase (LDH) 285 IU/l, creatinine 0.55 mg/dl, ferritin 1,679 ng/ml, and normal urine values. Serological tests were negative for rheumatoid factor (RF) and antinuclear antibodies (ANA). The serum IL-6, TNF-α and soluble IL-2 receptor levels were 398 pg/ml (normal range <4.0 pg/ml), 3.2 pg/ml (normal range 0.6–2.8 pg/ml) and 1,090 U/ml (normal range 220–530 U/ml), respectively. Computed tomography revealed cervical lymphadenopathy and mild splenomegaly. Laboratory evaluations ruled out infections and malignancies, and she was therefore given a diagnosis of AOSD based on Yamaguchi's classification criteria [2].

Because the patient refused to take corticosteroids, we tried etanercept (50 mg/week) first, since there are some case reports on anti-TNF agents' efficacy for treating AOSD [6, 7]. However, there was no improvement in patient's clinical symptoms (Fig. 1).

Therefore, we administered 8 mg/kg of TCZ, which rapidly mitigated symptoms and lowered inflammatory marker levels. Although the third infusion was planned at week 6, CRP and leukocyte count slightly increased and her symptoms such as low grade fever and polyarthralgia appeared at week 4 (Fig. 1). Therefore, the third infusion

Table 1 Characteristics of patients

	Patient 1	Patient 2
Sex/Age (years)	Female/32	Male/18
Disease duration (months)	20	1
Yamaguchi criteria [Reference][2]		
Major criteria	Fever	Fever
	Arthralgia	Arthralgia
	Typical rash	Leukocytosis
	Leukocytosis	
Minor criteria	Sore throat	Sore throat
	Lymphadenopathy	Splenomegaly
	Splenomegaly	RF and ANA negative
	RF and ANA negative	
Laboratory data	CRP 33.8 mg/dl	CRP 25.6 mg/dl
	Ferritin 1,679 ng/ml	Ferritin 2,756 ng/ml
	IL-6 398 pg/ml	IL-6 18.8 pg/ml
Previous treatment	NSAIDs	NSAIDs
	Etanercept	
Tocilizumab dosing	8 mg/kg infusion at week 0, 2, 5, 8, 11, and subsequently every 4 weeks	8 mg/kg infusion at week 0, 3, and subsequently every 4 weeks
Outcome	Remission	Remission
Duration to fever response (<38.0°C) (days)	1	1
Duration to arthritis response (days)	3	3
Duration to CRP response (<0.5 mg/dl) (days)	7	7

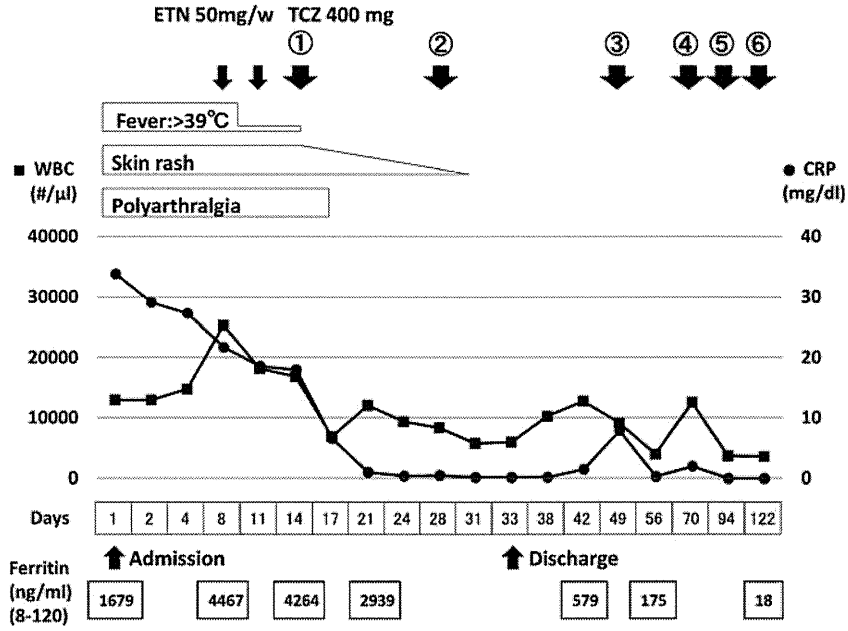

Fig. 1 Clinical course in case 1. Improvement in symptoms, WBC, CRP and ferritin levels in a patient with AOSD treated with TCZ monotherapy. Note that fever, skin rash, arthralgia and CRP levels rapidly normalized. *ETN* Etanercept

was administered at week 5 soon after. After the infusions at weeks 5, 8, and 11, all the symptoms and signs of AOSD disappeared, and the patient received TCZ subsequently every 4 weeks to remain in complete remission, which has lasted for more than 2 years. Serum IL-6 level also decreased to 5.4 pg/ml and ferritin level was normal. Corticosteroids were never administered throughout her disease course.

Case 2

An 18-year-old man was admitted to our hospital in August 2010 because of fever, polyarthralgia, sore throat, and myalgia. He had been well until about 3 weeks earlier. Two weeks prior to admission, he had received antibiotics, including azithromycin hydrate, levofloxacin hydrate, and panipenem/betamipron, but had shown no response. Laboratory test results on admission were as follows: leukocyte count 30,400/μl (neutrophils 25,500/μl), hemoglobin 12.5 g/dl, platelet count 521,000/μl, ESR 53 mm/h, prothrombin time INR 1.26, activated partial thromboplastin time 30.0 seconds, fibrinogen 700 mg/dl, D-dimer 1.96 μg/ml, CRP 25.6 mg/dl, AST 15 IU/l, ALT 26 IU/l, LDH 245 IU/l, serum creatinine 0.81 mg/dl, and ferritin 2,756 ng/ml. The urine values were all normal. Serological tests were negative for both RF and ANA. The serum IL-6 and soluble IL-2 receptor levels were, respectively, 18.8 pg/ml and 1,050 U/ml. Abdominal ultrasonography showed mild splenomegaly. We excluded infections, malignancies, and other rheumatic diseases by radiological examinations, gastrointestinal and colon endoscopy, and laboratory examinations that included repetitive culture for microorganisms as well as assays for various autoantibodies. The patient got a diagnosis of AOSD, but he and his family did not consent to corticosteroid therapy. We decided to administer 8 mg/kg of TCZ at weeks 0, 2, 6, and subsequently every 4 weeks. One day after the first TCZ infusion, fever and polyarthralgia abated markedly and serum CRP level returned to normal within 1 week. The second TCZ was administered at week 3 because of patient's affairs, and the patient was discharged 3 days later. TCZ has been continued every 4 weeks for 8 months and the patient is still in complete remission.

Discussion

This is the first case report, to our knowledge, demonstrating that TCZ monotherapy was effective for AOSD. Corticosteroids are usually required to improve clinical symptoms and laboratory abnormalities and are still a mainstay for inducing remission in AOSD. In fact, most (76%–95%) AOSD patients can be successfully treated with corticosteroids, and they respond dramatically [3]. However, consensus is lacking on a therapeutic corticosteroid tapering scheme after achieving clinical remission. Slow reduction is often necessary to maintain a good response and to avoid relapse. Corticosteroid dependence increases the risk for potentially serious mid- and long-term side effects caused by Cushing-like phenomena, diabetes, osteoporosis, and osteonecrosis. Anti-rheumatic drugs such as MTX are also reported to be effective [24, 25], but we are not aware of any randomized controlled trials.

Table 2 Literature review: Tocilizumab treatment for AOSD

Authors	Reference	Year	Sex	Age	Duration (years)	DMARDs before TCZ	Biologics before TCZ	Dose (Starting dose)	Outcome	Corticosteroid withdrawal
Iwamoto et al.	[10]	2002	Female	23	1.9	GST, MTX, CSA	None	4 mg/kg/2w	Good	Yes
De Bandt et al.	[11]	2009	Female	26	10	GST, MTX, LEF, thalidomide	anti-TNF (ETN, INF), ANK	8 mg/kg/2w	Good	Yes
Nakahara et al.	[12]	2009	Male	24	11.4	GST, SSZ, MTX, CSA, AZA	None	4 mg/kg/w	Good	Yes
Matsumoto et al.	[13]	2009	Female	29	0.1	CSA	None	8 mg/kg/2w	Good	Tapering
Perdan-Pirkmajer et al.	[14]	2010	Male	35	0.4	MTX	anti-TNF (ETN), ANK	8 mg/kg/4w	Good	Tapering
Naniwa et al.	[15]	2010	Female	64	1.7	CSA, tacrolimus, IvIg	None	8 mg/kg/2w	Good	Tapering
Sumida et al.	[16]	2010	Female	69	0.3	CSA, plasma exchange	anti-TNF (ETN)	8 mg/kg/4w	Good	Tapering
Yoshimura et al.	[17]	2010	Female	49	11	SSZ, bucillamine, MTX , LEF	anti-TNF (ETN, INF)	8 mg/kg/4w	Good	n.d.
Rech et al. case1	[18]	2011	Female	29	1	MTX	anti-TNF (ADA), ANK	8 mg/kg/4w	Good	Tapering
Rech et al. case2		2011	Male	73	3	MTX	anti-TNF (ADA, ETN), ANK	8 mg/kg/4w	Good	n.d.
Rech et al. case3		2011	Female	19	3	MTX	ANK	8 mg/kg/4w	Good	n.d.
Kishida et al.	[19]	2011	Male	40	22	MTX, SSZ, AZA, CY, CSA, tacrolimus	None	8 mg/kg/4w	Good	Yes
Thonhofer et al. case 1	[20]	2011	Female 1	24	1.3	MTX	anti-TNF (ADA, ETN)	8 mg/kg/4w	Good	Yes
Thonhofer et al. case 2		2011	Male 1	26	0.1	MTX	None	8 mg/kg/4w	Good	Yes
Kobayashi et al.	[21]	2011	Female	57	0.4	CSA	None	8 mg/kg/2w	Good	Tapering
Puéchal et al, 14 cases	[22]	2011	Male 5 Female 9	mean = 38.4 (23–68)	mean = 13.6 (3–27)	MTX (14), IvIg (7)	ANK (14), anti-TNF (12), abatacept (1), rituximab (1)	8 mg/kg/4w (n=9) 8 mg/kg/2w (n=4) 5 mg/kg/4w (n=1)	Good (n=11) Withdrawal (n=3; 2 AEs, 1 flare)	n.d.
Sabnis et al.	[23]	2011	Male	27	0.2	CSA	None	8 mg/kg/4w	Good	Tapering

ADA adalimumab, *AE* adverse event, *ANA* anakinra, *AZA*, azathioprine, *CSA* cyclosporine A, *CY* cyclophosphamide, *DMARDs* disease modified antirheumatic drugs, *ETN* etanercept, *GST* gold salts, *INF* infliximab, *IvIg* intravenous immunoglobulin, *LEF* leflunomide, *MTX* methotrexate, n.d. not described, *TCZ* tocilizumab, *SSZ* sulfasalazine, *TNF* tumor necrosis factor

The mechanisms underlying AOSD are not completely understood, but the role of proinflammatory cytokines may play a significant role in its pathogenesis because biological response modifiers targeted to these cytokines have been used successfully to treat AOSD. Promising results have been reported with TNF inhibitors such as etanercept and infliximab, but treatment failures and apparent loss of efficacy have also been described [6, 7]. A role for IL-1 has been also demonstrated by patient's response to anakinra [8, 9, 26].

IL-6 is also believed to play a crucial role in pathogenesis, and its serum levels correlate well with the AOSD's severity [4, 27]. There have been several other reports regarding TCZ's efficacy (Table 2) [10–23]. Fitzgerald et al. [8] proposed that TNF-α induces IL-1 production, which stimulates IL-6 expression. Therefore, Matsumoto et al. [13] stated that directly inhibiting IL-6 activity, which is the most downstream cytokine in the AOSD inflammatory cascade, may be better than blocking IL-1. In fact, AOSD patients who responded poorly to anakinra responded well to TCZ [11, 14, 18, 22]. Moreover, concomitant corticosteroid treatment described in 13 cases was successfully tapered in 7 [13–16, 18, 21, 23] and discontinued in 6 cases [10–12, 19, 20] after achieving TCZ-induced remission, suggesting that TCZ monotherapy may serve as first-line remission induction therapy.

In our present report, TCZ monotherapy rapidly improved each patient's health, and the patients have remained in complete remission. However, case 1 appeared to relapse after the second TCZ infusions. Although other cytokines such as IL-18 [4, 28, 29] might have been more important targets, TCZ may have been effective if biweekly infusion had been continued as used to treat systemic type juvenile idiopathic arthritis (classical Still's disease) [30]. Biweekly infusions of TCZ can be necessary for some AOSD patients to protect exacerbation completely.

TCZ was well tolerated by our patients. However, severe adverse events with macrophage activation syndrome (MAS) due to cytomegalovirus infection have been reported [11]. AOSD itself may cause MAS, and it may be difficult to distinguish TCZ's adverse effects from treatment insufficiency noted in the other case [21]. However, both patients were able to resume TCZ and continued after the MAS abated. Another concern is exacerbation of AOSD or MAS due to a transient increase of serum levels of target cytokine right after the cytokine blockade [31]. However, no exacerbation was seen in our cases, and was not described in all the reported cases treated with TCZ except one case [22]. We should be watchful for this kind of adverse event when we treat AOSD patients with cytokine blockade, but it seems to be rare in TCZ therapy.

In conclusion, TCZ monotherapy may be effective in some patients with AOSD.

Conflicts of interest H.K. has received honoraria from Mitsubishi-Tanabe Pharma, Pfizer, Abbott, Eisai Pharma, and Bristol-Myers KK. T.T. has received research support and consulting or lecture fees from Chugai Pharma. K.A. received research grants from Tanabe-Mitsubishi, Astellas, and Chugai pharmaceutical companies. The other authors have declared no conflicts of interest.

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EXTENDED REPORT

Golimumab monotherapy in Japanese patients with active rheumatoid arthritis despite prior treatment with disease-modifying antirheumatic drugs: results of the phase 2/3, multicentre, randomised, double-blind, placebo-controlled GO-MONO study through 24 weeks

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ABSTRACT

Objective To evaluate the efficacy and safety of golimumab 50 and 100 mg monotherapy in Japanese patients with active rheumatoid arthritis (RA) despite treatment with disease-modifying antirheumatic drugs (DMARDs).

Methods A total of 316 patients were randomised to receive subcutaneous injections every 4 weeks of placebo (group 1), golimumab 50 mg (group 2) or golimumab 100 mg (group 3); group 1 crossed over to golimumab 50 mg at week 16. The primary end point was the proportion of patients achieving ≥20% improvement in the American College of Rheumatology criteria (ACR20) at week 14. ACR50 and ACR70 response rates were also measured. Adverse events (AEs) were monitored throughout the study.

Results Demographics were similar across groups; the mean age was 52 years and 81.8% of patients (252/308) were female. Week 14 ACR20 response rates were significantly greater in groups 2 (51/101 (50.5%)) and 3 (60/102 (58.8%)) than in group 1 (20/105 (19.0%); $p<0.0001$ for both), as were ACR50 and ACR70 response rates. After placebo crossover at week 16, week 24 ACR response rates were similar in groups 1 and 2. Through week 16, 63.8% of patients in group 1, 62.4% in group 2 and 60.8% in group 3 had AEs and 1.9%, 1.0% and 2.0% had serious AEs. After week 16, one malignancy was reported (breast cancer, group 3). Infections were the most common AEs. No deaths or cases of tuberculosis were reported through week 24.

Conclusions Golimumab monotherapy (50 and 100 mg) was effective in reducing the signs and symptoms of RA in Japanese patients with active disease despite DMARD treatment.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterised by dysregulation of several cytokines, including tumour necrosis factor (TNF).[1 2] The bone and cartilage damage in the joints can significantly affect physical function[3] and the chronic inflammation of RA is associated with significant morbidity and mortality.[4] In observational studies, the anti-TNF agents infliximab[5] and etanercept[6] reduced disease activity in Japanese patients with RA.

Golimumab is a monoclonal antibody that binds with high affinity and specificity to TNF.[7] In large, phase 3, randomised, placebo-controlled trials, golimumab demonstrated efficacy in methotrexate (MTX)-naïve[8] and MTX-experienced patients with RA.[9] In these studies, many patients were treated with concomitant MTX. Some patients cannot tolerate MTX treatment[10]; therefore, it is clinically relevant to evaluate the safety and efficacy of golimumab monotherapy in Japanese patients with active RA who were previously treated with disease-modifying antirheumatic drugs (DMARDs).

PATIENTS AND METHODS

Patients

Patients (20–75 years) had to have a diagnosis of RA according to the American College of Rheumatology (ACR) criteria[11] for ≥3 months and active disease, despite previous DMARD treatment, defined as six or more swollen joints and six or more tender joints and two or more of the following: C-reactive protein (CRP) ≥2.0 mg/dl or erythrocyte sedimentation rate ≥28 mm/h using the Westergren method, morning stiffness ≥30 min, investigator-documented evidence of bone erosion on radiographs, or positive for anti-cyclic citrullinated peptide antibodies or rheumatoid factor. Patients were screened for latent and active tuberculosis (see also online supplementary text). All DMARDs were discontinued ≥4 weeks before the first study agent administration. Concomitant oral corticosteroids (stable dose ≤10 mg of prednisolone/day or equivalent) were permitted.

Study design

This was a phase 2/3 multicentre, randomised, double-blind, placebo-controlled trial carried out at 102 sites in Japan. Patients were randomly assigned (1:1:1) to receive subcutaneous injections every 4 weeks of placebo (group 1), golimumab 50 mg (group 2) or golimumab 100 mg (group 3). Concomitant DMARD treatment, including MTX, was prohibited in all treatment groups (a 4-week washout period was required). At week 16, all patients in group 1 crossed over to receive golimumab 50 mg in a double-blinded fashion.

The study was conducted according to the Declaration of Helsinki and in compliance with good clinical practice guidelines. The protocol was reviewed and approved by the institutional review board at each site. All patients provided written informed consent before any study-related procedures.

Study end points

Response to treatment was evaluated using the ACR criteria, the 28-joint count disease activity score (DAS28) using erythrocyte sedimentation rate and the ACR index of improvement in disease activity (ACR-N); physical function was evaluated with the Health Assessment Questionnaire-Disability Index (HAQ-DI). The primary end point was the proportion of patients achieving ≥20% improvement in ACR criteria (ACR20) at week 14. Due to ethical concerns about the potential for an inadequate response to placebo, week 14 was chosen for the primary efficacy assessment. Secondary end points included ACR50/70/90 response rates at weeks 14 and 24, changes from baseline at weeks 14 and 24 in DAS28 and HAQ-DI scores, ACR-N scores at weeks 14 and 24 and changes from baseline to week 24 in van der Heijde/Sharp (vdH–S) scores. Also the proportions of patients achieving a good or moderate DAS28 score[12 13] or DAS28 remission (score<2.6) were determined at weeks 14 and 24.

Radiographs of the hands and feet were obtained at baseline and week 24 or at the time of study discontinuation, if applicable, and scored by two independent readers (see online supplementary text). Radiographic progression was evaluated as changes from baseline to week 24 in the vdH–S score.[14] Erosion, joint space narrowing and total vdH–S scores are reported. All radiographs were scored by BioClinica Corporation (Newtown, Pennsylvania, USA) and readers were blinded to patient identity, treatment group and time point.

Patients were monitored for adverse events (AEs), including injection-site reactions and abnormal routine laboratory values.

Pharmacokinetic analyses and immunogenicity

Blood samples for the measurement of serum golimumab concentrations were obtained at weeks 0, 4, 8, 12, 14, 16, 20 and 24, with one additional sample between weeks 4 and 12. Blood samples for evaluation of antibodies to golimumab were obtained at weeks 0, 12 and 24. Antibodies to golimumab were detected using a previously described validated antigen bridging enzyme immunoassay.[15] Blood samples were drawn before administration of the study agent.

A post hoc analysis evaluated week 24 ACR20, ACR50 and ACR70 response rates for patients stratified according to the following serum golimumab concentration quartiles: <0.24 μg/ml, ≥0.24–<0.63 μg/ml, ≥0.63–<1.29 μg/ml and ≥1.29 μg/ml.

Statistical analyses

All patients who received at least one study agent injection and had efficacy data available were included in the efficacy analysis. All patients who received at least one study agent injection were included in the safety analysis. Patients who received one or more golimumab injection and had pharmacokinetic data available were included in the pharmacokinetic analysis. Descriptive statistics are reported. Differences between the treatment groups in ACR and DAS28 response rates were assessed using a χ^2 test. Type I error at the 0.05 level of significance was preserved with a hierarchical approach to control for multiplicity, in which a comparison between groups 3 and 1 was performed first and a comparison between groups 2 and 1 was performed only if the difference between groups 3 and 1 was significant. For changes in continuous variables, treatment group differences were assessed using analysis of covariance (ANCOVA) for HAQ-DI, DAS28 and vdH–S scores or analysis of variance (ANOVA) for ACR-N scores. Least-squares means and 95% CIs are reported. ACR response rates, ACR-N and HAQ-DI were calculated using the last observation carried forward method for the week 14 and week 24 time points. In the analysis of DAS28 response at weeks 14 and 24, observed data were used with no imputation for missing data, with the exception of the DAS28 remission analysis, in which patients with missing data were counted as non-responders. Observed data were used in the pharmacokinetic analysis.

Changes from baseline in vdH–S scores were compared between each golimumab group and placebo using two methods. ANCOVA was the prespecified method in the protocol and was chosen for consistency with the analyses of other continuous variables. A post hoc ANOVA based on van der Waerden normal scores was undertaken to account for the non-normal data distribution due to one patient in group 3 with an atypically large change in vdH–S score. Additionally, a cumulative probability plot of the changes in vdH–S scores from baseline to week 24 for each treatment group was constructed.

Assuming that 5% of patients would be excluded from the efficacy analysis owing to study discontinuation, the target total sample size of 300 patients provided >90% power to detect a difference between groups 2 and 3 and group 1 in ACR20 response rates at week 14 (α=0.05).

RESULTS

Patient disposition and baseline characteristics

A total of 316 patients were randomised; eight withdrew consent before administration of any study agents (figure 1). Therefore, 308 patients received one or more study agent administration (group 1, n=105; group 2, n=101; group 3, n=102). Patient demographics and baseline disease characteristics were well balanced across all groups (table 1). Among all patients, 82% were female, the mean age was 52 years, the mean disease duration was 8.9 years and the mean CRP level was 2.5 mg/dl. Most (73.7%) patients received prior MTX treatment.

Efficacy results

Clinical response and physical function

At week 14, significantly greater proportions of patients in groups 2 (50.5%) and 3 (58.8%) achieved an ACR20 response in comparison with group 1 (19.0%; p<0.0001 for both) (table 2). Likewise, significantly higher ACR50 and ACR70 response rates were seen in groups 2 and 3 than in group 1. While no patient in group 1 had an ACR90 response at week 14, three patients in group 2 and two in group 3 achieved an ACR90 response; however, statistical significance from placebo was not attained.

At week 24, after placebo crossover to golimumab 50 mg at week 16, patients in group 1 generally had ACR response rates

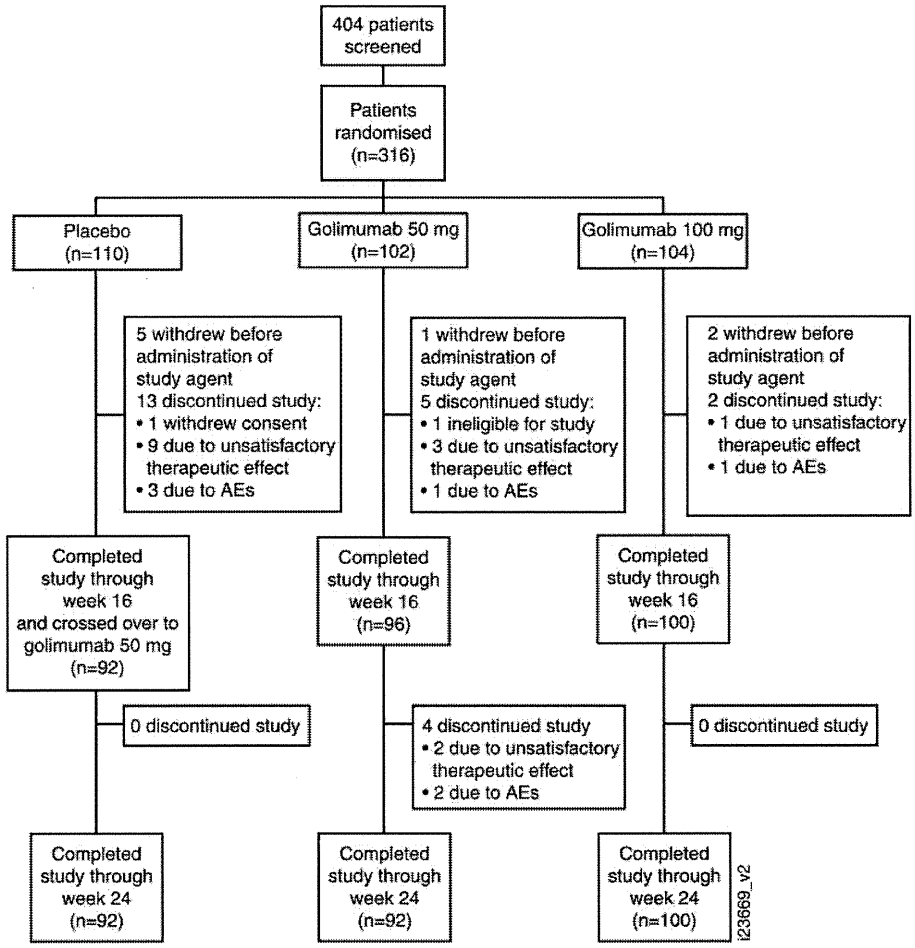

Figure 1 Patient disposition through week 24. AE, adverse event.

similar to those for patients who were initially assigned to group 2 from baseline (table 2). In group 3, week 14 ACR response rates were maintained at week 24.

Mean ACR-N scores at week 14 were significantly greater in groups 2 (30.5) and 3 (33.0) than in group 1 (9.1; $p<0.0001$ for both) (table 2). Mean improvements from baseline to week 14 in DAS28 scores were also significantly greater in groups 2 and 3 than in group 1 and significantly greater proportions of patients in groups 2 and 3 achieved a moderate or good DAS28 response or DAS28 remission. Improvements from baseline in physical function (HAQ-DI) were also significantly greater in groups 2 and 3 than in group 1.

Patients in group 1 had ACR-N scores at week 24 and mean improvements in DAS28 and HAQ-DI scores from baseline to week 24 that were similar to those seen in patients who were initially randomised to group 2. In group 3, week 14 ACR-N, DAS28 and HAQ-DI responses were maintained at week 24.

Radiographic progression

Two patients did not have complete radiographic data available (missing baseline data for one patient in group 3 and missing week 24 data for one patient in group 2) and changes from baseline in vdH–S score for these patients were substituted with the median change for all patients. Agreement between the two primary readers was good, with intraclass correlation coefficients of 0.98 at baseline and week 24 and 0.80 for the change at week 24. The proportion of patients with a change in total vdH–S score greater than the smallest detectable change was 22.1% (group 1, n=27; group 2, n=21; group 3, n=20).

At week 24, increases in erosion, joint space narrowing and total vdH–S scores were seen in all three groups (table 2), with smaller changes in erosion and total scores in groups 2 and 3, indicating less radiographic progression than in group 1, as shown in the probability plot (figure 2). In the a priori analysis (ANCOVA), no significant differences were seen in mean changes between groups 2 and 3 and group 1 at week 24. In the post hoc ANOVA using normalised scores, no significant differences were seen between groups 2 and 1. Although increases from baseline were observed in both groups 3 and 1, the mean changes in erosion and total vdH–S scores in group 3 were statistically significantly smaller than those in group 1 (1.1 vs 1.3, $p=0.0316$ and 2.1 vs 2.6, $p=0.0043$, respectively). Also, the median changes in total vdH–S scores followed a trend, showing less radiographic progression in groups 2 and 3 than in group 1 (0.5 and 0.0, respectively, vs 1.0).

Golimumab pharmacokinetics and antibodies to golimumab

Through week 16, serum golimumab levels increased in a dose-proportional manner; steady state was reached at week 12. Median serum golimumab concentrations for groups 2 and 3, respectively, were 0.52 μg/ml and 1.17 μg/ml at week 12 and

Table 1 Baseline patient demographics and disease characteristics

Characteristics	Group 1: Placebo	Group 2: Golimumab 50 mg	Group 3: Golimumab 100 mg	Total
Patients, n	105	101	102	308
Female, n (%)	86 (81.9)	81 (80.2)	85 (83.3)	252 (81.8)
Age, years	52.4 (11.1)	52.9 (11.3)	51.6 (11.9)	52.3 (11.4)
Body weight, kg	54.4 (10.4)	56.2 (12.4)	53.9 (9.8)	54.8 (10.9)
Duration of RA, years	9.2 (8.6)	8.1 (8.4)	9.4 (8.5)	8.9 (8.5)
Swollen joint count (0–66)	13.1 (6.9)	12.6 (5.8)	12.9 (6.7)	12.9 (6.5)
Tender joint count (0–68)	14.9 (8.5)	15.5 (9.0)	16.6 (10.2)	15.7 (9.3)
Patient's assessment of pain (VAS; 0–100 mm)	55.2 (24.5)	55.6 (22.3)	57.5 (23.1)	56.1 (23.3)
Patient's global assessment (VAS; 0–100 mm)	54.3 (25.4)	54.3 (23.7)	53.9 (24.5)	54.2 (24.5)
Physician's global assessment (VAS; 0–100 mm)	58.8 (17.8)	58.4 (18.1)	59.6 (18.3)	58.9 (18.0)
CRP, mg/dl	2.5 (2.5)	2.2 (2.5)	2.6 (2.8)	2.5 (2.6)
DAS28-ESR	5.9 (1.0)	5.8 (1.1)	6.0 (1.0)	5.9 (1.0)
HAQ-DI (0-3)	1.0 (0.6)	1.1 (0.6)	1.0 (0.6)	1.0 (0.6)

Data are presented as mean (SD) unless otherwise noted.
Results include data for all randomised patients who received at least one administration of the study agent and had available efficacy data.
CRP, C-reactive protein; DAS28-ESR, 28-joint Disease Activity Score using erythrocyte sedimentation rate; HAQ-DI, Health Assessment Questionnaire-Disability Index; RA, rheumatoid arthritis; VAS, visual analogue scale.

0.46 μg/ml and 1.04 μg/ml at week 16. Median serum concentrations at week 24 were 0.35 μg/ml in group 1, 0.43 μg/ml in group 2 and 0.99 μg/ml in group 3. Week 24 ACR20, ACR50 and ACR70 response rates were evaluated according to serum golimumab concentration, with patients stratified by the following quartiles: <0.24 μg/ml (n=45), ≥0.24–<0.63 μg/ml (n=50), ≥0.63–<1.29 μg/ml (n=49) and ≥1.29 μg/ml (n=48). Overall, response rates were lowest in patients with serum golimumab concentrations <0.24 μg/ml and increased with increasing serum golimumab concentration (figure 3).

At week 12, two patients (2.0%) each in groups 2 and 3 tested positive for antibodies to golimumab. At week 24, three patients each in group 1 (3.3%) and group 2 (3.2%) and four patients (4.0%) in group 3 tested positive for antibodies to golimumab. No antibody-positive patient demonstrated an ACR response.

Adverse events

Through week 16 (placebo-controlled period), AEs occurred in 63.8% of patients in group 1, 62.4% in group 2 and 60.8% in group 3 (table 3). Most AEs were mild. The most common AEs were infections (group 1 (23.8%); group 2 (26.7%); group 3 (28.4%)). The most common infections among all golimumab-treated patients were nasopharyngitis (16.3%), pharyngitis (3.4%) and gastroenteritis (2.0%). Three patients (2.9%) in group 1 (herpes zoster, atypical mycobacterial infection and abnormal liver function test), two patients (2.0%) in group 2 (liver disorder and cataract) and one patient (1.0%) in group 3 (transient cerebral ischaemic attack) discontinued the study agent owing to AEs. Serious AEs (SAEs) through week 16 were herpes zoster and organising pneumonia (n=1 each) in group 1, hydrocele (n=1) in group 2 and cellulitis and transient ischaemic attack (n=1 each) in group 3. When assessed by length of follow-up, the incidences (95% CI) of serious infection at week 24 were 3.30 (0.08 to 18.38), 1.69 (0.04 to 9.40) and 2.16 (0.05 to 12.01) for groups 1, 2 and 3, respectively.

After the placebo crossover at week 16, AEs occurred in 31 (33.7%) patients in group 1, 34 (35.4%) in group 2 and 33 (33.0%) in group 3 through week 24 (table 3). Infections were the most common AEs during this time period, consistent with results seen during the placebo-controlled period. AEs leading to discontinuation of the study agent after week 16 were ovarian neoplasm (non-malignant; n=1) and RA (n=1) in group 2 and breast cancer (n=1) in group 3. After week 16, SAEs occurred in three patients in group 2 (non-malignant ovarian neoplasm and dental pulpitis, each in one patient; paroxysmal tachycardia and RA in one patient) and in two patients in group 3 (breast cancer, between weeks 20 and 24 and organising pneumonia, one patient each); no SAEs were reported in group 1 during this period.

The incidence of injection-site reactions through week 16 was similar among all groups (group 1, 7/105 (6.7%); group 2, 8/101 (7.9%); group 3, 8/102 (7.8%)). From week 16 through week 24, the rates of injection-site reactions were 3.3% (3/92) in group 1, 6.3% (6/96) in group 2 and 5.0% (5/100) in group 3. All injection-site reactions were mild.

There were no reports of anaphylactic reactions, serum sickness-like reactions, or deaths through week 24. No cases of tuberculosis were reported through week 24; however, one case of atypical mycobacterial infection occurred in group 1 before week 16.

DISCUSSION

In this phase 2/3 study of golimumab 50 mg and 100 mg in Japanese patients with active RA despite DMARD treatment, those treated with golimumab monotherapy had significant improvements from baseline to week 14 in clinical measures of efficacy, including ACR20, ACR50 and ACR70 response rates and DAS28 and ACR-N scores, in comparison with those who received placebo. Physical function was also significantly improved from baseline in the golimumab groups compared with placebo. These significant improvements were seen despite the overall study population displaying relatively mild disease at study outset (mean swollen/tender joint counts of 13/16). However, clinical response to golimumab monotherapy was relatively modest in comparison with golimumab+MTX treatment in another Japanese population.[16]

Patients with active RA despite previous MTX treatment were evaluated previously in the large phase 3 GO-FORWARD trial.[9] While concomitant MTX was included in GO-FORWARD golimumab 100 mg monotherapy was also evaluated. ACR responses were also evaluated at week 14 in both trials and while significantly greater ACR response rates were achieved in group 3 in this study in comparison with placebo,

Table 2 Clinical efficacy and radiographic results† through week 24

	Placebo-controlled period			Placebo crossover period		
	Week 14			Week 24		
	Group 1: Placebo (n=105)	Group 2: Golimumab 50 mg (n=101)	Group 3: Golimumab 100 mg (n=102)	Group 1: Placebo→Golimumab 50 mg (n=105)	Group 2: Golimumab 50 mg (n=101‡)	Group 3: Golimumab 100 mg (n=102)
Clinical efficacy results						
ACR20 response	20 (19.0)	51 (50.5) p<0.0001	60 (58.8) p<0.0001	18 (17.1)	47 (46.5) p<0.0001	71 (69.6) p<0.0001
ACR50 response	6 (5.7)	29 (28.7) p<0.0001	33 (32.4) p<0.0001	8 (7.6)	28 (27.7) p=0.0001	43 (42.2) p<0.0001
ACR70 response	1 (1.0)	13 (12.9) p=0.0007	12 (11.8) p=0.0013	2 (1.9)	17 (16.8) p=0.0002	22 (21.6) p<0.0001
ACR90 response	0 (0.0)	3 (3.0) p=0.0752	2 (2.0) p=0.1493	0	5 (5.0) p=0.021	3 (2.9) p=0.0767
ACR-N	9.1 (4.3 to 14.0)	30.5 (25.6, 35.5) p<0.0001	33.0 (28.1, 38.0) p<0.0001	9.3 (3.9, 14.7)	30.9 (25.4, 36.4) p<0.0001	40.0 (34.6, 45.5) p<0.0001
DAS28-ESR						
Change from baseline	n=94 −0.3 (−0.6 to −0.1)	n=97 −1.5 (−1.8, −1.3) p<0.0001	n=100 −1.9 (−2.1 to −1.7) p<0.0001	n=91 −1.5 (−1.8, −1.2)	n=93 −1.6 (−1.9 to −1.4)	n=100 −1.9 (−2.1, −1.6)
Moderate response	n=93 27 (29.0)	n=97 69 (71.1) p<0.0001	n=100 74 (74.0) p<0.0001	n=91 56 (61.5)	n=93 65 (69.9)	n=100 78 (78.0)
Good response	n=93 4 (4.3)	n=97 23 (23.7) p=0.0001	n=100 32 (32.0) p<0.0001	n=91 21 (23.1)	n=93 21 (22.6)	n=100 31 (31.0)
Remission	n=94 2 (2.1)	n=97 13 (13.4) p=0.0025	n=100 23 (23.0) p<0.0001	n=92 8 (8.7)	n=93 16 (17.2)	n=100 19 (19.0)
HAQ-DI						
Change from baseline	−0.03 (−0.12 to 0.06)	0.24 (0.15 to 0.34) p<0.0001	0.33 (0.24 to 0.42) p<0.0001	−0.03 (−0.13 to 0.07)	0.23 (0.13 to 0.33) p=0.0003	0.33 (0.23 to 0.43) p<0.0001
Radiographic results						
vdH–S score, baseline						
Total	–	–	–	56.1 (62.2)	43.8 (50.6)	56.9 (57.0)
Joint space narrowing	–	–	–	25.9 (30.2)	19.9 (24.0)	25.3 (26.2)
Erosion	–	–	–	30.2 (33.8)	23.9 (28.3)	31.7 (33.0)
vdH–S score, change from baseline to week 24						
Total				n=105 2.6 (4.7) 1.0 (−2.5 to 29.8)	n=100 1.9 (4.1) 0.5 (−1.8 to 23.0) p=0.5091* p=0.1802**	n=102 2.1 (10.4) 0.0 (−2.5 to 102.5) p=0.6573* p=0.0043**
Joint space narrowing				n=92 0.9 (1.9) 0.0 (−1.0 to 9.5)	n=93 1.0 (2.8) 0.0 (−1.5 to 17.5) p=0.7530* p=0.3373**	n=99 1.0 (5.1) 0.0 (−2.0 to 48.5) p=0.9353* p=0.0832**

Continued

Table 2 Continued

	Placebo-controlled period			Placebo crossover period		
	Week 14			Week 24		
	Group 1: Placebo (n=105)	Group 2: Golimumab 50 mg (n=101)	Group 3: Golimumab 100 mg (n=102)	Group 1: Placebo→Golimumab 50 mg (n=105)	Group 2: Golimumab 50 mg (n=101‡)	Group 3: Golimumab 100 mg (n=102)
Erosion						
n				n=92	n=93	n=99
Mean (SD)				1.3 (2.5)	1.0 (2.1)	1.1 (5.7)
Median (range)				0.5 (−2.5 to 14.5)	0.5 (−1.5 to 11.5)	0.0 (−2.5 to 54.0)
p Value*					p=0.6272*	p=0.7614*
p Value**					p=0.5895**	p=0.0316**

*p Values based on analysis of covariance on least-squares mean and two-sided 95% CIs with treatment and baseline value as covariates.
**p Values based on analysis of variance on van der Waerden normal scores.
†Clinical efficacy data are presented as n (%) or least-squares mean (95% CI). Radiographic data are presented as mean (SD) and median (range).
‡Data from one patient who discontinued the study before week 24 were included in these analyses because the timing of the study termination visit fell within the prespecified time period for week 24 data collection.
ACR20/50/70/90, 20%/50%/70%/90% improvement in the American College of Rheumatology criteria; ACR-N, American College of Rheumatology index of improvement; DAS28-ESR, 28-joint Disease Activity Score using erythrocyte sedimentation rate; HAQ-DI, Health Assessment Questionnaire-Disability Index; vdH–S, van der Heijde/Sharp.

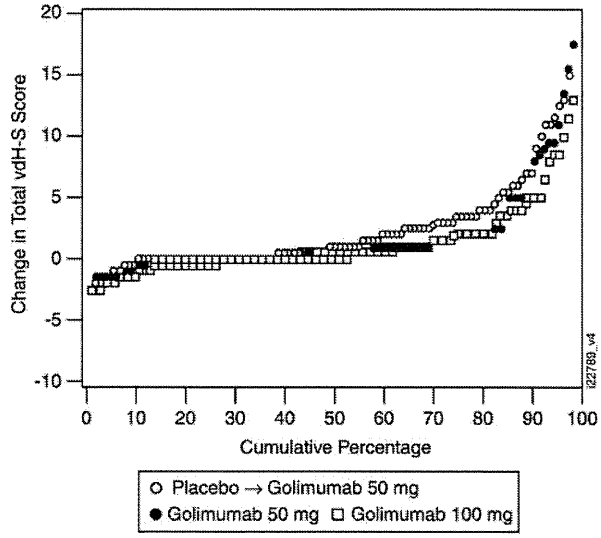

Figure 2 Cumulative probability plot of changes in van der Heijde-Sharp (vdH–S) scores from baseline to week 24 Data from one patient in the golimumab 100 mg group who had an atypically large change in vdH–S score were excluded.

the primary end point was not achieved in the golimumab 100 mg monotherapy group in the GO-FORWARD trial. Possible explanations for the non-statistically significant response in the GO-FORWARD 100 mg monotherapy group were previously described (eg, the relatively low disease activity in the trial population and the high response rate in the MTX monotherapy group).[9] However, factors such as patient body weight, which is known to affect the pharmacokinetic properties of monoclonal antibodies,[17–19] may also account for the difference in response seen in the two trials. While a previous study found no apparent differences in the pharmacokinetic parameters of golimumab in healthy body-weight-matched Caucasian and Japanese male subjects,[20] it is possible that the body weights of patients in 100 mg monotherapy groups in

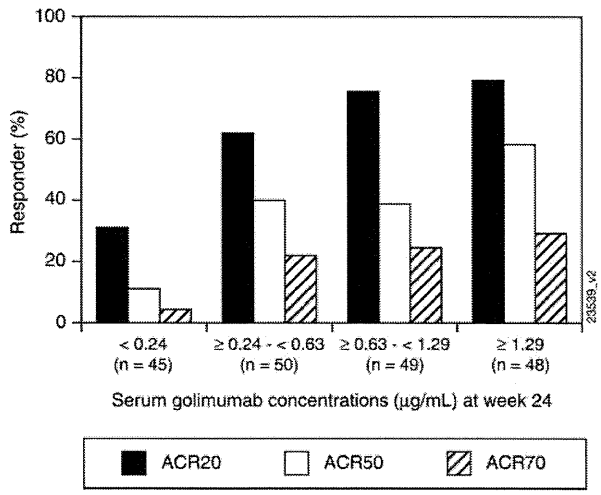

Figure 3 The proportions of patients achieving an ACR20, ACR50 and ACR70 responses stratified by serum golimumab concentration quartiles (µg/ml) at week 24. ACR20/50/70, 20%/50%/70% improvement in the ACR criteria.

Table 3 Week 16 and week 24 safety results

	Placebo-controlled period			Placebo crossover period			Cumulative	
	Weeks 0–16			Weeks 16–24			Weeks 0–24	
	Group 1: Placebo	Group 2: Golimumab 50 mg	Group 3: Golimumab 100 mg	Group 1: Placebo→Golimumab 50 mg	Group 2: Golimumab 50 mg	Group 3: Golimumab 100 mg	Group 2: Golimumab 50 mg	Group 3: Golimumab 100 mg
Patients, n	105	101	102	92	96	100	101	102
Patients with AEs	67 (63.8)	63 (62.4)	62 (60.8)	31 (33.7)	34 (35.4)	33 (33.0)	72 (71.3)	72 (70.6)
Patients with SAEs	2 (1.9)	1 (1.0)	2 (2.0)	0 (0)	3 (3.1)	2 (2.0)	4 (4.0)	4 (3.9)
Patients with AEs leading to discontinuation of study agent	3 (2.9)	2 (2.0)	1 (1.0)	0 (0)	2 (2.1)	1 (1.0)	4 (4.0)	2 (2.0)
Patients with infections	25 (23.8)	27 (26.7)	29 (28.4)	5 (5.4)	11 (11.5)	7 (7.0)	33 (32.7)	34 (33.3)
Patients with serious infections	1 (1.0)	0 (0)	1 (1.0)	0 (0)	1 (1.0)	0 (0)	1 (1.0)	1 (1.0)
Patients with abnormal LFTs	3 (2.9)	0 (0)	4 (3.9)	0 (0)	0 (0)	0 (0)	0 (0)	4 (3.9)
Patients with injection-site reactions	7 (6.7)	8 (7.9)	8 (7.8)	3 (3.3)	6 (6.3)	5 (5.0)	12 (11.9)	10 (9.8)
Patients with neoplasms (benign, malignant and unspecified)	0 (0)	0 (0)	0 (0)	0 (0)	2 (2.1)	1 (1.0)	2 (2.0)	1 (1.0)
Breast cancer	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.0)	0 (0)	1 (1.0)
Skin papilloma	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.0)	0 (0)	1 (1.0)	0 (0)
Ovarian neoplasm	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.0)	0 (0)	1 (1.0)	0 (0)

Data are presented as n (%) unless otherwise noted.
AEs, adverse events; LFT, liver function test; SAEs, serious adverse events.

this trial and in GO-FORWARD might have varied considerably[21] given that Japanese patients are generally more slight, and the resulting dose per unit mass would be higher than in other populations. Indeed, treatment effects on radiographic progression appear to be related to serum golimumab concentrations, as patients receiving golimumab 50 mg+MTX in the GO-FORTH trial in Japanese patients with RA (week 16 median serum golimumab concentration=0.73 μg/ml) demonstrated significantly less radiographic progression than placebo-treated patients,[16] while such a difference was not seen in this study, in which patients receiving golimumab 50 mg had a week 16 median serum golimumab concentration of 0.46 μg/ml.

Radiographic progression was evaluated at week 24, at which point patients randomised to group 1 had been receiving golimumab 50 mg since week 16. The a priori ANCOVA did not show significant differences in radiographic progression between either groups 2 or 3 and group 1; however, in a post hoc analysis using normalised data, significantly smaller changes from baseline in erosion and total vdH–S scores were seen in group 3 than in group 1. This significant difference was confirmed by an additional ANCOVA that excluded a single group 3 patient with an atypically large change in vdH–S score (p=0.01; data not shown). Biological monotherapy with the anti-interleukin 6 agent tocilizumab has also demonstrated radiographic benefit in patients with RA with inadequate response to DMARD treatment.[22] In this study, the mean baseline CRP level, which is a good predictor of radiographic progression,[23] was moderately raised and 22.1% of patients had a change in total vdH–S that exceeded the smallest detectable change. In contrast, only 4.3% of patients in GO-FORWARD had such a change in total vdH–S score.[24] Thus, patients in our study probably had higher disease activity than patients in GO-FORWARD. This may account for the observation that radiographic progression in this study was greater than expected based on the clinical response seen at similar time points in earlier golimumab trials, including GO-FORWARD.[24] Our results suggest that golimumab 100 mg monotherapy may prevent further joint damage in Japanese patients with active radiographic progression, which is consistent with the golimumab package insert approved by the Japanese Pharmaceuticals and Medical Devices Agency.[25]

Golimumab was generally well tolerated. Infections were the most common AEs. Serious infections were reported in two patients through week 16 and one patient between weeks 16 and 24; the week 24 incidences per 100 patient-years of follow-up indicated no increase in serious infection versus placebo. Most AEs were mild and few patients discontinued due to AEs. Rates of SAEs, serious infections and malignancies were low. No deaths and one malignancy (breast cancer) occurred through week 24. Of note, this study was not powered to detect rare events and these findings are limited also by the short-term nature of the analysis.

This was the first golimumab monotherapy study to demonstrate that Japanese patients with active RA despite prior DMARD treatment had significantly improved signs and symptoms of RA after 14 weeks of treatment with 50 or 100 mg golimumab in comparison with placebo. Group 3 had significantly less radiographic progression than group 1 when analysed post hoc using normalised scores, and median changes in total vdH–S scores suggested a dose-dependent trend. Additional long-term analyses are needed to further explore the effect of golimumab monotherapy on joint destruction and fully assess its safety profile in Japanese patients with RA.

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マイクロアレイを用いた自己免疫疾患の解析

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KEY WORDS
●マイクロアレイ
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●発現解析
●機能解析
●遺伝子

Analysis of autoimmune diseases using microarray.
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はじめに

体がもつ多様な生体防御反応には，初期の免疫応答として非特異的に働く自然免疫，そして特異性が高い防御反応である獲得免疫がある。この過程には，マクロファージ，樹状細胞，好酸球，好塩基球，マスト細胞およびリンパ球（NK細胞，T細胞，B細胞）などの多種の細胞群が互いに関与しており，機能性発現のためのさまざまなシグナル伝達経路が存在している。そこでは，非常に多くの分子が，受容体，シグナル伝達分子，転写因子，転写調節因子などとしての役割を果たしている。

自己免疫疾患は，多数の遺伝的背景があるところに，感染，紫外線，化学物質などの環境要因が加わることにより免疫寛容の破綻が生じ，全身性もしくは臓器特異的に自己免疫現象が生じることにより引き起こされる疾患と考えられてきた。今世紀に入りヒトにおいてゲノムワイド関連解析（genome wide association study：GWAS）が急速に進み，ゲノムレベルでの遺伝的要因が次々と解明されてきている。この流れと並行して，転写レベル，蛋白発現レベルでもさまざまな解析手法の進歩がなされ，より疾患に近いレベルでの病態解析の研究が進んでいる。特に非常に複雑な生体反応を同時に網羅的に捉えることができる技術であるマイクロアレイ法の開発とその普及は，生体現象を理解するためにもはや不可欠な存在となっている。

本稿では，マイクロアレイに関する最近の状況とその自己免疫疾患の解析への応用例について概説する。

1．マイクロアレイとは[1)-3)]

多数の対象物を微細な（マイクロ）基盤上に固定化，配列（アレイ）することで，一度に多くの対象を網羅的に

表．マイクロアレイの種類

プローブ	アレイ	主な用途
核酸	DNAマイクロアレイ	配列解析，発現解析，変異解析，多型解析
蛋白質（抗原，抗体，受容体）	プロテインマイクロアレイ	抗原，抗体，受容体，蛋白質相互作用
ペプチド	ペプチドマイクロアレイ	抗原，抗体，受容体
糖鎖	糖鎖マイクロアレイ	分類，受容体
低分子化合物	低分子化合物マイクロアレイ	受容体，化合物のスクリーニング
細胞	細胞マイクロアレイ	抗原，受容体
組織	組織マイクロアレイ	抗原，受容体

扱う解析技術をさす用語である。核酸を対象とするDNAマイクロアレイ（DNAチップ）が最初に普及し，最近では蛋白質などのさまざまな対象物でのマイクロアレイが可能となっている（表）。

1．DNAマイクロアレイ

DNAマイクロアレイでは，固定化してあるDNAと試料を蛍光標識した遺伝子プローブとのハイブリダイゼーションの有無を専用の読み取り装置で検出し，目的遺伝子の発現量を算出する。異なる試料との比較によるシグナル強度の増減で遺伝子発現量の変化を解析する方法が多く用いられている。対象の細胞や組織におけるメッセンジャーRNAなどの発現量を検出し，各種疾患との相関をみる解析に加え，変異DNAを配列し一塩基多型（single nucleotide polymorphism；SNP）を調べることにより（SNPsアレイ），癌を中心とした疾患の分子レベルでの診断や，薬物の有効性や副作用の予見にも応用され始めている。DNAアレイを研究の端緒とし，得られた知見を基にさらに機能解析へつなげていくことで，テーラーメイド医療や新規の医薬品開発へ期待が高まっている。

DNAマイクロアレイ技術も年々進歩しており，標準的なDNAアレイは1枚数万円程度とチップが低価格化するとともに，一度に測定できる種類も数万程度あるいはそれ以上と高密度化が進んでいる。さらに非特異的な反応の抑制，ばらつき補正，感度上昇などの技術的な改良も進められている。DNAマイクロアレイそのものの進歩とともに，大量のデータを解析するバイオインフォマティクス技術の進歩も急速で，差次的発現量解析の他にもGene Ontology解析，パスウェイ解析，ネットワーク解析などが可能となり，システムバイオロジーのための必須ツールとなっている。

2．プロテインマイクロアレイ（プロテインチップ）

プロテインマイクロアレイ（プロテインチップ）は，古典的なセントラルドグマにおけるDNAからRNA，そして蛋白質への遺伝情報の流れにおいて，最終産物である蛋白質の発現を網羅的に測定する手法として用いられている。プロテインチップは用途により種類と量を測定・検出する分析プロテインチップと蛋白質の機能解析を行う機能（解析型）プロテインチップがある。抗体プロテインチップとして，特に腫瘍関連バイオマーカーとしての研究や，疾患関連蛋白質と相互作用する蛋白質，DNA，薬物の同定により治療および副作用発現などの分野についての応用が試みられている。

3．低分子化合物マイクロアレイ

低分子化合物をアレイし，相互作用のある蛋白質を同定するために用いられる。特にその蛋白質が疾患と関連があった場合には，新規治療薬のシーズ（種）となる可能性がある。低分子化合物マイクロアレイにより薬剤のハイスループットスクリーニングが可能となりつつある。

Ⅱ．マイクロアレイを用いた自己免疫疾患の解析

疾患研究におけるマイクロアレイ技術は，当初は癌研究の分野を中心に発展してきたが，その後自己免疫疾患の分野にも多く応用されてきた。疾患に特徴的な遺伝子発現の変化を見いだすために，モデル動物，ヒト末梢血などの試料とした，DNAマイクロアレイを用いた遺伝子発現量の網羅的解析が中心であった。

最近ではマイクロRNAをはじめとするノンコーディングRNAの遺伝子発現の調節へ重要性が明らかとなり，自己免疫疾患での検討が行われはじめている。関節リウマチ（rheumatoid arthritis；RA），シェーグレン症候群

(sjögren syndrome；SjS)，全身性エリテマトーデス(systemic lupus erythematosus；SLE)，多発性筋炎/皮膚筋炎(polymyositis；PM/dermatomyositis；DM)，1型糖尿病(type 1 diabetes；T1D)などの自己免疫疾患と関連するマイクロRNAのマイクロアレイを用いた網羅的解析の報告が相次いでいる。

自己抗体応答を測定する自己抗原マイクロアレイに関する研究も進んでいる。Robinsonらによる報告では，SLE，PM，SjS，混合性結合組織病(mixed connective tissue disease；MCTD)，原発性胆汁性肝硬変(primary biliary cirrhosis；PBC)，強皮症(systemic scleroderma；SSc)由来の試料を用いたアレイの結果が，蛍光抗体法による自己抗体パターンとよく一致していることが明らかとなった[4]。

マイクロアレイを用いた自己免疫疾患の解析への応用例の報告は非常に多数に渡るため，本稿では，次に疾患ごとにごく最近の一部の例を紹介する。

1．RA

RAは関節滑膜における慢性，持続的な炎症の結果，骨破壊とともに身体機能の低下，障害が起こりうる疾患である。RAおよび変形性関節症(osteoarthritis；OA)例の人工関節置換術時に採取された関節液を用いたDNAマイクロアレイの結果，RAにおいてはシグナル伝達性転写因子1(signal transducer and activator of transcription 1；STAT1)やインターフェロン制御因子1(interferon regulatory factor 1；IRF1)，Th1免疫反応を促進させるケモカインCXCL9やCXCL10，CCL5がOAに比べて高発現していることが報告されている[5]。

RAとOAの骨髄細胞を用いたDNAマイクロアレイを用いた発現解析およびパスウェー解析の結果，細胞増殖，DNA複製クロモソーム，DNA代謝に分類される遺伝子の発現低下，抗原提示に関わるMHCクラスⅠ分子(HLA-E，HLA-F，HLA-G)やタパシン(TAP)とTAP結合蛋白，インターフェロンγ，インターロイキン(IL)-8の高発現が報告されている[6]。

治療薬との関連への応用[7-9]としては，筆者らのグループはTNF阻害薬であるインフリキシマブの有効，効果不十分例におけるDNAマイクロアレイ解析での遺伝子発現の差異を基に，薬剤の効果予測について報告している。

2．SLE

SLEは全身性に臓器障害をきたす疾患であり，抗核抗体や抗dsDNA抗体などが診断や病勢の評価に用いられているが，新規バイオマーカー探索への応用例について紹介する。

SLE，RAおよび健常人の末梢血中のマイクロRNA発現の網羅的解析から，SLEではDNAメチル化に関連するマイクロRNAであるmiR-126の増加，miR-21，miR-451，miR-223，miR-16のSLEおよびRAでの増加，そしてSLEでのmiR-125a-3p，miR-155，miR-146aの減少などが報告された[10]。また，プロテインマイクロアレイによる血清中の自己抗体スクリーニングの検討において，細胞内のチャネル蛋白であるCLIC2がSLEの新たな自己抗体として見いだされ，抗CLIC2抗体は疾患活動性を反映し診断に有用である可能性が報告されている[11]。

3．SjS

マウスとヒトの唾液腺におけるRNA発現を，疾患関連および機能について加重遺伝子共発現解析(weight-gene co-expression network analysis)を行い，SjSではIL-4やTNF経路と関わる遺伝子発現との正の相関，酸化還元，エネルギーの代謝産物およびコファクターの代謝過程に関わる遺伝子発現との負の相関が報告されている[12]。

4．SSc

SScは微小血管障害とともに皮膚や消化管の線維化をきたす膠原病であるが，ペプチドマイクロアレイを用い，centromere protein A(CENPA)のN末端のlinear epitopeとして知られるGP-R/S-RRを共通のモチーフとする3つのペプチドが検出されている[13]。

5．多発性硬化症(multiple sclerosis；MS)

MSは自己免疫機序を介した慢性炎症性脱髄疾患であるが，抗アクアポリン4(AQP4)抗体の関与が明らかになりつつある。MS患者の全血からのmicroRNAの網羅的検討において，miR-22およびmiR-422aの増加が認められ，miR-422aはコレステロールから胆汁酸へ異化する際の律速因子となる遺伝子であるCYP7A1mRNAの発現の抑制により，中枢神経の炎症性脱髄疾患に関連するとの報告がある[14]。疾患の再燃や予後との関連ではG蛋白質共役受容体3をコードする遺伝子GPR3や，サイトカインであるIL-17受容体Cをコードする遺伝子IL17RCの発現低下は疾患活動性が高く，予後が不良である可能性があると報告されている[15]。

6. T1D

自己免疫性に，インスリン産生を行う膵β細胞の破壊が原因で起こる糖尿病である。T1Dの末梢血単球でプロモーター/エンハンサー領域にあるhistone H3-lysine 9 acetylationが単球のHLA-DRB1およびHLA-DQB1の上流にあるH3K9Acの変動により生じていることで，エピジェネティックにT1Dへ関連する可能性が報告されている[16]。また，自己免疫的機序が関わらない2型糖尿病との比較において，T1Dでは血清CLCL1分子の上昇が認められており，自己免疫素因となる可能性について報告されている[17]。

7. グレーブス病 (Graves' disease；GD)

甲状腺に対する自己抗体により甲状腺機能亢進症を起こす疾患であるが，末梢単核細胞のmicroRNAの検討にて，miR-154*，miR-376b，miR-431*はGD患者で抑制され，治療により寛解状態となっている患者においては抑制から回復しており，バイオマーカーとなる可能性が報告されている[18]。

おわりに

最近の自己免疫疾患解析への応用例を中心に述べた。少数の分子，遺伝子による検討しか行うことができなかった時代と比べると，マイクロアレイを用いた網羅的遺伝子発現解析技術の普及は自己免疫疾患研究に大きな前進をもたらしたことは間違いない。しかしながら，マイクロアレイ解析により取得された大量のデータの解釈や検証に関しては，いまだ必ずしも容易でないため，その適応と限界を認識し，解析技術の工夫や経験に裏打ちされた注意深い検討を加えることが重要であると考えている。今後は，遺伝子のみならず蛋白，糖鎖などへのアレイ技術の応用，また微量検体からの解析も可能となり，自己免疫疾患の病態解明，分子標的治療薬の創薬へとつながることが強く期待される。

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CCL18 Activates Fibroblast-like Synoviocytes in Patients with Rheumatoid Arthritis

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