Mod Rheumatol (2011) 21:458–468
DOI 10.1007/s10165-011-0425-8

ORIGINAL ARTICLE

Efficacy and safety of additional use of tacrolimus in patients with early rheumatoid arthritis with inadequate response to DMARDs—a multicenter, double-blind, parallel-group trial

Shinichi Kawai · Tsutomu Takeuchi · Kazuhiko Yamamoto · Yoshiya Tanaka · Nobuyuki Miyasaka

Received: 17 November 2010 / Accepted: 28 January 2011 / Published online: 24 February 2011

Abstract In this trial, we investigated the safety and efficacy of tacrolimus used in addition to standard anti-rheumatic drugs in patients with rheumatoid arthritis. Tacrolimus 3 mg or placebo was orally administered once daily for 52 weeks in a double-blind manner to patients with early active rheumatoid arthritis receiving other disease-modifying antirheumatic drugs (DMARDs). A total of 123 patients were randomized to the tacrolimus group (61 patients) and to the placebo group (62 patients). In the tacrolimus group, 70.5% achieved a clinical response according to American College of Rheumatology (ACR) 20 criteria, whereas 45.2% in the placebo group did so ($P = 0.005$). The tacrolimus group also showed significant improvement in terms of the European League Against Rheumatism (EULAR) response criteria of "good or moderate" versus the placebo group (86.9 vs. 56.5%, respectively). Likewise, significantly more patients in the tacrolimus group versus the placebo group achieved remission of the Disease Activity Score in 28 joints (DAS28) (45 vs. 21%). The mean changes in the Total Sharp Score and erosion score were lower in the tacrolimus group, but the differences between the two groups were not significant. There was no significant difference between the two groups in the incidence of adverse events. Based on these results, we can conclude that the additional use of tacrolimus in patients with early rheumatoid arthritis with inadequate response to other DMARD treatments is useful, and this could become one of the treatment options for these rheumatoid arthritis patients.

Keywords DMARD · Randomized controlled trial · Rheumatoid arthritis · Tacrolimus

S. Kawai (✉)
Division of Rheumatology, Department of Internal Medicine, Toho University School of Medicine, 6-11-1 Omori-Nishi, Ota-ku, Tokyo 143-8541, Japan
e-mail: skawai@med.toho-u.ac.jp

T. Takeuchi
Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan

K. Yamamoto
Tokyo University, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

Y. Tanaka
University of Occupational and Environmental Health, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu, Fukuoka 807-8555, Japan

N. Miyasaka
Tokyo Medicine and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8510, Japan

Introduction

Tacrolimus is an immunosuppressive agent with a macrolide structure; it is produced by the streptomycete *Streptomyces tsukubaensis* and it specifically suppresses T-cell activation by inhibiting calcineurin. In Japan, this agent is approved for various indications in transplantations and autoimmune diseases as an injectable drug, as encapsulated formulations, and as a granulated powder. It is also approved as an ointment for the indication of atopic dermatitis, and as an ophthalmic solution for spring catarrh.

Rheumatoid arthritis is a disease characterized by destructive synovial joint inflammation, which causes not only pain but also interference with the activities of daily living and decreased quality of life because of functional impairment. The involvement of immunocompetent T cells

has been reported as the pathogenic mechanism of rheumatoid arthritis [1] and the activation of autoreactive T cells is one cause of inflammatory cytokine production.

Tacrolimus, by inhibiting T-cell activation, inhibits the production of tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, and IL-6, which are inflammatory cytokines that participate in the pathogenesis of rheumatoid arthritis [2–4]. The efficacy of tacrolimus against collagen arthritis and adjuvant arthritis, which are animal models of rheumatoid arthritis, has also been ascertained [5, 6]. Furthermore, this efficacy has been confirmed in various clinical trials in patients with rheumatoid arthritis; all the clinical trials in Japanese patients with rheumatoid arthritis were implemented as monotherapy trials [7, 8]. In North America, trial results have also suggested that this drug used additionally with methotrexate (MTX) is effective [9]. When a single disease-modifying antirheumatic drug (DMARD) has been found to be insufficient in rheumatoid arthritis patients, combined therapy with other antirheumatic drugs has been recommended [10]. Furthermore, because joint destruction due to rheumatoid arthritis has been reported to occur during the early stages of the disease [11], treatments are required to start at an earlier stage than has previously been reported.

We thus conducted a double-blind, placebo-controlled trial to investigate the efficacy and safety of tacrolimus used in addition to MTX, salazosulfapyridine, or bucillamine, which are the standard antirheumatic drugs used in Japan. We also investigated the efficacy of tacrolimus against the progression of joint destruction during a double-blind trial in patients with early rheumatoid arthritis with significant progression of bone destruction.

Patients and methods

Patients

Our inclusion criteria included patients aged between 20 and 65 years diagnosed with rheumatoid arthritis based on the definition (1987) [12] of the American College of Rheumatology (ACR), with a disease duration of at least 6 months but no more than 3 years, with at least 6 tender joints and at least 3 swollen joints, a C-reactive protein (CRP) level higher than 1.0 mg/dL, and an erythrocyte sedimentation rate higher than 30 mm/h, despite continuous administration of either MTX (6–8 mg/week), salazosulfapyridine (1 g/day), or bucillamine (100–300 mg/day). Our criteria also included patients with erosions that had been observed in more than 1 joint by X-ray films of hands and feet.

Our main exclusion criteria included patients who had previously received tacrolimus; patients corresponding to Steinbrocker's functional classification class 4; patients who had received biological products with an inhibitory effect on the progression of joint destruction (such as infliximab or etanercept) or leflunomide within 12 weeks before administration of the study drug; patients whose daily oral glucocorticoid dose exceeded 7.5 mg (prednisolone equivalent) within 4 weeks before administration of the study drug; patients who used at least two nonsteroidal anti-inflammatory drugs (NSAIDs) (oral or suppository) concomitantly within 4 weeks before administration of the study drug; and patients with the complications of renal dysfunction, pancreatitis/glucose intolerance, hyperkalemia, advanced liver dysfunction, cardiac disorders (such as ischemic cardiac disease, arrhythmia requiring treatment or cardiac failure), severe respiratory disorders, severe infectious disease, severe drug hypersensitivity disorders, or a malignant tumor.

Study protocol

This trial was conducted in 32 facilities from April 2006 to October 2008. All participating institutions received the approval of their governing institutional board or equivalent, and the trial was implemented in accordance with the ethical principles of the Declaration of Helsinki and the good clinical practice (GCP) guidelines, as well as relevant laws or regulations promulgated by the Institutional Review Boards for clinical trials. This trial is registered at ClinicalTrials.gov (NCT00319917).

After obtaining written consent, we allocated rheumatoid arthritis patients who met the inclusion criteria and did not fall under the exclusion criteria in a double-blind manner, and administered tacrolimus 3 mg or placebo orally once daily after dinner. The treatment period was 52 weeks.

As a general rule, we did not make any changes to the allocated dosage of MTX, salazosulfapyridine, or bucillamine, or to the dosage of oral glucocorticoid and/or NSAID (oral or suppository) during the treatment period. However, oral glucocorticoid doses were allowed to be changed if they were equal to or less than 7.5 mg prednisolone equivalent daily. The average prednisolone equivalent doses during the study period fluctuated between 4.3 and 4.5 mg daily in the tacrolimus group and between 4.9 and 5.7 mg daily in the placebo group. The differences in the glucocorticoid doses between these 2 groups were not statistically significant. The new administration of antirheumatic drugs or oral glucocorticoid was not allowed during the treatment period.

Clinical response was evaluated based on the ACR criteria (ACR20, ACR50, or ACR70) [13], Disease Activity Score in 28 joints (DAS28), and the European League Against Rheumatism (EULAR) response criteria [14, 15].

Remission was defined as DAS28 <2.6, in accordance with the EULAR definition [16]. Furthermore, the Modified Health Assessment Questionnaire (MHAQ) Score [17] was determined, from answers to the questionnaire given by the patients.

Joint destruction was assessed based on the change from baseline in the Total Sharp Score (0–448), erosion score, and joint-space narrowing score, using the modified Sharp van der Heijde scoring system [18, 19]. We obtained X-ray films of the hands and feet before the first administration of the study drug, then at week 28, and at week 52 (or at the time of discontinuation). Interpretation of the radiographic images was conducted by two people, who evaluated each radiographic image independently, without knowing the time the radiograph was taken or information relating to the disease activity or the treatment group. These scores were calculated based on the average of both readers' results.

Safety was evaluated by determining the incidence of adverse events (AEs) and laboratory abnormalities. We also measured the concentration of tacrolimus in whole blood, using the microparticle enzyme immunoassay technique in a blind manner, within a period (mean ± SD) of 12 ± 4 h after every administration of the study drug.

Statistical analysis

Efficacy analyses were based on the full analysis set (FAS), which consisted of all randomized subjects with rheumatoid arthritis who received at least one dose of the randomized study drug and who had at least one set of post-randomization data. Safety analyses were performed for all patients who received at least one dose of the randomized study drug.

All reported *P* values are two-sided; those less than 0.05 were considered to indicate statistical significance. ACR20, ACR50, and ACR70 responses, EULAR response criteria (good or moderate response), and DAS28 remission (cut-off point of DAS28 <2.6) were compared between the treatment groups using logistic regression analyses, adjusted for additional MTX use. For the radiographic end points, changes from baseline in the modified Sharp Scores (Total Sharp Score, erosion score, joint-space narrowing score) over 52 weeks were compared. The radiographic progression was extrapolated to impute 52-week values if patients had discontinued treatment after 28 weeks of study drug administration. Analyses of covariance were performed with the additional use of MTX as the covariate. For the other efficacy endpoints, means and proportions were compared using analyses of covariance and logistic regression analyses, respectively. Frequencies of adverse events (AEs) were compared with the use of Fisher's exact test. The coding dictionary for this study was the medical dictionary for regulatory activities (MedDRA), version 11.1. It was used to summarize AEs by system organ class and preferred term.

Results

Patient characteristics

Figure 1 shows the patient disposition in this trial. Of the 157 patients who gave their consent, 123 patients (61 in the tacrolimus group, 62 in the placebo group) were randomized. All of them received the study drug to be analyzed for safety and efficacy. Ninety-five patients (56 in the tacrolimus group, 39 in the placebo group) completed the treatment.

Twenty-eight patients discontinued the study drug (5 in the tacrolimus group, 23 in the placebo group). Reasons for

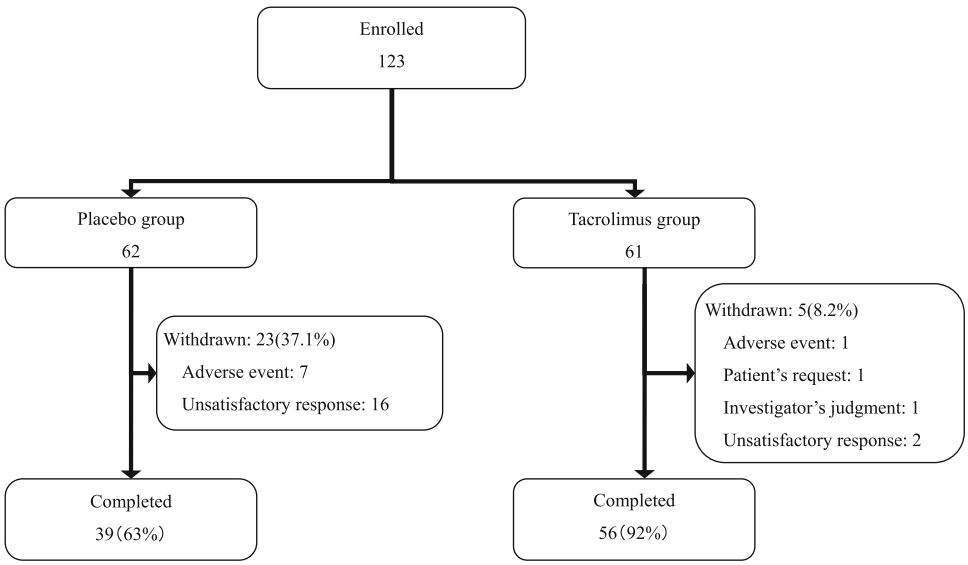

Fig. 1 Randomization, reasons for withdrawal, and numbers of patients who completed the trial

Table 1 Baseline characteristics of the patients

	Placebo ($n = 62$)	Tacrolimus ($n = 61$)	P value
Gender [number, female (%)]	50 (80.6)	55 (90.2)	0.202[a]
Age (years)	50.0 ± 11.6	47.1 ± 9.9	0.144[b]
Weight (kg)	56.4 ± 12.7	53.4 ± 8.1	0.130[b]
Disease duration (years)	1.7 ± 0.7	1.6 ± 0.7	0.276[b]
Steinbrocker stage [number (%)]			
I	1 (1.6)	0 (0.0)	0.792[c]
II	43 (69.4)	42 (68.9)	
III	16 (25.8)	19 (31.1)	
IV	2 (3.2)	0 (0.0)	
Steinbrocker class [number (%)]			
1	14 (22.6)	12 (19.7)	0.970[c]
2	45 (72.6)	48 (78.7)	
3	3 (4.8)	1 (1.6)	
4	0 (0.0)	0 (0.0)	
Concomitant therapy at baseline			
Glucocorticoids [number (%)]	31 (50.0)	32 (52.5)	0.857[a]
Prednisolone equivalent dose (mg/day)	4.9 ± 1.6	4.4 ± 1.9	0.328[b]
MTX [number (%)]	42 (67.7)	42 (68.9)	1.000[a]
Dose (mg/week)	7.3 ± 1.0	7.2 ± 1.0	
SASP [number (%)]	12 (19.4)	14 (23.0)	0.664[a]
Dose (mg/day)	1.0 ± 0.0	1.0 ± 0.0	
BUC [number (%)]	8 (12.9)	5 (8.2)	0.559[a]
Dose (mg/day)	150 ± 54	190 ± 55	
Tender joint count	11.7 ± 4.9	13.3 ± 7.5	0.152[b]
Swollen joint count	9.7 ± 5.1	10.9 ± 5.7	0.225[b]
CRP (mg/dL)	2.2 ± 2.1	1.8 ± 1.6	0.186[b]
ESR (mm/h)	46.3 ± 25.1	44.9 ± 21.7	0.738[b]
MHAQ	0.5 ± 0.4	0.5 ± 0.5	0.473[b]
Total Sharp Score	17.2 ± 20.6	17.7 ± 18.7	0.889[b]
Erosion score	8.3 ± 10.1	10.1 ± 10.9	0.354[b]
Joint-space narrowing score	8.8 ± 12.9	7.6 ± 9.5	0.537[b]
Yearly progression	10.8 ± 12.2	12.7 ± 15.2	0.433[b]
Rheumatoid factor (IU/mL)	128.6 ± 140.2	105.3 ± 137.4	0.353[b]

Plus-minus values are means ± SD

MTX methotrexate, *SASP* salazosulfapyridine, *BUC* bucillamine, *CRP* C-reactive protein, *ESR* erythrocyte sedimentation rate, *MHAQ* modified health assessment questionnaire

[a] Fisher's exact test

[b] *t*-test

[c] Wilcoxon rank sum test

discontinuation in the tacrolimus group were "AE", "patient's request", and "investigator's judgment" in one patient each, and "unsatisfactory response" in two patients. In the placebo group, seven patients discontinued the trial due to "AEs" and 16 patients discontinued due to "unsatisfactory response".

Table 1 shows the patient characteristics. The age range (mean ± SD) was 47.1 ± 9.9 years in the tacrolimus group and 50.0 ± 11.6 years in the placebo group; disease duration (mean ± SD) was 1.6 ± 0.7 years in the tacrolimus group, and 1.7 ± 0.7 years in the placebo group. The Total Sharp Score according to the modified Sharp van der Heijde scoring system was 17.7 ± 18.7 in the tacrolimus group, and 17.2 ± 20.6 in the placebo group; yearly progression was 12.7 ± 15.2 in the tacrolimus group, and 10.8 ± 12.2 in the placebo group. There were no major differences between the two groups.

Of the antirheumatic drugs used, MTX was the most commonly used: in 42 patients (68.9%) in the tacrolimus group, and 42 patients (67.7%) in the placebo group; salazosulfapyridine was used by 14 patients (23.0%) in the tacrolimus group, and 12 patients (19.4%) in the placebo group; bucillamine was used by five patients (8.2%) in the tacrolimus group and eight patients (12.9%) in the placebo group. Oral glucocorticoids (prednisolone, <7.5 mg per day) were used in 32 patients (52.5%) patients in the tacrolimus group and 31 patients (50.0%) in the placebo group.

Efficacy

Clinical responses

In the tacrolimus group, 70.5% (43/61 patients) achieved an ACR20 response at the end of treatment, compared with 45.2% (28/62 patients) in the placebo group; with a significantly higher response rate in the tacrolimus group ($P = 0.005$). Although the ACR50 and ACR70 response rates were higher in the tacrolimus group, the difference between the two groups was not statistically significant ($P = 0.085$, $P = 0.166$, Table 2).

According to the EULAR criteria, the incidence of a "good or moderate" response at the end of treatment was 86.9% (53/61 patients) in the tacrolimus group, and 56.5% (35/62 patients) in the placebo group, with the incidence being significantly higher ($P < 0.001$) in the tacrolimus group. Likewise, for a "good" response according to the EULAR criteria, the rate was 55.7% (34/61 patients) in the tacrolimus group, compared with 29.0% (18/62 patients) in the placebo group. Furthermore, the incidence of a "good" and that of a "good or moderate" response according to the EULAR criteria was significantly higher in the tacrolimus group ($P < 0.001$ to $P = 0.009$, Fig. 2) at any time after 8 weeks during the assessment period.

The percentage of patients achieving DAS28 remission (DAS28 < 2.6) after study drug administration was 45.0% (27/60 patients) in the tacrolimus group and 21.0% (13/62 patients) in the placebo group; there was a significantly greater response in the tacrolimus group ($P = 0.005$, Table 3).

Regarding MHAQ Scores, improvement was observed after the start of study drug administration in the tacrolimus group. The improvement effect at the end of treatment was significantly higher in the tacrolimus group than in the placebo group ($P < 0.05$) (Fig. 3).

The efficacy parameters, including Total Sharp Score, were compared in the patients with and without glucocorticoid use in the tacrolimus group. There were no significant differences between these 2 groups in any of the parameters (data not shown). The efficacy parameters were also compared in the patients in the tacrolimus group receiving MTX, salazosulfapyridine, or bucillamine therapies. We could not find any significant differences among these 3 groups either (data not shown).

Table 2 ACR20, ACR50, and ACR70 response rates at week 28, week 52, and end of the treatment

	Placebo $n = 62$ n (%)	Tacrolimus $n = 61$ n (%)
ACR20		
Week 28	22 (44.0)	39 (67.2)*
Week 52	23 (59.0)	42 (75.0)
End of treatment	28 (45.2)	43 (70.5)**
ACR50		
Week 28	15 (30.0)	19 (32.8)
Week 52	16 (41.0)	29 (51.8)
End of treatment	20 (32.3)	29 (47.5)
ACR70		
Week 28	7 (14.0)	10 (17.2)
Week 52	9 (23.1)	16 (28.6)
End of treatment	10 (16.1)	16 (26.2)

ACR American College of Rheumatology

* $P < 0.05$, ** $P < 0.01$ compared with placebo (logistic regression analysis)

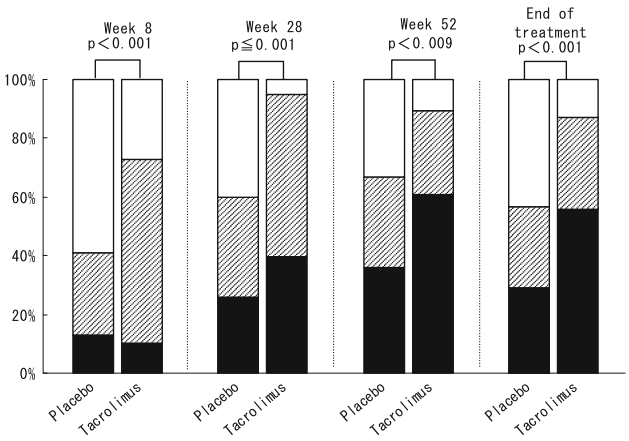

Fig. 2 European League Against Rheumatism (EULAR) response criteria. *Open bars* indicate "no response", *shaded bars* indicate "moderate response", and *solid bars* indicate "good response". EULAR response criteria (good or moderate response) were compared between treatment groups, using logistic regression analyses

Joint destruction

The change from baseline in the Total Sharp Score (mean value ± SD) at 52 weeks was 6.16 ± 10.84 in the tacrolimus group and 7.73 ± 12.23 in the placebo group. Although the score was lower in the tacrolimus group, this −1.44 difference between the two groups was not statistically significant ($P = 0.485$). The change from baseline in

Table 3 The proportions of patients in DAS28 remission

	Placebo	Tacrolimus	*P* value
Week 28	7 (14.0)	16 (28.1)	0.084
Week 52	11 (28.2)	27 (49.1)	0.041
End of treatment	13 (21.0)	27 (45.0)	0.005

No. of patients (%)

Disease Activity Score in 28 Joints (DAS28) remission, defined as DAS28 <2.6, was compared between treatment groups using logistic regression analyses

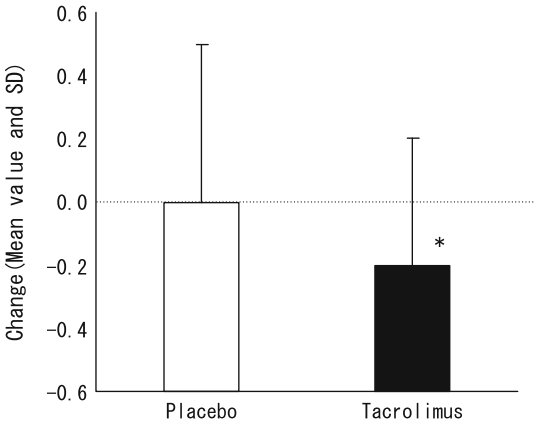

Fig. 3 Change in the Modified Health Assessment Questionnaire (MHAQ) Score from baseline to end of treatment. $*P < 0.05$, analysis of covariance (ANCOVA)

the erosion score (mean value ± SD) at 52 weeks was 2.50 ± 4.56 in the tacrolimus group and 4.27 ± 7.53 in the placebo group; although the score was lower in the tacrolimus group, this −1.91 difference between the groups was not significant ($P = 0.090$). The joint-space narrowing scores were 3.67 ± 7.03 in the tacrolimus group and 3.46 ± 5.33 in the placebo group; there was no significant difference between these groups. ($P = 0.665$, Table 4).

Figure 4 shows the cumulative probability plots for the modified Sharp Scores (Total Sharp Score, erosion score, joint-space narrowing score). The proportion of patients with change from baseline of the Total Sharp score of ≤0 over 52 weeks was 14/58 patients (24.1%) in the tacrolimus group, higher than the 7/50 patients (14.0%) in the placebo group. Similarly, the proportion of patients with change from baseline of the erosion score of ≤0 over 52 weeks was 21/58 patients (36.2%) in the tacrolimus group, and 11/50 patients (22.0%) in the placebo group, also being higher in the tacrolimus group.

Safety

The incidence of AEs was 86.9% (53/61 patients) in the tacrolimus group, and 79.0% (49/62 patients) in the placebo group; the incidence of discontinuation of the study drug due to AEs was 3.3% (2/61 patients) in the tacrolimus group and 11.3% (7/62 patients) in the placebo group. There was no significant difference between the two groups ($P = 0.338$ and $P = 0.163$, respectively). Furthermore, the incidence of serious AEs (SAEs) was significantly higher in the placebo group ($P = 0.017$, Table 5).

Table 4 Radiographic analysis

	Placebo ($n = 50$)	Tacrolimus ($n = 58$)	*P* value
Total Sharp Score			
Mean ± SD	7.73 ± 12.23	6.16 ± 10.84	0.485
Median (IQR)	3.50 (0.75–8.00)	3.25 (0.50–7.00)	
Erosion score			
Mean ± SD	4.27 ± 7.53	2.50 ± 4.56	0.090
Median (IQR)	2.00 (0.50–5.00)	1.00 (−0.50 to 4.50)	
Joint-space narrowing score			
Mean ± SD	3.46 ± 5.33	3.67 ± 7.03	0.665
Median (IQR)	1.75 (0.00–4.00)	0.50 (0.00–5.50)	

Changes from baseline to end of treatment in radiographic outcomes. *P* values for between-group differences in change were calculated by analysis of covariance (ANCOVA)

IQR interquartile range

One SAE was observed in the tacrolimus group (benign giant cell bone tumor), with nine SAEs (in nine patients) observed in the placebo group (peritonitis, deep vein thrombosis, thrombotic stroke, organizing pneumonia, pyoderma gangrenosum, subarachnoid hemorrhage, herpes zoster, retinal break, and colon cancer). All the events (with the exception of organizing pneumonia and colon cancer with unchanged outcome) that were observed in the placebo group were resolved by appropriate treatment. The benign giant cell bone tumor that occurred in a patient in the tacrolimus group was identified as multiple microcystic lesions in the left patella during an X-ray examination on day 85 after the first administration of the study drug. In this patient, administration of tacrolimus had been withdrawn since day 57 due to the occurrence of other AEs, and administration was discontinued after the tumor was identified. We then conducted nidus curettage and bone cement filling and the patient recovered on day 234. The investigator determined that the possibility of a causal relationship with the study drug could not be ruled out.

Regarding AEs which led to discontinuation of the trial for other reasons, two events (vomiting and high blood pressure) in four patients in the tacrolimus group and four events (hypoglycemia, headache, glucose-positive urine, tendon rupture) in two patients in the placebo group were observed. All events observed in the tacrolimus group were resolved by discontinuation of the study drug.

Regarding AEs in the tacrolimus group, the highest incidence was observed in "infectious and parasitic diseases" , followed by "laboratory data" and "gastrointestinal

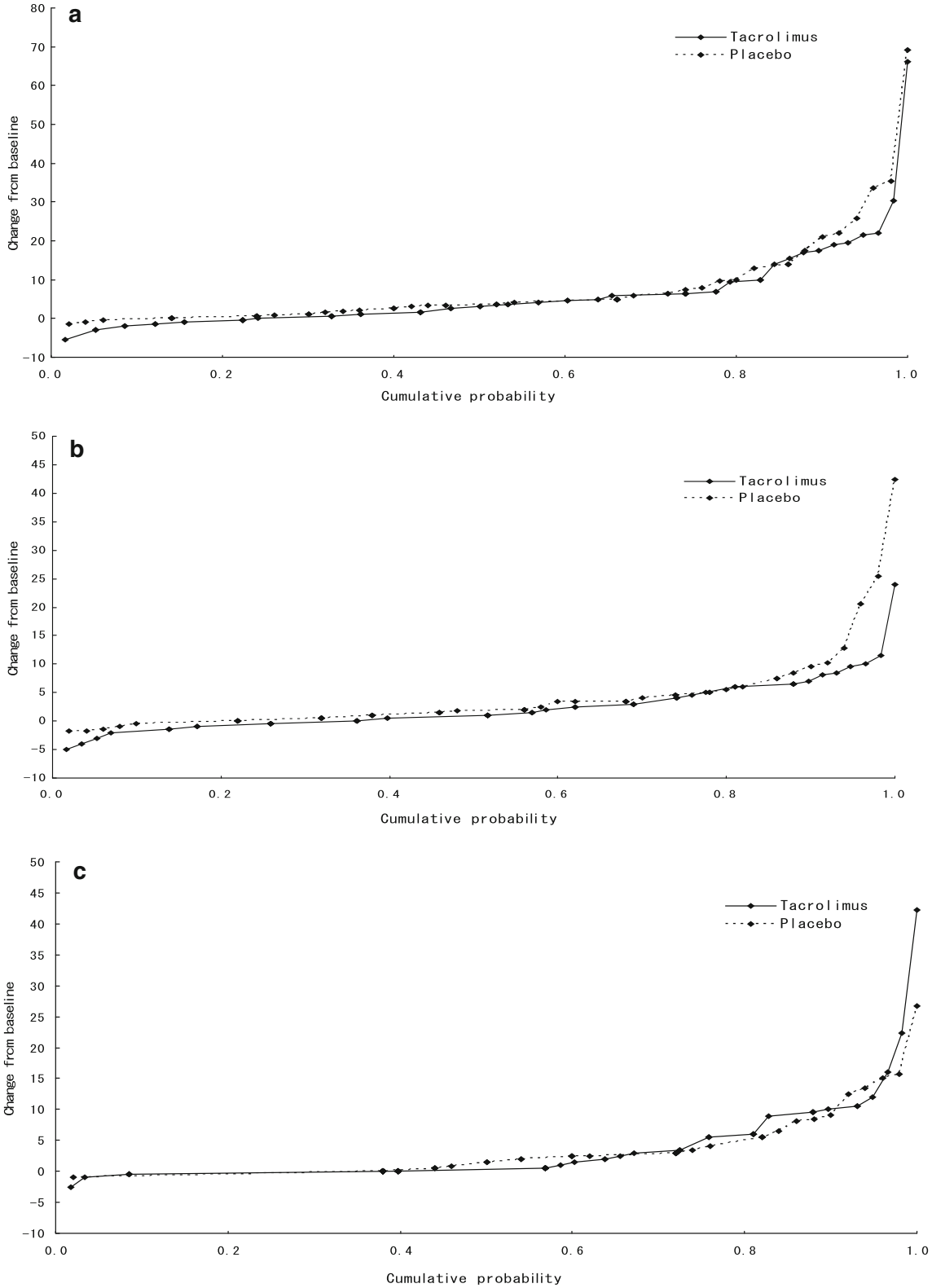

Fig. 4 Cumulative probability plots of radiographic changes from baseline to week 52 for patients treated with tacrolimus or with placebo. The space between the curves indicates the different treatment effects with a considerable difference in favor of the tacrolimus group. **a** Total Sharp Score, **b** Erosion score, **c** Joint-space narrowing score

Table 5 Treatment-related adverse events (AEs)

	Placebo ($n = 62$)	Tacrolimus ($n = 61$)
All	49 (79.0)	53 (86.9)
Serious AEs	9 (14.5)*	1 (1.6)
Infections and infestations	22 (35.5)	26 (42.6)
Vascular disorders	3 (4.8)	3 (4.9)
Respiratory, thoracic, and mediastinal disorders	9 (14.5)	8 (13.1)
Gastrointestinal disorders	10 (16.1)	25 (41.0)**
Hepatobiliary disorders	1 (1.6)	0 (0.0)
Skin and subcutaneous tissue disorders	10 (16.1)	14 (23.0)
Musculoskeletal and connective tissue disorders	3 (4.8)	4 (6.6)
Renal and urinary disorders	5 (8.1)	2 (3.3)
Neoplasms: benign, malignant, and unspecified (including cysts and polyps)	1 (1.6)	1 (1.6)
Reproductive system and breast disorders	0 (0.0)	3 (4.9)
Congenital, familial, and genetic disorders	0 (0.0)	1 (1.6)
General disorders and administration-site conditions	3 (4.8)	4 (6.6)
Laboratory data	16 (25.8)	26 (42.6)
Injury, poisoning, and procedural complications	6 (9.7)	2 (3.3)
Blood and lymphatic system disorders	1 (1.6)	5 (8.2)
Metabolic and nutritional disorders	0 (0.0)	3 (4.9)
Psychiatric disorders	1 (1.6)	0 (0.0)
Nervous system disorders	4 (6.5)	7 (11.5)
Visual disorders	1 (1.6)	5 (8.2)

Data are numbers of patients (%). * $P < 0.05$ compared with tacrolimus, ** $P < 0.01$ compared with placebo (Fisher's exact test)

disorders". Aside from these events, only "gastrointestinal disorders" was observed at a significantly higher incidence than in the placebo group ($P = 0.003$). In addition, nasopharyngitis, which occurred in 24.6% (15/61 patients), was the AE with the highest incidence in the tacrolimus group. The incidence of nasopharyngitis in the placebo group was 29.0% (18/62 patients). Events observed in at least five patients were diarrhea (seven patients), upper respiratory tract inflammation (seven patients), oral inflammation (six patients), upper abdominal pain (five patients), alanine and aminotransferase elevations (five patients), and abnormality in liver function tests (five patients)in the tacrolimus group , and upper respiratory tract inflammation (six patients) in the placebo group.

Serum creatinine had increased by more than 40% after study drug administration compared with the pre-dose value in 12/61 patients (19.7%) in the tacrolimus group, and in 1/62 patients (1.6%) in the placebo group ($P = 0.001$). This number was higher in the tacrolimus group, but no patient discontinued the trial for this reason. Changes in blood pressure and laboratory data results did not show any notable differences between the two groups.

Blood concentration of tacrolimus

The mean and median tacrolimus blood concentration values in each evaluation period (weeks 2–52) were in the range of 4.9–6.1 ng/mL and 4.5–5.5 ng/mL, respectively. Though the blood concentration in one patient increased to 20 ng/mL or higher (day 253 after administration of the study drug, 23.8 ng/mL), it was only a transient increase and the value measured at any other time point was not higher than 10 ng/mL. Moreover, in the patients with AEs or side effects, there was no trend in which the blood concentrations prior to the onset of the AEs or side effects, or the mean blood concentration values during the treatment period were higher than those in the patients without AEs or side effects.

Discussion

The efficacy of tacrolimus monotherapy in Japanese patients with rheumatoid arthritis has been demonstrated previously [7, 8]. On the contrary, in this double-blind experiment, we showed for the first time sufficient efficacy and safety of tacrolimus administered in addition to the standard DMARDs used in Japan (MTX, salazosulfapyridine, or bucillamine) in patients with early rheumatoid arthritis with an inadequate response to previous treatment.

Regarding efficacy in terms of disease activity, 70.5% of the tacrolimus group in the present study achieved an ACR20 response, compared with 45.2% in the placebo

group; the incidence of a "good or moderate" response according to the EULAR criteria was 86.9% in the tacrolimus group, and 56.5% in the placebo group at the end of treatment; furthermore, 45.0% of the tacrolimus group and 21.0% of the placebo group achieved DAS28 remission (DAS28 < 2.6). The patients in the tacrolimus group exhibited significantly better results in comparison with the placebo group. The difference between the group with the additional use of tacrolimus and the placebo group (DMARD only) was significant, and sufficient efficacy of the additional use of tacrolimus is considered to be proved.

It has been shown previously that the use of biological products in addition to MTX is more effective than MTX monotherapy [20–22]. In the present study, the difference in the DAS28 remission rate between the tacrolimus group and the placebo group (the existing therapy group) was found to be comparable to that in studies using biological products. In recent years, combined treatment with antirheumatic drugs has been recommended for rheumatoid arthritis patients with a poor prognosis[10] and it has been noted that the ultimate goal of antirheumatic treatment is to approach clinical remission [23–25]. Considering these facts, the result of the present trial is clinically significant.

The inhibition of joint destruction has been reported mainly with biological products, including TNF inhibitors, and also with other products such as oral antirheumatic MTX, leflunomide, and salazosulfapyridine [20, 26–30]. During the present trial, there was no significant difference in Total Sharp Score, erosion score, or joint-space narrowing score for joint destruction between the tacrolimus group and the placebo group. Regarding the change in erosion score from before study drug administration, the erosion score showed a lower value at 52 weeks in the tacrolimus group than that in the placebo group ($P = 0.090$), and this suggested the possibility that the progress of erosion was delayed. Furthermore, 24.1% (14/58 patients) of patients in the tacrolimus group showed inhibition of joint destruction over 52 weeks (Total Sharp Score ≤ 0). This was higher than the 14.0% (7/50 patients) in the placebo group. In addition, 36.2% (21/58 patients) of patients in the tacrolimus group and 22.0% (11/50 patients) in the placebo group had a change in the erosion score of ≤ 0 from baseline ; this percentage was also higher in the tacrolimus group. The inhibitory effect of joint destruction with the additional use of tacrolimus was unclear partly because the number of the patients was small. However, it appears that the use of tacrolimus with other DMARDs can delay erosion, and further investigation is considered to be necessary.

In the present study, the incidence of AEs was 86.9% (53/61 patients) in the tacrolimus group and 79.0% (49/62 patients) in the placebo group; there was no significant difference between the two groups. When classifying the AEs by organ, "infectious and parasitic diseases" occurred with a high incidence in the tacrolimus group, followed by "laboratory data" and "gastrointestinal disorders". The incidence of "gastrointestinal disorders" was significantly higher in the tacrolimus group than that in the placebo group ($P = 0.003$), but there were no patients who discontinued the trial because of AEs or severe events. This result is not significantly different from those of tacrolimus monotherapy trials [7, 8, 31, 32] in patients with rheumatoid arthritis, and in the present study, good tolerability of tacrolimus was shown in patients with early rheumatoid arthritis who also received other DMARDs. Moreover, the main side effects, such as infections, gastroenterological disorders, abnormal variations in renal function test valuesand abnormal variations in glucose tolerance test valueshave been observed in previous studies [7, 8, 32]. However, in the present trial, no significant abnormal variations in renal function test values or abnormal variations in glucose tolerance test values were observed. The number of patients with an increased creatinine level after study drug administration was larger in the tacrolimus group, which is similar to results previously reported from trials conducted elsewhere [33–35]. However, there was no serious increase in serum creatinine in patients who discontinued tacrolimus administration in our study.

Tacrolimus is a drug metabolized in the liver, yet hepatic dysfunction has never been a major problem in trials of tacrolimus monotherapy . However, hepatic dysfunction is known as a side effect of MTX [36, 37]. Because MTX was used together with tacrolimus in about 70% of patients in the present trial, we looked closely at the occurrence of hepatic disorders due to the additional use of tacrolimus. Regarding abnormal liver function test values and variations in various liver function test values (alanine aminotransferase [ALT] increased, aspartate aminotransferase [AST] increased, γ-guanosine triphosphate [GTP] increased, alkaline phosphatase [ALP] increased), 15 events occurred in 12 patients in the tacrolimus group, and seven events occurred in 10 patients in the placebo group among patients who concomitantly received MTX. The tacrolimus group had a slightly higher incidence of events, but most of the events were "slight". These results suggest a low possibility of perturbation of liver function even with the additional use of tacrolimus, suggesting that, in this respect, combined tacrolimus and MTX therapy is not significantly different from tacrolimus monotherapy. Therefore, it is concluded that the combination therapy can be used without major problems.

The safety profile of the additional use of tacrolimus with DMARDs (MTX, salazosulfapyridine, or bucillamine) was almost the same as that of the tacrolimus monotherapy which was reported previously [7, 8, 32, 33]. Based on the results mentioned above, we can conclude that the

additional use of tacrolimus in patients with early rheumatoid arthritis with an inadequate response to other DMARD treatments is useful, and this could become one of the treatment options for these rheumatoid arthritis patients.

Acknowledgments The authors wish to thank the members of the present study group, who were as follows: Kou Katayama (Katayama Orthopedic Rheumatology Clinic, Hokkaido, Japan); Hirofumi Osaki (Osaki Medical Clinic, Hokkaido, Japan); Kouichi Kitamura (Hakodate Goryokaku Hospital, Hokkaido, Japan); Akio Suda (Suda Memorial Orthopedic Clinic); Shuji Ohta (Taga General Hospital HITACHI Ltd, Ibaraki, Japan); Makoto Nishinarita (Nishinarita Clinic, Ibaraki, Japan) Takehiko Ayabe (Ayabe Medical Clinic, Ibaraki, Japan); Takayuki Sumida (Tsukuba University Hospital, Ibaraki, Japan); Hitoaki Okazaki (Jichi Medical University Hospital, Tochigi, Japan); Hiroshi Inoue (Inoue Hospital, Gunma, Japan); Toshihide Mimura (Saitama Medical University Hospital, Saitama, Japan); Tsutomu Takeuchi (Saitama Medical Center, Saitama, Japan); Shuji Ohno (Ohno Clinic, Saitama, Japan); Kazuhiko Inoue (Tokyo Women's Medical University Medical Center East, Tokyo, Japan); Kaichirou Saigo (Nihon University Itabashi Hospital, Tokyo, Japan); Kazuhiko Yamamoto (The University of Tokyo Hospital, Tokyo, Japan); Hitoshi Kohsaka (Tokyo Medical and Dental University Hospital Faculty of Medicine, Tokyo, Japan); Naoyuki Kamatani (Institute of Rheumatology Tokyo Women's Medical University, Tokyo, Japan); Kunio Matsuda (Matsuda Clinic, Tokyo, Japan); Shouhei Nagaoka (Yokohama Minami Kyosai Hospital, Kanagawa, Japan); Katsumitsu Arai (Niigata University Medical and Dental Hospital, Niigata, Japan); Akio Suzuki (Marunouchi Hospital, Nagano, Japan); Kazunori Sugimoto (Fukui General Hospital, Fukui, Japan); Shigeru Honjo (Saiseikai Takaoka Hospital, Toyama, Japan); Takashi Ikawa (Osaka Rehabilitation Hospital, Osaka, Japan); Tsukasa Matsubara (Matsubara Clinic, Hyogo, Japan); Kaoru Atsui (Matsubara Mayflower Hospital, Hyogo, Japan); Seizo Yamana (Higashi Hiroshima Memorial Hospital, Hiroshima, Japan); Yuji Yamanishi (Hiroshima City Hospital, Hiroshima, Japan); Hiroaki Nishizaka (Kitakyushu Municipal Medical Center, Fukuoka, Japan); Yoshiya Tanaka (University of Occupational and Environmental Health, Fukuoka, Japan); Hisaaki Miyahara (National Kyushu Medical Center, Fukuoka, Japan); Eiichi Suematsu (National Kyushu Medical Center, Fukuoka, Japan); Shouichi Kondo (Kondo Clinic, Fukuoka, Japan); Eisuke Shono (Shono Rheumatism Clinic, Fukuoka, Japan); Koji Kuroda (Kuroda Orthopedic Hospital, Fukuoka, Japan) Hiroshi Tsurugami (Tsurugami Orthopedic Clinic, Kumamoto, Japan); Michishi Tsukano (Kumamoto Orthopedic Hospital, Kumamoto, Japan); Toshihiko Hidaka (Zenjinkai Shimin-no-mori Hospital, Miyazaki, Japan). This study was funded by a grant from Astellas Pharma Inc. (Tokyo, Japan).

Conflict of interest S. Kawai, Y. Tanaka, T. Takeuchi, K. Yamamoto, and N. Miyasaka are affiliated with Astellas Pharma Inc.

References

1. Panayi GS, Lanchbury JS, Kingsley GH. The importance of the T cell in initiating and maintaining the chronic synovitis of rheumatoid arthritis. Arthritis Rheum. 1992;35(7):729–35.
2. Sakuma S, Kato Y, Nishigaki F, Sasakawa T, Magari K, Miyata S, et al. FK506 potently inhibits T cell activation induced TNF-alpha and IL-1beta production in vitro by human peripheral blood mononuclear cells. Br J Pharmacol. 2000;130(7):1655–63.
3. Sakuma S, Kato Y, Nishigaki F, Magari K, Miyata S, Ohkubo Y, et al. Effects of FK506 and other immunosuppressive anti-rheumatic agents on T cell activation mediated IL-6 and IgM production in vitro. Int Immunopharmacol. 2001;1(4):749–57.
4. Kino T, Hatanaka H, Miyata S, Inamura N, Nishiyama M, Yajima T, et al. FK-506, a novel immunosuppressant isolated from a *Streptomyces* II. Immunosuppressive effect of FK-506 in vitro. J Antibiot (Tokyo). 1987;40(9):1256–65.
5. Miyahara H, Hotokebuchi T, Arita C, Arai K, Sugioka Y, Takagishi K, et al. Comparative studies of the effects of FK506 and cyclosporin A on passively transferred collagen-induced arthritis in rats. Clin Immunol Immunopathol. 1991;60(2):278–88.
6. Sakuma S, Nishigaki F, Magari K, Ogawa T, Miyata S, Ohkubo Y, et al. FK506 is superior to methotrexate in therapeutic effects on advanced stage of rat adjuvant-induced arthritis. Inflamm Res. 2001;50(10):509–14.
7. Kondo H, Abe T, Hashimoto H, Uchida S, Irimajiri S, Hara M, et al. Efficacy and safety of tacrolimus (FK506) in treatment of rheumatoid arthritis: a randomized, double blind, placebo controlled dose-finding study. J Rheumatol. 2004;31(2):243–51.
8. Kawai S, Hashimoto H, Kondo H, Murayama T, Kiuchi T, Abe T. Comparison of tacrolimus and mizoribine in a randomized, double-blind controlled study in patients with rheumatoid arthritis. J Rheumatol. 2006;33(11):2153–61. Epub 2006 Sep 1.
9. Kremer JM, Habros JS, Kolba KS, Kaine JL, Borton MA, Mengle-Gaw LJ, et al. Tacrolimus in rheumatoid arthritis patients receiving concomitant methotrexate: a six-month, open-label study. Arthritis Rheum. 2003;48(10):2763–8.
10. Saag KG, Teng GG, Patkar NM, Anuntiyo J, Finney C, Curtis JR et al. American College of Rheumatology 2008 recommendations for the use of nonbiologic and biologic disease-modifying anti-rheumatic drugs in rheumatoid arthritis. Arthritis Rheum. 2008; 59(6):762–84.
11. Fuchs HA, Kaye JJ, Callahan LF, Nance EP, Pincus T. Evidence of significant radiographic damage in rheumatoid arthritis within the first 2 years of disease. J Rheumatol. 1989;16(5):585–91.
12. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum. 1988;31(3):315–24.
13. Felson DT, Anderson JJ, Boers M, Bombardier C, Furst D, Goldsmith C, et al. American College of Rheumatology. Preliminary definition of improvement in rheumatoid arthritis. Arthritis Rheum. 1995;38(6):727–35.
14. Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum. 1995;38(1):44–8.
15. van Gestel AM, Prevoo ML, van 't Hof MA, van Rijswijk MH, van de Putte LB, van Riel PL. Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis. Comparison with the preliminary American College of Rheumatology and the World Health Organization/International League Against Rheumatism Criteria. Arthritis Rheum. 1996;39(1):34–40.
16. Fransen J, Creemers MC, Van Riel PL. Remission in rheumatoid arthritis: agreement of the disease activity score (DAS28) with the ARA preliminary remission criteria. Rheumatology (Oxford). 2004;43(10):1252–5.
17. Pincus T, Summey JA, Soraci SA Jr, Wallston KA, Hummon NP. Assessment of patient satisfaction in activities of daily living using a modified Stanford Health Assessment Questionnaire. Arthritis Rheum. 1983;26(11):1346–53.
18. van der Heijde DM. Plain X-rays in rheumatoid arthritis: overview of scoring methods, their reliability and applicability. Baillieres Clin Rheumatol. 1996;10(3):435–53.

19. van der Heijde D, Simon L, Smolen J, Strand V, Sharp J, Boers M, et al. How to report radiographic data in randomized clinical trials in rheumatoid arthritis: guidelines from a roundtable discussion. Arthritis Rheum. 2002;47(2):215–8.
20. Klareskog L, van der Heijde D, de Jager JP, Gough A, Kalden J, Malaise M, et al. Therapeutic effect of the combination of etanercept and methotrexate compared with each treatment alone in patients with rheumatoid arthritis: double-blind randomised controlled trial. Lancet. 2004;363(9410):675–81.
21. Weinblatt ME, Keystone EC, Furst DE, Moreland LW, Weisman MH, Birbara CA, et al. Adalimumab, a fully human anti-tumor necrosis factor alpha monoclonal antibody, for the treatment of rheumatoid arthritis in patients taking concomitant methotrexate: the ARMADA trial. Arthritis Rheum. 2003;48(1):35–45.
22. Maini R, St Clair EW, Breedveld F, Furst D, Kalden J, Weisman M, et al. Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial ATTRACT Study Group. Lancet. 1999;354(9194):1932–9.
23. Smolen JS, Aletaha D, Koeller M, Weisman MH, Emery P. New therapies for treatment of rheumatoid arthritis. Lancet. 2007;370(9602):1861–74.
24. Goekoop-Ruiterman YP, de Vries-Bouwstra JK, Kerstens PJ, Nielen MM, Vos K, van Schaardenburg D, et al. DAS-driven therapy versus routine care in patients with recent-onset active rheumatoid arthritis. Ann Rheum Dis. 2010;69(1):65–9.
25. Smolen JS, Aletaha D, Bijlsma JW, Breedveld FC, Boumpas D, Burmester G, et al. Treating rheumatoid arthritis to target: recommendations of an international task force. Ann Rheum Dis. 2010;69(4):631–7.
26. Nishimoto N, Hashimoto J, Miyasaka N, Yamamoto K, Kawai S, Takeuchi T, et al. Study of active controlled monotherapy used for rheumatoid arthritis, an IL-6 inhibitor (SAMURAI): evidence of clinical and radiographic benefit from an X-ray reader-blinded randomised controlled trial of tocilizumab. Ann Rheum Dis. 2007;66(9):1162–7.
27. St Clair EW, van der Heijde DM, Smolen JS, Maini RN, Bathon JM, Emery P, et al. Combination of infliximab and methotrexate therapy for early rheumatoid arthritis: a randomized, controlled trial. Arthritis Rheum. 2004;50(11):3432–43.
28. Sharp JT, Strand V, Leung H, Hurley F, Loew-Friedrich I. Treatment with leflunomide slows radiographic progression of rheumatoid arthritis: results from three randomized controlled trials of leflunomide in patients with active rheumatoid arthritis. Leflunomide Rheumatoid Arthritis Investigators Group. Arthritis Rheum. 2000;43(3):495–505.
29. Kerstens PJ, Boerbooms AM, Jeurissen ME, de Graaf R, Mulder J, van de Putte LB. Radiological and clinical results of longterm treatment of rheumatoid arthritis with methotrexate and azathioprine. J Rheumatol. 2000;27(5):1148–55.
30. van der Heijde DM, van Riel PL, Nuver-Zwart IH, Gribnau FW, van de Putte LB. Effects of hydoxychloroquine and sulphasalazine on progression of joint damage in rheumatoid arthritis. Lancet. 1989;1(8646):1036–8.
31. Akimoto K, Kusunoki Y, Nishio S, Takagi K, Kawai S. Safety profile of tacrolimus in patients with rheumatoid arthritis. Clin Rheumatol. 2008;27(11):1393–7.
32. Kawai S, Tanaka K, Ohno I, Utsunomiya K, Seino Y. Safety of long-term tacrolimus therapy for rheumatoid arthritis: an open-label, uncontrolled study in non-elderly patients. Mod Rheumatol. 2008;18(4):345–53.
33. Furst DE, Saag K, Fleischmann MR, Sherrer Y, Block JA, Schnitzer T, et al. Efficacy of tacrolimus in rheumatoid arthritis patients who have been treated unsuccessfully with methotrexate: a six-month, double-blind, randomized, dose-ranging study. Arthritis Rheum. 2002;46(8):2020–8.
34. Yocum DE, Furst DE, Bensen WG, Burch FX, Borton MA, Mengle-Gaw LJ, et al. Safety of tacrolimus in patients with rheumatoid arthritis: long-term experience. Rheumatology (Oxford). 2004;43(8):992–9.
35. Yocum DE, Furst DE, Kaine JL, Baldassare AR, Stevenson JT, Borton MA, Tacrolimus Rheumatoid Arthritis Study Group, et al. Efficacy and safety of tacrolimus in patients with rheumatoid arthritis: a double-blind trial. Arthritis Rheum. 2003;48(12):3328–37.
36. Morgan SL, Baggott JE, Vaughn WH, Young PK, Austin JV, Krumdieck CL, et al. The effect of folic acid supplementation on the toxicity of low-dose methotrexate in patients with rheumatoid arthritis. Arthritis Rheum. 1990;33(1):9–18.
37. Shiroky JB, Neville C, Esdaile JM, Choquette D, Zummer M, Hazeltine M, et al. Low-dose methotrexate with leucovorin (folinic acid) in the management of rheumatoid arthritis. Results of a multicenter randomized, double-blind, placebo-controlled trial. Arthritis Rheum. 1993;36(6):795–803.