possible to discontinue ADA but maintain LDA status without biological DMARDs, even in patients with established RA. Further studies are warranted to confirm this possibility.

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Appendix

The following rheumatologists are the members of the BRIGHT Study Investigators Group: T. Koike (Hokkaido University); H. Takahashi (Sapporo Medical University); M. Mukai (Sapporo City General Hospital); S. Ohta (Taga General Hospital); T. Sumita (Tsukuba University); N. Watanabe (Chiba University); R. Matsumura (NHO Chiba East Hospital); T. Mimura (Saitama Medical University); T. Hanyu (Nagaoka Red Cross Hospital); T. Kasama (Showa University); H. Yamagata (NHO Murayama Medical Center); H. Yamada (St. Marianna University School of Medicine); S. Nagaoka (Yokohama Minami Kyousai Hospital); S. Toma (NHO Sagamihara National Hospital); S. Hirohata (Kitasato University); H. Taki (Toyama University); K. Sugimoto (Fukui General Hospital); T. Tsuji (Fukui Onsen Hospital); N. Ishiguro (Nagoya University); Y. Eto (NHO Nagoya Medical Center); T. Fujii (Kyoto University); Y. Kawahito (Kyoto Prefectural University); S. Kumagai (Kobe University); H. Wakabayashi (Okayama University); E. Suematsu (NHO Kyusyu Medical Center); T. Fukuda (Kurume University); R. Koike, T. Nanki, M. Tanaka, K. Watanabe, H. Yamazaki, R. Sakai (Tokyo Medical and Dental University).

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Clinical Significance of Cartilage Biomarkers for Monitoring Structural Joint Damage in Rheumatoid Arthritis Patients Treated with Anti-TNF Therapy

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Abstract

Purpose: With the current use of biologics in rheumatoid arthritis (RA), there is a need to monitor ongoing structural joint damage due to the dissociation of articular cartilage damage from disease activity of RA. This study longitudinally analyzed levels of serum cartilage biomarkers during 54 weeks of infliximab therapy, to evaluate the feasibility of biomarkers for monitoring structural joint damage.

Methods: Subjects comprised 33 patients with early RA and 33 patients with established RA. All patients received 3 mg/kg of infliximab and methotrexate for 54 weeks. Levels of the following serum cartilage markers were measured at baseline and at weeks 14, 22, and 54: hyaluronan (HA); cartilage oligometric matrix protein (COMP); type II collagen (CII)-related neoepitope (C2C); type II procollagen carboxy-propeptide (CPII); and keratin sulfate (KS). Time courses for each biomarker were assessed, and relationships between these biomarkers and clinical or radiographic parameters generally used for RA were investigated.

Results: Levels of CRP, MMP-3, DAS28-CRP, and annual progression of TSS were improved to similar degrees in both groups at week 54. HA and C2C/CPII were significantly decreased compared to baseline in the early RA group ($p<0.001$), whereas HA and COMP, but not C2C/CPII, were decreased in the established RA group. Strikingly, serum C2C/CPII levels were universally improved in early RA, regardless of EULAR response grade. Both ΔHA and ΔC2C/CPII from baseline to week 54 correlated significantly with not only ΔCRP, but also ΔDAS28 in early RA. Interestingly, when partial correlation coefficients were calculated by standardizing CRP levels, the significant correlation of ΔHA to ΔDAS28 disappeared, whereas correlations of ΔC2C/CPII to ΔDAS28, ΔJNS, and ΔHAQ remained significant. These results suggest a role of ΔC2C/CPII as a marker of ongoing structural joint damage with the least association with CRP, and that irreversible cartilage damage in established RA limits restoration of the C2C/CPII level, even with tight control of joint inflammation.

Conclusion: The temporal course of C2C/CPII level during anti-TNF therapy indicates that CII turnover shifts toward CII synthesis in early RA, but not in established RA, potentially due to irreversible cartilage damage. ΔC2C/CPII appears to offer a useful marker reflecting ongoing structural joint damage, dissociated from inflammatory indices such as CRP and MMP-3.

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Introduction

Anti-tumor necrosis factor (TNF) therapy is considered the global standard in the treatment of rheumatoid arthritis (RA), originally with the purpose of achieving clinical remission and now extending to structural remission at the radiographic level. Mounting evidence has accumulated that anti-TNF therapy not only inhibits radiographic progression of joint space narrowing, but also promotes joint space widening, particularly in patients with early RA, in whom annual changes in total modified van der Heijde (vdH)-Sharp score (TSS) are negative [1,2]. These observations allow clinicians to expect that TNF-blockade is capable of regenerating cartilage. However, 2-dimensional radiographic assessments based on TSS have not yet confirmed whether ongoing cartilage damage can be precisely evaluated. Ultrasonography and magnetic resonance imaging have recently been reported to allow detection of subclinical joint damage in patients showing clinical remission, suggesting a dissociation between clinical remission and structural joint deterioration [2,3]. Alternative tools that can assess ongoing joint destruction more easily than these imaging modalities should facilitate the evaluation of anti-rheumatic therapy with the potential to target structural remission. Molecular-marker technology (i.e., biomarkers) reportedly offer

Table 1. Baseline characteristics of the patients with early and established RA enrolled in this study*.

	Early RA (<9 months)	Established RA (>10 yrs)
No. of patients	33	33
Mean age	46.2 (19–75)	55.6 (34–80)
Gender (male/female)	10/23	6/27
Disease duration [months]	5.5 (2–9)	285 (122–516)
Swollen joint counts	10.3 (3–25)	10.3 (0–23)
Tender joint counts	8.8 (1–24)	8.6 (0–27)
CRP [mg/dl]	4.3 (0.2–11.0)	3.2 (0.1–10.9)
MMP-3 [ng/ml]	367 (31–1378)	302 (37–1292)
Rate of anti-CCP antibody [%]	82	85
DAS28-CRP	5.24 (3.11–7.75)	4.8 (2.54–6.83)
HAQ score	1.68 (0.75–2.38)	2.12 (0.75–3.00)
corticosteroid administration [%(cases)]	9 (3)	18 (6)

*Except where indicated otherwise, values are expressed as the mean (range).
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greater reliability and sensitivity than 2-dimensional radiography in clinical applications [4–6] and may offer a potential alternative to evaluate ongoing cartilage destruction in RA.

Alteration of articular cartilage turnover under arthritic conditions finally depends on the balance between the synthesis and degradation of cartilage matrix [7,8]. This can be monitored by measuring cartilage-derived synthesis and degradation molecules released into biological fluids, such as synovial fluid, serum and urine. These cartilage-derived biomarkers have been shown to reflect structural joint damage in RA and allow assessment of therapeutic efficacy in candidate anti-rheumatoid therapy. Existing biomarkers include cartilage oligometric matrix protein (COMP), human cartilage glycoprotein-39 (YKL-40), type II collagen (CII)-related neoepitope (C2C), carboxy-terminus of three-quarter peptide from cleavage of type I collagen and CII (C1,2C), type II procollagen carboxy-propeptide (CPII), C-telopeptide of type II collagen (CTX-II), keratin sulfate (KS-5D4), and aggrecan neoepitope (CS-846). Although controversy remains about which of the biological fluids offers the best sampling source and about diurnal and activity-related variations in each biomarker [9], a fundamental principle is that markers for cartilage degradation generally increase with the progression of joint destruction, whereas markers for cartilage synthesis increase following successful treatment with anti-TNF therapy [10]. The current use of biologics in RA makes it increasingly important to identify useful and simple blood tests that can precisely reflect responses to treatment, particularly in terms of cartilage turnover and systemic inflammation resulting from RA.

Despite the advantages of technical simplicity, the practical application of serum cartilage-derived biomarkers to date has remained limited. This is due, in part, to the fact that superiority over traditional laboratory markers has not been studied in a longitudinal fashion. The present study analyzed time courses for serum levels of cartilage markers during 54 weeks of infliximab therapy in two different cohorts of early and established RA, and compared the results with other laboratory, clinical and radiographic parameters generally used for RA. This study also estimated the feasibility of using cartilage biomarkers as a potential indicator of structural joint deterioration in RA.

Materials and Methods

All study protocols were approved by the institutional review board at Saitama Medical Center. All participants were informed about the goals and methods of the study and written consent was obtained prior to enrolment.

Patients

In this study, a total of 66 patients were enrolled from the Division of Rheumatology and Clinical Immunology at Saitama Medical Center, and all patients fulfilled the diagnostic criteria for RA according to American College of Rheumatology criteria [11]. Thirty-three patients with arthritis symptoms of <9 months duration were classified as having early RA, and 33 patients with disease duration >10 years were classified as showing established RA. Baseline characteristics of patients are shown in Table 1. All patients had clinically active disease, despite administration of conventional first-level disease-modifying anti-rheumatic drugs, and the mean 28-joint disease activity score (DAS28)-CRP at baseline was 5.24 for early RA and 4.8 for established RA. The rate of anti-cyclic citrullinated peptide (anti-CCP) antibody was 82% (27 patients) for the early RA group and 85% (28 patients) for the established RA group. Infliximab was administered at 3 mg/kg dose in weeks 0, 2, and 6, and then every 8 weeks. MTX was concomitantly administered at 6–10 mg/week in all patients. Patients were allowed to continue use of non-steroidal anti-inflammatory drugs and oral glucocorticoids (prednisolone-equivalents <10 mg/day) that they had been taking at study entry.

Patients were evaluated for therapeutic response at baseline and 14, 22, and 54 weeks after starting infliximab. At each evaluation, blood samples were obtained and sera were separated and stored at −80°C until needed for biomarker analysis.

Clinical evaluation of therapeutic response

The following clinical and laboratory parameters were longitudinally examined in each patient: CRP; stromelysin 1 (MMP-3); modified Health Assessment Questionnaire (HAQ) score; and DAS28-CRP. Scores for DAS28-CRP are reportedly lower than the original DAS28 assessments using the erythrocyte sedimentation rate [12] and were defined as follows: ≥4.1, high activity; ≥2.7 to <4.1, moderate activity; ≥2.3 to <2.7, low activity; and <2.3, remission. In terms of radiographic analysis, radiographs of

both hands and feet at baseline and 54 weeks were available for 26 patients in the early RA group and 23 patients in the established RA group. Two expert readers independently scored articular damage and progression in a blinded fashion according to the modified vdH-Sharp scoring method. Progression of TSS from baseline to week 54 (ΔTSS) was determined, and the proportion of patients with ΔTSS≤0 was calculated.

Cartilage biomarker analyses

The neoepitope resulting from collagenase cleavage of CII (i.e., C2C) and the c-propeptide cleaved from procollagen type II (i.e., CPII) were used as indicators of the degradation and synthesis of CII, respectively. Serum levels of each marker were measured using enzyme-linked immunosorbent assay (ELISA) (IBEX Technologies, Montreal, Quebec, Canada). The ratio C2C/CPII was used as an indicator of CII turnover, as previously reported [13,14]. Serum COMP levels were determined by sandwich ELISA (BioVendor Laboratory, Brno, Czech Republic), using 2 monoclonal antibodies against separate antigenic determinants of human COMP molecules. Serum HA levels were determined using an HA Assay Kit (IBA method; Seikagaku, Tokyo, Japan) utilizing HA-binding protein. KS was determined by high-performance liquid chromatography (HPLC) after digestion with keratanase II (Seikagaku), as reported previously [15,16]. Serum samples were treated with actinase E (Kaken Pharmaceutical, Tokyo, Japan) and the negatively charged substance containing KS was fractionated by Q sepharose and digested by keratanase II. These sequential enzymatic digestions yielded KS-derived β-galactosyl-(1–4)-6-O-sulfo-N-acetylglucosamine (m-ks) and β-6-O-sulfo-garactosyl-(1–4)-6-O-sulfo-N-acetylglucosamine (d-ks), which were measured using HPLC. Total KS was calculated as the sum of m-ks and d-ks values.

Statistical analysis

Analysis of our data revealed that most of the clinical, radiographic, and laboratory results were non-parametric. Statistical comparisons of laboratory parameters or cartilage biomarker levels at each time point with those at baseline were performed using Wilcoxon's matched-pairs signed-ranks test (two-tailed). Spearman's rank correlation coefficient was used to analyze relationships between changes in individual biomarkers and changes in laboratory or functional or radiographic parameters of RA. To remove the effects of decreased inflammation (i.e., CRP level) resulting from anti-TNF therapy on cartilage turnover, partial correlation coefficients controlling for CRP level were calculated to examine the relationship between cartilage biomarkers and measures of RA disease activity. Subgroup analysis was conducted based on European League of Associations for Rheumatology (EULAR) response criteria, such as good response, moderate response, and no response. As an indicator of CII turnover, C2C/CPII ratios in each response group were analyzed longitudinally, and changes from baseline to week 54 (i.e., C2C/CPII improvement) were compared between the three subgroups using the Kruskal-Wallis test. Statistical analyses were performed using SPSS version 17.0 software (SPSS, Chicago, IL). Values of $p<0.05$ were considered significant.

Sample size analysis for Wilcoxon's signed-ranks test was performed to demonstrate differences between serum level at baseline and at week 54 under the effect size given in each biomarker or laboratory index. In post-hoc analysis for early RA, 11, 60, 8, 5, 13, and 22 patients would be required to demonstrate a difference with an alpha level of 0.05 and 80% power, for C2C/CPII, HA, CRP, DAS28, MMP3, and HAQ, respectively. Similarly, for established RA, 29, 30, 20, 13, 7, and 18 patients would be required to demonstrate a difference, with an alpha level of 0.05 and 80% power, for KS, HA, COMP, CRP, DAS28, and MMP3, respectively. Sample sizes for correlation analysis were also analyzed to detect a moderate to large correlation coefficient ($r>0.4$) that was significantly different from the presence of no correlation ($r = 0$) with an alpha level of 0.05 and 80% power. In post-hoc analysis for early RA at week 54, 29 and 26 patients would be required to represent a given bivariate correlation coefficient with 80% power for ΔC2C/ΔCPII vs. ΔCRP and ΔC2C/ΔCPII vs. ΔDAS28, respectively. Similarly, 31 patients would be required to detect a given partial correlation coefficient with 80% power for ΔC2C/ΔCPII vs. ΔDAS28. In the established RA at week 54, 27 and 20 patients would be required to represent a given bivariate correlation coefficient with 80% power for ΔC2C/ΔCPII vs. ΔJNS and ΔC2C/ΔCPII vs. ΔHAQ, respectively. Regarding the partial correlation coefficient, 31 patients each would be required both for ΔC2C/ΔCPII vs. ΔJNS and for ΔC2C/ΔCPII vs. ΔHAQ. Taken together with these data, the projected sample size offering sufficient statistical power was 30 patients each in the early and established RA groups.

Results

Clinical evaluation

Of the 33 patients in the early RA group, 1 patient achieved clinical remission and 1 patient exhibited secondary loss of efficacy after 6-month infliximab therapy. These 2 patients discontinued infliximab, and the latter patient switched to tocilizumab. One patient experienced anaphylactic reaction at week 38 and switched to etanercept. Overall, 3 patients withdrew from the study, and the remaining 30 patients in the early RA group completed 54 weeks of infliximab therapy. In the established RA group, 5 patients exhibited secondary loss of efficacy and switched to etanercept ($n = 3$) or tocilizumab ($n = 2$). One patient discontinued infliximab at week 22, because she was planning to become pregnant. Overall, 6 patients were excluded and the remaining 27 patients in the established RA group completed 54 weeks of infliximab therapy.

As expected, laboratory indices for RA disease activity, such as CRP, MMP-3 and DAS28-CRP, had decreased significantly by week 54 in both groups (Table 2). The decrease in DAS28-CRP was prominent in patients with early RA, with mean score at week 54 below the level of clinical remission. Mean HAQ score was significantly decreased at week 54 in the early RA group, but remained unchanged in the established RA group. When DAS28-CRP scores were assessed using EULAR response criteria, 90% and 78% of patients were categorized as showing good or moderate response in the early and established RA groups, respectively, with no significant difference apparent between groups. Radiographic structural assessment using the TSS revealed that mean ΔTSS per year (annual progression) was 3.7 in the early RA group and 4.0 in the established RA group, while the proportion with ΔTSS≤0 exceeded 70% in both groups, suggesting that our clinical study using infliximab yielded successful clinical results comparable to those in a previous study in Japan [17].

Temporal changes in cartilage biomarkers during 54-week infliximab therapy

In the early RA group, serum levels of HA and C2C/CPII gradually decreased over time during 54-week infliximab therapy, and levels of HA at weeks 14, 22 and 54, and C2C/CPII at weeks 22 and 54 were significantly lower than each baseline level ($p<0.001$). These two biomarkers appeared to synchronize with

Table 2. Time-course changes in biochemical, clinical, radiographic, and functional measures during 1-year infliximab therapy.

	Time after starting infliximab			
	0W (baseline)	**14W**	**22W**	**54W**
Early RA (n = 30)				
CRP [mg/dl]	4.12†	1.43**	1.02**	0.45**
DAS28-CRP	5.16	3.13**	2.74**	2.2**
MMP-3 [ng/ml]	342	167	116*	105*
HAQ score†	1.46	0.92**	0.9**	0.8**
TSS (SD) (n = 26)	10.5 (18.7)	n.d.***	n.d.	14.2 (20.1)
JNS (SD) (n = 26)	4.8 (7.6)	n.d.	n.d.	7.2 (10.3)
ΔTSS (mean/median)				3.7/0
Rate of ΔTSS≤0 [%(cases)]				73 (19)
EULAR category of response [% (cases)]				
Good				63 (19)
Moderate				27 (8)
No response				10 (3)
Established RA (n = 27)				
CRP [mg/dl]	2.91	0.68**	0.66*	0.66*
DAS28-CRP	5.11	2.96**	2.76**	2.80**
MMP-3 [ng/ml]	298	92	98*	91*
HAQ score	1.88	1.7	1.71	1.73
TSS (SD) (n = 23)	211.2 (90.2)	n.d.	n.d.	215.4 (96.3)
JNS (SD) (n = 23)	85.8 (43.6)	n.d.	n.d.	88.1 (44.2)
ΔTSS (mean/median)				4.0/0
Rate of ΔTSS≤0 [%(cases)]				70 (16)
EULAR category of response [% (cases)]				
Good				41 (11)
Moderate				37 (10)
No response				22 (6)

†Except where indicated otherwise, values are expressed as the mean.
*$p<0.05$ versus baseline levels.
**$p<0.001$ versus baseline levels.
***n.d., not determined.
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decreasing CRP level over the 54 weeks of infliximab therapy. In contrast, COMP level remained constant during infliximab therapy (Table 3, Fig. 1A). Serum KS level slightly increased at week 14, followed by a gradual decrease to the baseline level at week 54. In the established RA group, serum level of HA was significantly decreased at week 14 ($p<0.05$) and became constant, demonstrating a quite similar pattern to that of CRP, whereas C2C/CPII remained unchanged during 54 weeks (Table 3, Fig. 1B). Level of serum COMP in established RA, which demonstrated a higher baseline level than in early RA, gradually decreased during the 54-week infliximab therapy with significant differences at week 54 ($p<0.05$). In contrast, level of serum KS in established RA, which also demonstrated a higher baseline level than in early RA, gradually increased with significant differences at weeks 22 and 54 compared to baseline ($p<0.05$).

Correlations between cartilage biomarkers and RA disease activity markers

Correlations between levels of cartilage biomarkers and degree of RA disease activity (e.g., CRP, MMP-3, and DAS-28), radiographic progression (e.g., ΔJNS) and patient function (e.g., HAQ score) were investigated at weeks 22 and 54. Several marker pairs with significant correlations are summarized in Table 4. Among the four cartilage biomarkers tested, only C2C/CPII and HA level yielded strong linear correlations with several disease activity measures of RA. Since the degree of structural joint damage, particularly in terms of cartilage destruction, is reportedly dissociated from the degree of joint inflammation, the present analysis focused on whether temporal changes in cartilage turnover were associated with the degree of CRP decrement. In the early RA group, ΔC2C/ΔCPII and ΔHA displayed significant correlations with ΔCRP at both weeks 22 and 54. Correlation with ΔDAS28 was observed at week 22 for ΔHA, and at both weeks 22 and 54 for ΔC2C/ΔCPII. Interestingly, according to partial correlation coefficients, the significant correlation between ΔHA and ΔDAS28 disappeared when the level of CRP was standardized. In contrast, the significant correlation between ΔC2C/ΔCPII and ΔDAS28 remained present even after standardization of CRP levels. In the established RA group, ΔC2C/ΔCPII correlated with neither ΔCRP nor ΔDAS28, whereas ΔHA did correlate with ΔCRP at both weeks 22 and 54. Of note is the finding that ΔC2C/ΔCPII significantly correlated with ΔJNS and

Table 3. Time-course changes in the levels of cartilage biomarkers during 1-year infliximab therapy.

	Time after starting infliximab			
	0W (baseline)	14W	22W	54W
Early RA (n = 30)				
HA [ng/ml]	420 (923) †	306 (852)*	134 (166)*	81 (69)**
KS [μg/ml]	0.87 (0.30)	0.96 (0.37)*	0.90 (0.31)	0.85 (0.22)
COMP [ng/ml]	545 (297)	549 (237)	561 (232)	570 (239)
C2C [ng/ml]	229 (47)	204 (45)	171 (46)*	156 (46)**
CPII [ng/ml]	733 (304)	858 (437)	875 (416)*	997 (489)*
C2C/CPII	0.34 (0.17)	0.32 (0.16)	0.20 (0.04)**	0.17 (0.05)**
Established RA (n = 27)				
HA [ng/ml]	335 (301)	199 (209)*	191 (196)*	193 (199)*
KS [μg/ml]	1.05 (0.34)	1.12 (0.43)	1.22 (0.38)*	1.25 (0.46)*
COMP [ng/ml]	845 (321)	788 (278)	734 (267)	669 (230)*
C2C [ng/ml]	224 (68)	231 (62)	211 (58)	264 (54)
CPII [ng/ml]	1039 (465)	1087 (439)	834 (306)	886 (243)*
C2C/CPII	0.28 (0.15)	0.27 (0.13)	0.3 (0.11)	0.31 (0.12)

†Values are expressed as mean (SD).
*$p<0.05$ versus baseline levels.
**$p<0.001$ versus baseline levels.
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ΔHAQ at week 54, and these significant correlations were present even after standardizing CRP level. These results suggest that ΔHA preferentially correlated with the level of CRP, while ΔC2C/ΔCPII represented a CRP-independent indicator of joint destruction reflecting radiographic joint space narrowing and patient function.

Association between balance of CII synthesis/degradation and efficacy of infliximab

As C2C/CPII preferentially reflected joint damage independent of changes in inflammatory indices, C2C/CPII was further analyzed for relationships with EULAR response grade after 54 weeks of infliximab therapy. Strikingly, in the early RA group, C2C/CPII was reduced (i.e., improved), regardless of responsiveness to infliximab, indicating that even in non-responders, the balance of CII synthesis/degradation became shifted toward synthesis (Fig. 2A). By contrast, C2C/CPII in the established RA group universally increased (i.e., worsened), regardless of responsiveness to infliximab, indicating that the net balance of CII synthesis/degradation was shifted toward degradation even in good responders (Fig. 2B). For all patients, C2C/CPII in non-responders was increased (i.e., worsened) compared to baseline, whereas C2C/CPII in moderate or good responders was reduced (i.e., improved) from baseline (Fig. 2C).

Discussion

RA is an inflammatory joint disease that predominantly involves the synovial tissues of joints and is characterized by variable disease onset and clinical course, ultimately resulting in structural joint destruction and subsequent physical disability. Early treatment with anti-TNF therapy is currently accepted as an effective strategy to achieve clinical and structural remission, potentially improving physical disability. In the present study, 54-week treatment with infliximab achieved satisfactory results according to the levels of CRP, MMP-3, and DAS28, EULAR response criteria, and the rate of ΔTSS≤0. Although these clinical measures for RA were similarly improved in both early and established RA, C2C/CPII as an indicator of CII turnover was significantly improved from baseline in early RA, but not in established RA. Strikingly, C2C/CPII was universally improved and shifted toward CII regeneration in early RA, regardless of EULAR response grade. In contrast, C2C/CPII was universally shifted toward CII degradation in established RA, regardless of

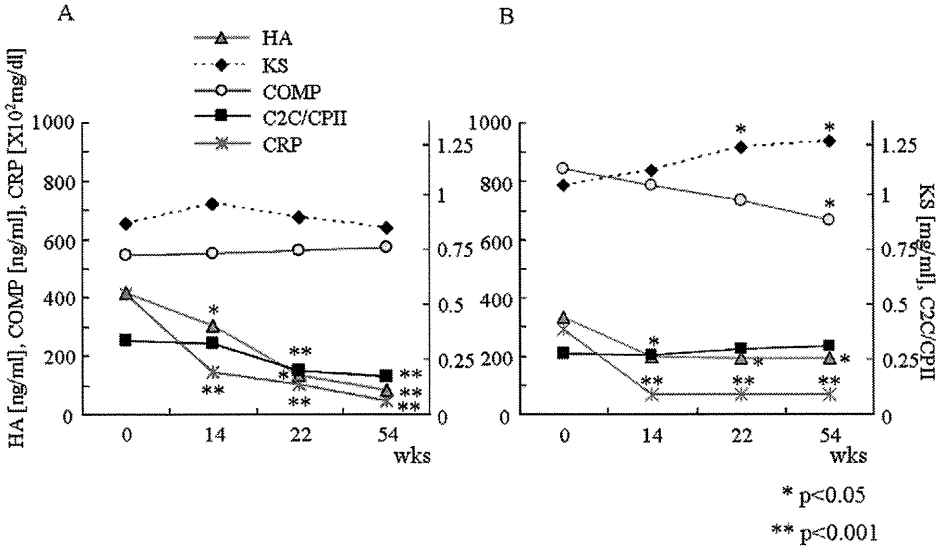

Figure 1. Temporal course of cartilage biomarker levels during 54-week infliximab therapy. Data for each time point represent mean levels of serum CRP, HA, COMP, KS, and C2C/CPII in early RA (A) and established RA (B). Standard deviation (SD) error bars are not plotted in these graphs for clarity and are shown in Table 3. Statistical analyses were performed using Wilcoxon's matched-pairs signed-ranks test, two-tailed. *$p<0.05$ versus level at baseline. **$p<0.001$ versus level at baseline.
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Table 4. Spearman's correlation coefficients and partial correlation coefficients of cartilage markers vesus RA disease markers.

		22W		54W	
		Spearman's r	partial r**	Spearman's r	partial r
Early RA					
ΔC2C/ΔCPII	vs. ΔCRP	0.44 (0.02)*	n.a.¶	0.50 (0.01)	n.a.
	vs. ΔDAS28	0.49 (0.03)	0.48 (0.04)	0.52 (0.01)	0.49 (0.02)
	vs. ΔKS	n.s.	n.s.†	−0.46 (0.03)	n.s.
ΔHA	vs. ΔCRP	0.37 (0.04)	n.a.	0.45 (0.03)	n.a.
	vs. ΔDAS28	0.52 (0.02)	n.s.	0.43 (0.04)	n.s.
	vs. ΔMMP-3	0.63 (0.02)	n.s.	0.74 (0.002)	0.48 (0.04)
Established RA					
ΔC2C/ΔCPII	vs. ΔCRP	n.s.	n.a.	n.s.	n.a.
	vs. ΔDAS28	n.s.	n.s.	n.s.	n.s.
	vs. ΔKS	n.s.	n.s.	−0.49(0.03)	n.s.
	vs.ΔJNS	n.d.	n.d.‡	0.51 (0.03)	0.48 (0.04)
	vs.ΔHAQ	n.d.	n.d.	0.58 (0.02)	0.49 (0.03)
ΔHA	vs. ΔCRP	0.56 (0.02)	n.a.	0.43 (0.04)	n.a.
	vs. ΔDAS28	n.s.	n.s.	n.s.	n.s.
	vs. ΔMMP-3	0.49 (0.04)	n.s.	0.51 (0.04)	n.s.

*Values are correlation coefficients calculated using Spearman's rank correlation. P values are expressed in parentheses. $p<0.05$ is considered as statistically significant.
**Partial correlation coefficients were obtained after controlling CRP level for each marker pair.
¶n.a., not applicable.
†n.s., not significant.
‡n.d., not determined.
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EULAR response grade. From the perspective of CII turnover, anti-TNF therapy should clearly be initiated while the patient is still in the early phase, while the regenerative capacity of articular cartilage is maintained and before irreversible structural joint damage occurs. Past clinical trials, such as the Best study, have demonstrated that patients initially treated with infliximab exhibited persistent low disease activity even after the cessation of infliximab, suggesting the clinical significance of early introduction of aggressive treatment in early RA with poor prognostic factors [18].

A noteworthy finding was that annual changes in cartilage biomarker levels correlated with annual progression of joint destruction and physical function after anti-TNF therapy. To the best of our knowledge, no previous studies have provided such insights. Significant correlations were found between ΔC2C/CPII and ΔHAQ ($r=0.58$, $p=0.02$) and between ΔC2C/CPII and ΔJNS ($r=0.51$, $p=0.03$) in our established RA cohort. The fact that joint space narrowing on radiography largely reflects loss of cartilage rather than bony erosion may explain the close relationship between ΔJNS and ΔC2C/CPII. Although determining whether HAQ improvement is a cause or consequence of decreased ΔC2C/CPII is difficult, one potential explanation for this correlation is that high activity and subsequent mechanical

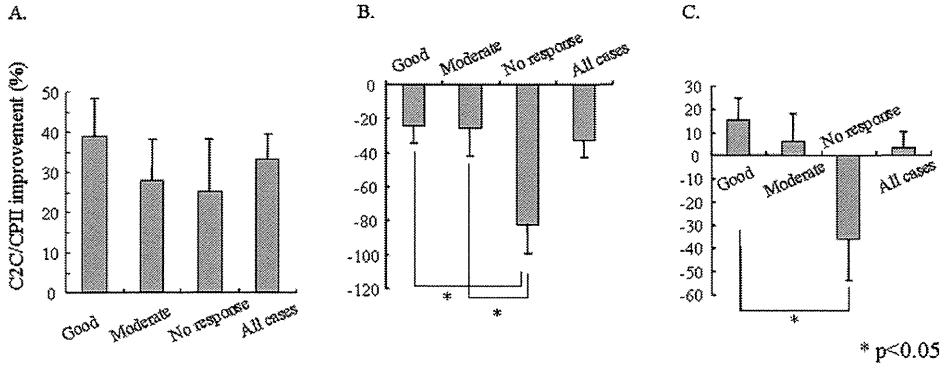

Figure 2. Improvement of C2C/CPII from baseline to week 54 was assessed in early RA (A), established RA (B), and all patients (C). Data are expressed as mean (± SD) percentage of baseline. Patients were divided into three subgroups according to the degree of clinical response at week 54 using EULAR response criteria. Positive values signify that the balance of CII synthesis/degradation is biased toward synthesis, while negative values indicate that the balance is biased toward degradation. Statistical analysis was performed using the Kruskal-Wallis test. *$p<0.05$.
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loading on cartilage either resulted in or is attributed to improved CII turnover, as reported by Roos et al. [19].

Serum levels of KS have been reported as an indirect measure of aggrecan turnover in articular cartilage and further analyzed for a role as a predisposing factor for osteoarthritis (OA) with a polyarticular, progressive phenotype [20]. KS level is elevated not only in patients with cartilage degeneration, but also in healthy individuals with higher sports activity [21], indicating that KS level can universally elevate in cases with increased cartilage turnover, even in normal cartilage. Wakitani et al. have reported that serum KS level in the knee OA is elevated more in the early stage than in the advanced stage, suggesting that KS reflects aggrecan turnover rather than the degree of joint destruction [16]. Similarly, levels of serum CS-846 epitope, as the marker for newly synthesized aggrecan, have been shown to increase in slowly progressive RA and signify an ability or attempt to repair damaged cartilage matrix [22,23]. From this perspective, gradually increased KS turnover in established RA was potentially attributable to not only persistent aggrecan release from cartilage, but also the fact that newly synthesized aggrecan cannot be incorporated into cartilage matrix that has been inherently damaged at baseline. In cases of early RA, KS levels were increased in week 14, but stabilized thereafter due to the inhibitory effects of TNF blockade on cartilage degradation, leading to normalization of cartilage turnover.

Contrasting results were obtained regarding the temporal course of serum COMP levels between early and established RA. Numerous studies have proposed the feasibility of serum COMP levels in monitoring articular cartilage damage or predicting the efficacy of anti-TNF therapy in RA [24–26]. In our established RA cohort, serum COMP levels were high at baseline, and gradually decreased during the course of infliximab therapy, as previously reported [24]. However, in early RA, serum COMP levels at baseline were low, and remained unchanged over 54-week infliximab therapy, despite fully exertion of the therapeutic effects of infliximab. Given the evidence that serum COMP levels elevate with increasing physical activity [27], constant levels of COMP over time in early RA might theoretically be explained if the decrement in COMP levels induced by infliximab is balanced by increased physical activity as evidenced from decreased HAQ scores.

Most measures of RA disease activity, such as the simplified disease activity index, Boolean criteria, and DAS28, exhibit correlations with CRP, because CRP is involved in each definition. As for cartilage biomarkers, this study showed that ΔHA and ΔC2C/CPII correlated significantly with not only ΔCRP, but also ΔDAS28 in early RA. Interestingly, when partial correlation coefficients were calculated by standardizing CRP levels, the significant correlation of ΔHA with ΔDAS28 disappeared, whereas correlations of ΔC2C/CPII with ΔDAS28, ΔJNS, and ΔHAQ were still significant. These results suggest a role of ΔC2C/CPII as a marker of ongoing structural joint damage with the least association to markers for systemic inflammation, such as CRP and erythrocyte sedimentation rate. Indeed, serum cytokine profile among the patients with established RA in this study revealed that levels of most inflammatory cytokines, including IL-6, TNF, and IL-17, were decreasing with decreasing CRP level over 54-week of infliximab therapy, whereas C2C/CPII level deteriorated over time (unpublished data, Fig. S1).

Significant concerns remain as to the differences in cartilage regenerative capacity between early and established RA. C2C/CPII was universally improved and shifted toward CII regeneration in early RA, but not in established RA, regardless of responsiveness to infliximab. Restoration of C2C/CPII balance and the resulting cartilage regeneration is likely to be relevant to the degree to which the cartilage matrix has been damaged before starting anti-TNF therapy, rather than the magnitude of the suppression of systemic inflammation during anti-TNF therapy. A previous experimental study showed that mice with antigen- or zymosan-induced arthritis displayed reversible cartilage damage only when levels of collagen degradation were low [28]. This finding was corroborated by a human study using cartilage explants culture in vitro, in which aggrecanase-mediated aggrecan degradation did not influence the regenerative capacity of cartilage, but was markedly impaired after MMP-mediated aggrecan and collagen type II degeneration were initiated [29]. MMP-mediated aggrecan and collagen type II degeneration might thus represent a turning point for the reversibility of cartilage degradation. Therefore, whether RA is in the early or established phase (i.e., disease duration) does not appear critical.

In conclusion, ΔC2C/CPII offers a useful marker reflecting ongoing cartilage damage, which appears dissociated from inflammatory indices. As most measures of RA disease activity generally correlate with CRP, C2C/CPII appears to be of great clinical value as a CRP-independent marker, particularly when ongoing structural joint damage is evaluated during biological therapy in RA. The temporal course of C2C/CPII level during anti-TNF therapy indicated that CII turnover shifted toward CII synthesis in early RA, but not in established RA, potentially due to irreversible cartilage damage. The clinical significance of C2C/CPII should be further investigated in large-scale prospective studies to evaluate the feasibility of using this ratio as a surrogate marker for monitoring ongoing structural joint damage during the course of anti-rheumatoid therapy.

Supporting Information

Figure S1 Temporal course of the serum levels of various cytokines in patients with established RA during 54-week infliximab therapy. The data were measured using a Luminex® multiplex beads cytokine assay. Values were expressed as a proportion of each baseline value. Of note is the finding that serum levels of most inflammatory cytokines, including IL-6, TNF, and IL-17, were decreasing with decreasing CRP level over 54-week of infliximab therapy, whereas C2C/CPII level deteriorated over time.
(TIF)

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Author Contributions

Conceived and designed the experiments: YN TT. Performed the experiments: MN HN TK. Analyzed the data: YN TT. Contributed reagents/materials/analysis tools: HY YT. Wrote the paper: YN TM. Proof check of English: YT TM.

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ORIGINAL ARTICLE

Phase II dose–response study of abatacept in Japanese patients with active rheumatoid arthritis with an inadequate response to methotrexate

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Abstract
Objective The objective of this study was to assess the response to abatacept at doses of 2 mg/kg and 10 mg/kg compared to placebo in patients with active rheumatoid arthritis (RA) with an inadequate clinical response to methotrexate (MTX).
Methods In this multicenter, placebo-controlled, double-blind, parallel-group, dose–response study, 195 Japanese patients with active RA with an inadequate response to MTX were randomized 1:1:1 to receive 10 mg/kg or 2 mg/kg abatacept plus MTX, or placebo plus MTX, for 24 weeks.
Results Abatacept demonstrated a dose–response relationship when given at 2 and 10 mg/kg. Based on the American College of Rheumatology criteria (20, 50, and 70 %), the responses to 10 mg/kg abatacept were significantly greater than those to placebo at week 24 ($p < 0.001$). Smaller yet statistically significant responses were also seen in the 2 mg/kg abatacept group. Overall rates of adverse events, serious adverse events, and treatment discontinuations because of adverse events were comparable in all three groups.
Conclusions Abatacept (2 mg/kg and 10 mg/kg) showed a dose–response relationship in Japanese patients with active RA with an inadequate clinical response to MTX. Administration of abatacept in combination with MTX for 24 weeks was well tolerated.

Keywords Abatacept · Active rheumatoid arthritis · Clinical response · Japan · Methotrexate

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Introduction

Rheumatoid arthritis (RA) is a chronic, systemic, inflammatory autoimmune disease that is characterized by progressive joint damage and disability, which severely affects quality of life [1, 2]. Increased understanding of the pathogenesis of RA and the proinflammatory cytokines that underlie its progression has led to the development of disease-modifying, anti-rheumatic drugs (DMARDs) [3]. These biological agents target T cells, B cells and proinflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1 and IL-6, and have had a profound impact on the treatment of this debilitating condition [4–8]. However, treatment is not always effective as many patients fail to respond [6, 8, 9] or maintain a response [5] to the therapies. Some patients develop antibodies against the particular agent used [7], while others experience relatively severe adverse reactions. These disadvantages of existing DMARDs highlight the need for new therapeutic agents with a different mechanism of action and improved efficacy.

The underlying pathogenesis of RA is thought to involve activated T cells that produce proinflammatory cytokines such as TNF-α, IL-1, and IL-6 [10]. T cells are one of the most abundant cell types in the RA synovium, comprising up to 50 % of all cells present [11]. Activated T cells may also work together with other cells in the connective tissue of joints to activate other immune cells, leading to the production of inflammatory mediators and metalloproteinases, such as matrix metalloproteinase-3. This process results in the degradation of bone and cartilage, and contributes to joint destruction [2, 10]. Autoreactive T cells, which react to self-antigens, have also been implicated in autoimmune disorders such as RA [12]. Therefore, inhibition of T cell activation represents a potential therapeutic strategy for RA.

At least two signals from antigen-presenting cells (APCs) are required for full T cell activation: an antigen-specific signal and a second signal transduced by the binding of a co-stimulatory receptor on the T cell to a ligand on the APC. Activation is also facilitated by the binding of CD80 or CD86 on the surface of an APC to CD28 expressed on T cells [11]. Activation is then followed by the induction of cytotoxic T-lymphocyte antigen 4 (CTLA4), a naturally occurring inhibitory molecule expressed on the surface of T cells, which has a significantly greater affinity for CD80 and CD86 than does CD28 [1, 11].

Abatacept is a recombinant fusion protein consisting of the extracellular domain of human CTLA4. It is the first in a new class of agents for RA that selectively modulates the CD80 or CD86–CD28 co-stimulatory signal involved in full T cell activation. Abatacept binds to CD80 and CD86 on T cells and thereby inhibits the binding of these molecules to CD28, preventing T cell activation [13]. This approach has therapeutic benefits in individuals with RA [10, 13, 14] and was shown to be safe and efficacious in a Phase I study conducted in Japanese patients with RA [15]. Of note, abatacept was effective in patients with an inadequate response to methotrexate (MTX) [10, 16–18], those who are MTX-naive [19] and those with an inadequate response to TNF-α inhibition [14, 20]. Furthermore, a global Phase II study showed good efficacy of abatacept in patients with active RA despite MTX therapy [10, 17]. To date, however, there are limited data in Japanese patients with RA.

Here, we conducted a Phase II bridging study to assess the efficacy and dose–response of abatacept in Japanese patients with active RA despite MTX therapy. We also evaluated whether the results of Phase III studies in Western patients [14, 18, 21] can be extrapolated to Japanese patients.

Materials and methods

Objectives

The primary objective of this bridging study was to assess the efficacy and dose response of abatacept by comparing the administration of abatacept at 2 and 10 mg/kg with placebo. Japanese patients with active RA despite MTX therapy fulfilling the American College of Rheumatology 20 % response (ACR20) criteria received either abatacept or placebo for 12 weeks, while continuing MTX therapy. Secondary objectives included ACR50 and ACR70 response rates at week 24; ACR20, ACR50, and ACR70 responses within 24 weeks; improvement in Health Assessment Questionnaire (HAQ); Disease Activity Score 28 based on C-reactive protein concentrations (DAS28-CRP); and the safety and immunogenicity of abatacept.

Patients

The study enrolled Japanese males and females aged ≥20 years. Enrollment criteria included fulfillment of the ACR 1987 criteria for the diagnosis of RA with a functional status of Class I, II or III [22, 23]; previous treatment with MTX at 6–8 mg weekly for at least 12 weeks, with a stable dose for at least 4 weeks before registration; and one or more of the following: ≥10 swollen joints (66-joint count), ≥12 tender joints (68-joint count), or CRP ≥1.0 mg/dL.

Exclusion criteria included females of childbearing age who were unwilling or unable to use an acceptable method of contraception for the duration of the study and for

10 weeks after the study; females who were either pregnant or breastfeeding; active vasculitis of a major organ system other than rheumatoid nodules; current symptoms of severe, progressive, or uncontrolled renal, hepatic, hematologic, gastrointestinal, pulmonary, cardiac, neurologic or cerebral disease; evidence of HIV, hepatitis B or hepatitis C; evidence of opportunistic infections, serious infections (e.g., pneumonia, renal infection, sinusitis) or chronic infections within 3 months before preliminary or formal registration in this study; or active tuberculosis requiring treatment within 3 years before registration. Patients with severe asthma, cancer, or a history of cancer within 5 years before the study, body weight >125 kg, treatment with any investigational drug within 8 weeks before formal registration, or prior administration of abatacept were also excluded.

Study design

This multicenter, placebo-controlled, double-blind, parallel-group, dose–response study was conducted at 42 sites in Japan from June 2006 to November 2007 (ClinicalTrials.gov identifier: NCT00345748). The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines, applicable regulatory requirements, and the study protocol. Written informed consent was obtained from all patients.

All patients continued prior MTX therapy (6–8 mg/week) throughout the study. Patients were randomized 1:1:1 to receive 2 mg/kg abatacept, 10 mg/kg abatacept, or placebo. DMARDs other than MTX or biologic therapies at study enrollment were stopped with an appropriate wash out before randomization. Abatacept was intravenously infused in a fixed volume of 100 mL saline or 5 % glucose over 30 min on weeks 0, 2, 4, 8, 12, 16 and 20 of the study. Administration of other DMARDs was prohibited, but stable doses of corticosteroids (≤10 mg/day) or non-steroidal anti-inflammatory drugs were allowed. No change in the dose or mode of administration of MTX was permitted throughout the study, unless safety concerns necessitated dose reduction. Patients who discontinued the study were assessed at an early termination visit.

Evaluation of clinical efficacy

Clinical efficacy was assessed by the ACR response rate criteria at enrollment and at each visit before study drug administration during the double-blind treatment period. Briefly, an ACR20 response requires a 20 % reduction in the number of swollen and tender joints and in three of the following parameters: physician global assessment of disease, patient global assessment of disease, patient assessment of pain, CRP or erythrocyte sedimentation rate (ESR), and degree of disability on the HAQ score. The ACR50 and ACR70 responses are defined as reductions of 50 and 70 %, respectively [24, 25].

Response to treatment was assessed based on DAS28-CRP values. A response was defined as a reduction in DAS28 from week 0 to week 24 of ≥1.2. A DAS28 value of ≤3.2 at week 24 was classified as low disease activity and a DAS28 value of <2.6 was considered to indicate disease remission.

Safety

All adverse events (AEs) that occurred within the dosing period and within 8 weeks after the last dose of study drug were analyzed. All reported AEs and serious AEs (SAEs) were reviewed at each visit.

Immunogenicity evaluation

Immunogenicity of abatacept was assessed by measuring serum anti-abatacept and anti-CTLA4-T antibody titers using enzyme-linked immunosorbent assays. As none of the samples tested showed positive signals for either antibody after the first dose of the study drug, the neutralizing activity of these antibodies was not analyzed.

Statistical analyses

Frequency distribution or descriptive statistics of all demographic variables were summarized according to treatment group. The primary efficacy analysis was designed to test the non-zero slope of the dose–response relationship using the Cochran–Armitage χ^2 trend test for proportions. Differences in ACR20, ACR50, and ACR70 response rates between the abatacept groups and the placebo group were summarized using point estimates and 95 % confidence intervals (CI). For safety evaluation, summary statistics were tabulated, with frequency distribution and individual listing of all AEs generated for each treatment group. Immunogenicity was summarized using descriptive statistics for each group, and the positive immunogenicity response rate was calculated.

Results

Patient disposition is summarized in Fig. 1. Of 195 patients, 62 were randomized to 10 mg/kg abatacept, 67 to 2 mg/kg abatacept, and 66 to placebo. Of these patients, 194 received at least one dose of study medication (61 in the 10 mg/kg abatacept group, 67 in the 2 mg/kg abatacept group, and 66 in the placebo group). One patient in the 10 mg/kg abatacept group withdrew consent and

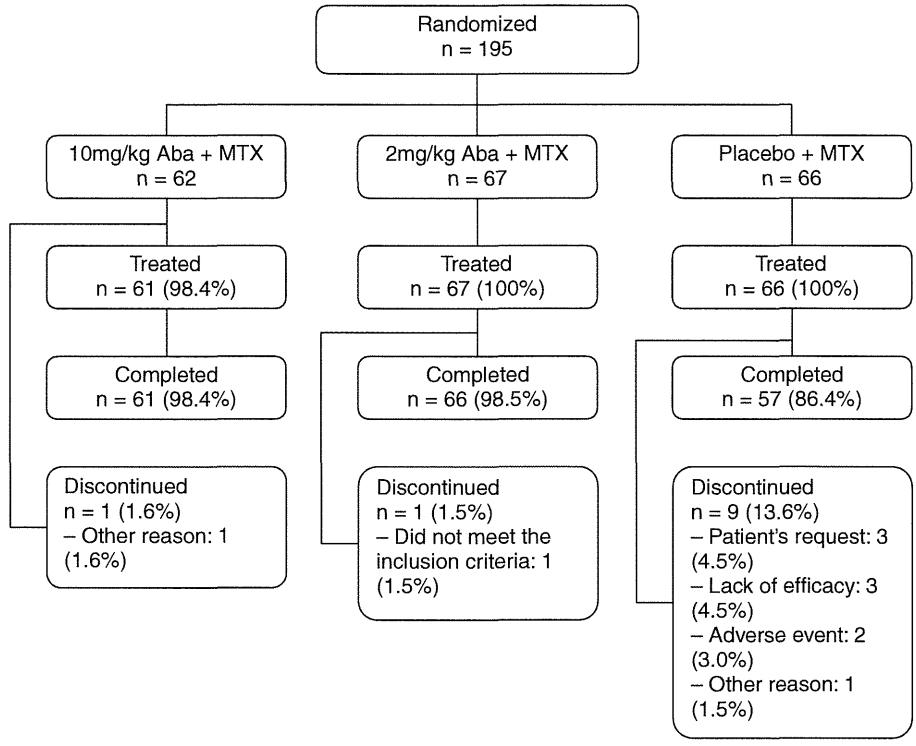

Fig. 1 Patient disposition. *Aba* abatacept, *MTX* methotrexate

discontinued the study before receiving the first dose of study medication. The rate of discontinuation during the 24-week treatment period was higher in the placebo group than in both abatacept groups (placebo 13.6 %, 10 mg/kg abatacept 1.6 % and 2 mg/kg abatacept 1.5 %). The main reasons for discontinuation included lack of efficacy, AEs, and withdrawal of consent. As few doses were missed in each treatment group, this was deemed unlikely to have affected either the administration period or dosage. There were no significant differences between baseline patient demographics, including duration of RA, painful joint count, swollen joint count, physical function, and DAS28-CRP across all three treatment groups. The majority of patients were female (Table 1).

Clinical efficacy

The study met its primary endpoint, with a dose–response relationship evident for the ACR20 response rate in the 10 and 2 mg/kg abatacept groups relative to the placebo group at week 24 (Fig. 2). Analysis using the Cochran-Armitage trend test confirmed that the ACR20 response rates at week 24 were significantly higher in the 10 mg/kg (77.0 %; 47/61 patients) and 2 mg/kg (62.7 %, 42/67 patients) abatacept-treated groups than in the placebo group (21.2 %; 14/66 patients) (Fig. 2). The differences in the ACR20 response rate between the abatacept and placebo groups were 55.8 % (95 % CI 41.4, 70.3) for 10 mg/kg abatacept and 41.5 % (95 % CI 26.3, 56.7) for 2 mg/kg abatacept (Fig. 2).

The Cochran-Armitage trend test also showed that the ACR50 and ACR70 were significantly greater in both abatacept groups compared with the placebo group at week 24 (Fig. 2). The ACR50 response rates at week 24 were 45.9 % (28/61 patients) for 10 mg/kg abatacept, 37.3 % (25/67 patients) for 2 mg/kg abatacept and 6.1 % (4/66 patients) for placebo. The corresponding ACR70 response rates were 21.3 % (13/61 patients), 16.4 % (11/67 patients) and 0 % (0/66 patients). The differences in ACR50 response rates between the abatacept and placebo groups were 39.8 % (95 % CI 26.1, 53.6 %) for 10 mg/kg abatacept and 31.3 % (95 % CI 18.3, 44.2 %) for 2 mg/kg abatacept, while the differences in ACR70 response rates were 21.3 % (95 % CI 11.0, 31.6 %) and 16.4 % (95 % CI 7.5, 25.3 %), respectively (Fig. 2). Both the ACR50 and ACR70 response rates showed a statistically significant dose–response relationship between the treatment groups at week 24, with the greatest efficacy in the 10 mg/kg abatacept group followed by the 2 mg/kg abatacept group, with the lowest response in the placebo group.

Analysis of the ACR response rates over time (with last observation carried forward) showed consistently higher ACR20 response rates in the 10 mg/kg abatacept group compared to the placebo group from week 2 to week 24, with a marked difference (41 %) as early as week 4. The 95 % CI for the difference between the 10 mg/kg abatacept

Table 1 Patient characteristics

	Abatacept (10 mg/kg)	Abatacept (2 mg/kg)	Placebo
Female, *n* (%)	49 (80.3)	57 (85.1)	52 (78.8)
Age (years)	53.4 ± 11.3	52.5 ± 11.1	53.4 ± 12.0
Weight (kg)	53.8 ± 8.0	56.2 ± 10.1	57.7 ± 9.6
Duration of RA, *n* (%)			
≤2 years	12 (19.7)	10 (14.9)	10 (15.2)
>2 to ≤5 years	14 (23.0)	26 (38.8)	18 (27.3)
>5 to ≤10 years	15 (24.6)	14 (20.9)	21 (31.8)
>10 years	20 (32.8)	17 (25.4)	17 (25.8)
Duration of RA (years)	7.4 ± 5.7	8.5 ± 9.0	7.3 ± 6.2
Tender joint count	21.8 ± 9.3	21.0 ± 8.2	21.6 ± 8.2
Swollen joint count	16.6 ± 6.7	17.6 ± 6.5	17.5 ± 6.1
HAQ physical function[a]	1.33 ± 0.59	1.24 ± 0.69	1.50 ± 0.73
CRP (mg/dL)	3.40 ± 2.74	2.98 ± 2.37	3.39 ± 2.28
DAS28-CRP	6.0 ± 0.7	5.8 ± 0.7	6.0 ± 0.7
Biologics-history, *n* (%)			
Prior use of infliximab (recombinant)	9 (14.8)	11 (16.4)	17 (25.8)
Prior use of etanercept (recombinant)	5 (8.2)	5 (7.5)	13 (19.7)
Prior use of adalimumab (recombinant) (study drug)	1 (1.6)	2 (3.0)	5 (7.6)
Prior use of tocilizumab (recombinant)	1 (1.6)	2 (3.0)	2 (3.0)
MTX dose (mg/week)	7.11 ± 1.00	7.11 ± 0.98	7.26 ± 0.96
Other DMARDs-history, *n* (%)			
Prior use of other DMARDs[a]	21 (34.4)	18 (26.9)	15 (22.7)
Concomitant adrenocorticosteroid[a], *n* (%)	47 (77.0)	54 (80.6)	56 (84.8)
Adrenocorticosteroid dose[b] (mg/day)	5.68 ± 2.21	5.81 ± 2.45	5.58 ± 2.47

Values are mean ± standard deviation or *n* (%)

CRP C-reactive protein, *DAS28* Disease Activity Score 28, *HAQ* Health Assessment Questionnaire, *MTX* methotrexate, *RA* rheumatoid arthritis

[a] other DMARDs = Salazosulfapyridine, Bucillamine, Tacrolimus hydrate, Auranofin, D-penicillamine, Gold sodium thiomalate, Mizoribine and Actaritused

[b] Oral adrenocorticosteroids were converted to the equivalent dose of prednisolone

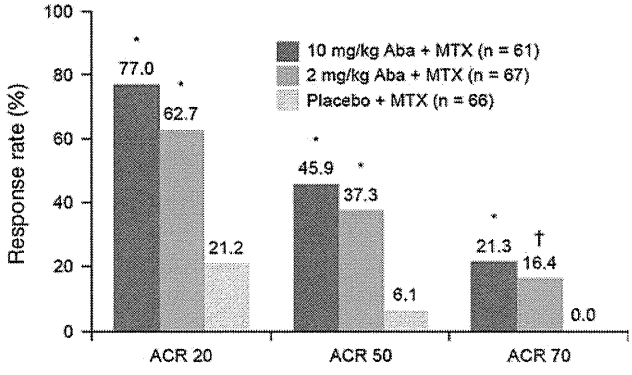

Fig. 2 ACR response rates at week 24. ACR20/50/70, 20, 50, or 70 % improvement from baseline in ACR score. Patients who discontinued treatment because of lack of efficacy were considered ACR non-responders at all subsequent time points. For all patients who discontinued treatment for other reasons, their last ACR response was carried forward. *$p < 0.001$ versus placebo (Cochran-Armitage χ^2 trend test); †$p = 0.002$ versus placebo (χ^2 test with continuous correction). *Aba* abatacept, *ACR* American College of Rheumatology, *MTX* methotrexate

group and the placebo group did not include 0 (Fig. 3a). A difference in ACR50 between the 10 mg/kg abatacept and placebo groups was also observed at week 4, with response rates of 13.1 and 1.5 %, respectively. The 10 mg/kg group showed higher ACR response rates than the placebo group that persisted until week 24 (Fig. 3b). The ACR70 response rate was 11.5 % in the abatacept 10 mg/kg group versus 0 % in the placebo group at week 12, which was maintained from week 12 to week 24 (Fig. 3c).

The 2 mg/kg abatacept group showed a clear improvement in the ACR20 response rate at week 8 compared to the placebo group (52.2 vs. 27.3 %) (Fig. 3a). At week 12, the 2 mg/kg abatacept group showed clear improvements in the ACR50 (23.9 vs. 6.1 %, respectively) and ACR70 (6.0 vs. 0 %, respectively) response rates (Fig. 3b, c) compared to the placebo group.

The DAS28-CRP values at baseline indicated high disease activity, with values of 6.0 ± 0.7, 5.8 ± 0.7, and 6.0 ± 0.7 in the 10 mg/kg abatacept, 2 mg/kg abatacept

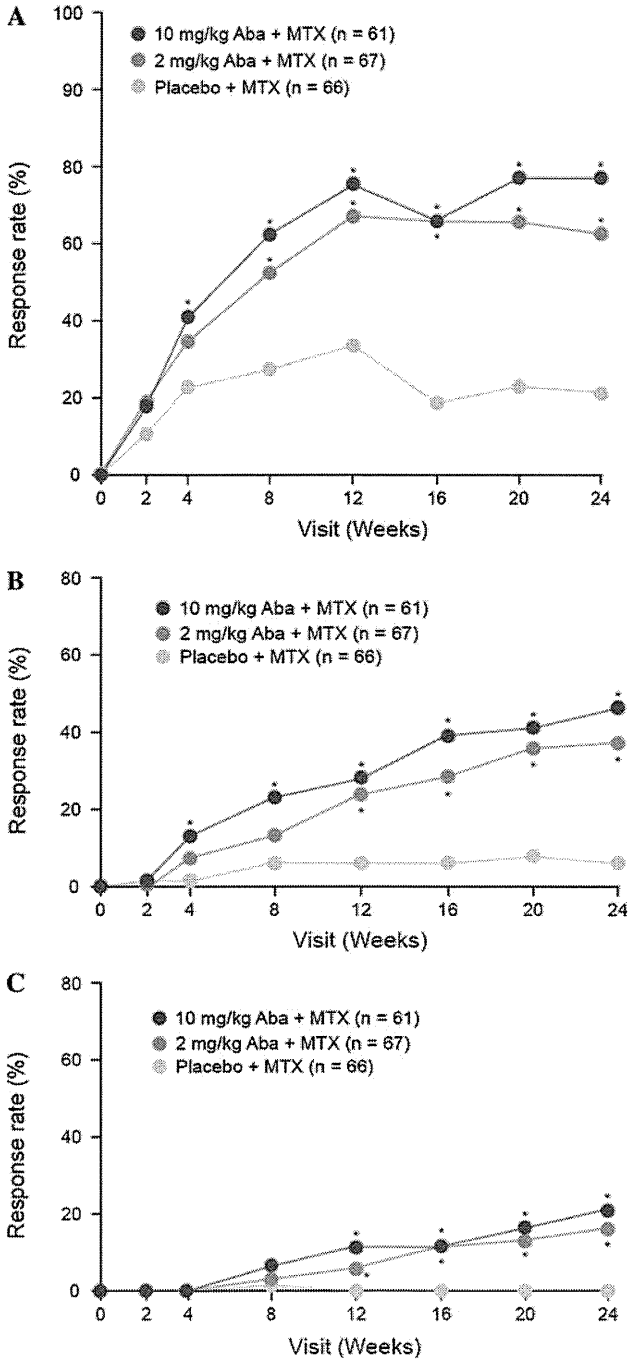

Fig. 3 ACR response rates over time (last observation carried forward). **a** ACR20, **b** ACR50, and **c** ACR70. ACR20/50/70, 20, 50,or 70 % improvement from baseline in ACR score. The 95 % confidence interval versus placebo did not include zero (*asterisk*). *Aba* abatacept, *ACR* American College of Rheumatology, *MTX* methotrexate

and placebo groups, respectively (Table 2). Individual components of the DAS28-CRP, including the number of swollen joints, number of tender joints, patient global assessment and serum CRP concentrations, showed similar trends. At week 24, DAS28-CRP decreased significantly in both abatacept groups compared with the placebo group (3.5 ± 1.3 in the 10 mg/kg abatacept group, 4.0 ± 1.2 in the 2 mg/kg abatacept group, and 5.3 ± 1.2 in the placebo group) (Table 2). The proportion of patients who achieved a response to the study drug, based on a reduction of DAS28-CRP of ≥1.2, by week 24 was 88.5 % (54/61 patients) in the 10 mg/kg abatacept group, 68.7 % (46/67 patients) in the 2 mg/kg abatacept group and 30.3 % (20/66 patients) in the placebo group (Fig. 4a). The proportions of patients with low disease activity (i.e., DAS28-CRP ≤3.2) were 41.0, 25.4, and 7.6 %, respectively, while the proportions of patients with remission (i.e., DAS28-CRP <2.6) were 24.6, 14.9, and 1.5 %, respectively (Fig. 4b). The rates of remission and low disease activity were greatest in the 10 mg/kg abatacept group (Fig. 4b).

The proportion of patients who showed an improvement in daily activities, defined as a reduction in HAQ score of ≥0.3 points, was greater in the 10 mg/kg abatacept group (60.7 %; 37/61 patients) than in the 2 mg/kg abatacept group (49.3 %; 33/67 patients), and the placebo group (24.2 %; 16/66 patients) (Fig. 5).

Safety

All of the patients (n = 194) who received at least one dose of study drug (61 in the 10 mg/kg abatacept group, 67 in the 2 mg/kg abatacept group, and 66 in the placebo group) were included in the safety evaluation.

SAEs were reported in 8.2 % (5/61), 3.0 % (2/67), and 9.1 % (6/66) of patients in the 10 mg/kg abatacept, 2 mg/kg abatacept, and placebo groups, respectively, (Table 3), and study drug-related SAEs were reported in 3.3 % (2/61), 0 % (0/67), and 1.5 % (1/66) of patients, respectively. Regarding SAEs, in the 10 mg/kg abatacept group, pure red cell aplasia, parvovirus infection and upper respiratory tract infection were reported in one patient, while abdominal pain and vomiting in a second. These SAEs resolved without treatment or with appropriate treatment. Discontinuation of the study drug because of AEs or SAEs occurred in the placebo group only. No deaths occurred during the study.

AEs were reported in 72.1 % (44/61), 73.1 % (49/67), and 62.1 % (41/66) of patients in the 10 mg/kg abatacept, 2 mg/kg abatacept and placebo groups, respectively, and study drug-related AEs were reported in 49.2 % (30/61), 59.7 % (40/67), and 34.8 % (23/66) of patients, respectively. The incidences of AEs and study drug-related AEs were similar in both abatacept groups, but were higher these groups compared with the placebo group. The most common AE was nasopharyngitis in each of the three treatment groups (Table 4). Most AEs were mild to moderate in intensity.

Table 2 Disease activity at baseline and at week 24

	Abatacept (10 mg/kg)		Abatacept (2 mg/kg)		Placebo	
	$n = 61$		$n = 67$		$n = 66$	
	Baseline	Week 24	Baseline	Week 24	Baseline	Week 24
Tender joint count	21.8 ± 9.3	8.2 ± 9.5	21.0 ± 8.2	8.8 ± 7.2	21.6 ± 8.2	15.8 ± 12.6
Swollen joint count	16.6 ± 6.7	5.2 ± 4.5	17.6 ± 6.5	6.6 ± 5.5	17.5 ± 6.1	13.7 ± 10.0
Patient global VAS	63.5 ± 20.0	33.4 ± 20.8	59.6 ± 19.5	37.4 ± 22.6	67.2 ± 17.5	54.9 ± 21.2
HAQ physical function	1.4 ± 0.6	0.8 ± 0.6	1.3 ± 0.6	0.9 ± 0.7	1.6 ± 0.7	1.4 ± 0.7
CRP (mg/dL)	3.4 ± 2.7	0.9 ± 1.5	3.0 ± 2.4	1.3 ± 1.4	3.4 ± 2.3	3.4 ± 2.7
DAS28-CRP	6.0 ± 0.7	3.5 ± 1.3	5.8 ± 0.7	4.0 ± 1.2	6.0 ± 0.7	5.3 ± 1.2

Values are mean ± standard deviation

CRP C-reactive protein, *DAS28* Disease Activity Score 28, *HAQ* Health Assessment Questionnaire

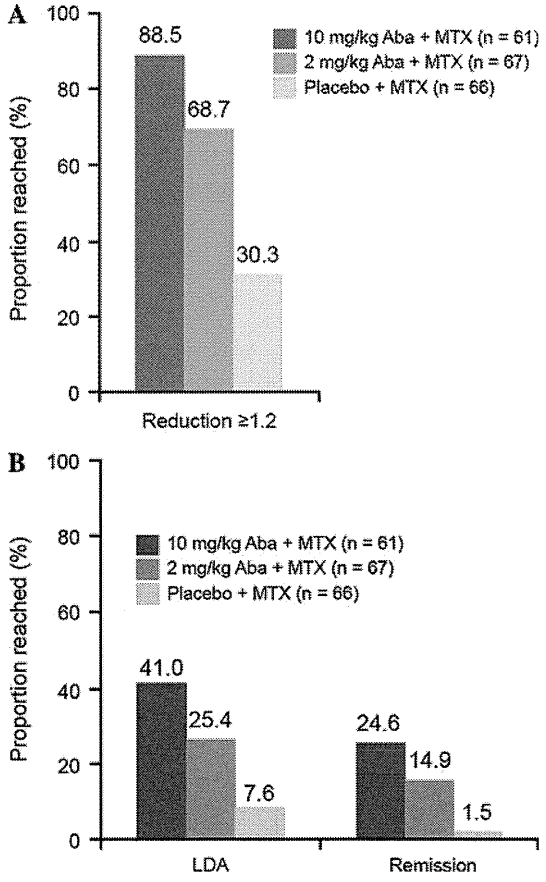

Fig. 4 Efficacy and disease status upon intravenous infusion of abatacept. The proportion of patients who improved based on a reduction of DAS28-CRP of ≥1.2 at week 24 is indicated in (**a**) and the proportion of patients with low disease activity and remission at week 24 are indicated in (**b**). Improved, DAS28-CRP change ≥1.2; LDA, low disease activity; DAS28-CRP ≤3.2; remission, DAS28-CRP <2.6. *Aba* abatacept, *CRP* C-reactive protein, *DAS28* Disease Activity Score 28, *LDAS* low disease activity, *MTX* methotrexate

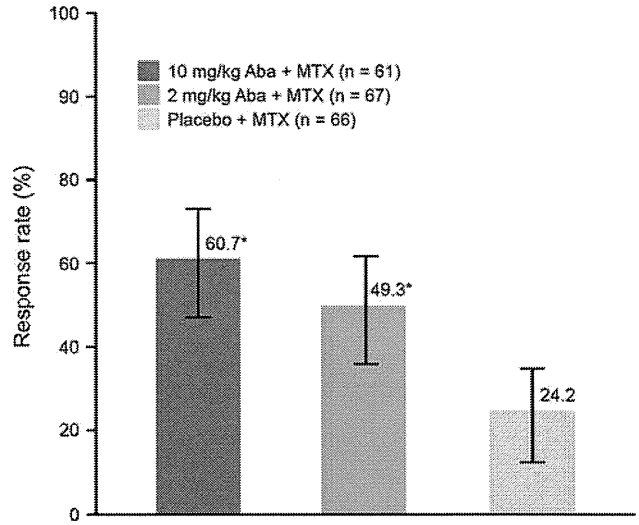

Fig. 5 HAQ response rates at week 24. The 95 % confidence interval versus placebo did not include zero (*asterisk*). *Aba* abatacept, *HAQ* Health Assessment Questionnaire, *MTX* methotrexate

Immunogenicity

The immunogenicity of abatacept was measured in 128 patients who received abatacept (61 in the 10 mg/kg abatacept group and 67 in the 2 mg/kg abatacept). None of these patients developed anti-abatacept or anti-CTLA4-T antibodies following administration of abatacept [26].

Discussion

The introduction of DMARDs and anti-TNF-α and anti-IL-6 agents has substantially revolutionized RA therapy. However, several limitations remain, including secondary failure of these drugs and discontinuation of treatment because of AEs, particularly in patients with RA with an inadequate response to conventional therapy. Abatacept is the first in a new class of RA treatments that selectively modulate the co-stimulatory signal required for full T cell activation. Phase II studies in Western populations have shown that treatment with abatacept is associated with significant reductions in disease activity and improvements

Table 3 Incidence of serious adverse events and adverse events

	Abatacept (10 mg/kg) $n = 61$	Abatacept (2 mg/kg) $n = 67$	Placebo $n = 66$
Deaths	0	0	0
Patients with SAEs	5 (8.2)	2 (3.0)	6 (9.1)
Patients with study drug-related SAEs	2 (3.3)	0	1 (1.5)
Patients who discontinued because of SAEs	0	0	2 (3.0)
Patients who discontinued because of AEs	0	0	2 (3.0)
Patients with AEs	44 (72.1)	49 (73.1)	41 (62.1)
Patients with study drug-related AEs	30 (49.2)	40 (59.7)	23 (34.8)

Values are *n* (%)

AE adverse event, *SAE* serious adverse event

Table 4 Adverse events occurring in ≥5 % of patients in any treatment group

System organ class and preferred term	Abatacept (10 mg/kg) $n = 61$	Abatacept (2 mg/kg) $n = 67$	Placebo $n = 66$
Gastrointestinal disorders	15 (24.6)	15 (22.4)	13 (19.7)
Stomatitis	5 (8.2)	2 (3.0)	3 (4.5)
Constipation	1 (1.6)	1 (1.5)	4 (6.1)
Infections and infestations	20 (32.8)	28 (41.8)	16 (24.2)
Nasopharyngitis	13 (21.3)	18 (26.9)	8 (12.1)
Cystitis	0	4 (6.0)	0
Investigations	7 (11.5)	7 (10.4)	5 (7.6)
Blood pressure increased	2 (3.3)	5 (7.5)	1 (1.5)
Nervous system disorders	5 (8.2)	8 (11.9)	6 (9.1)
Headaches	2 (3.3)	4 (6.0)	3 (4.5)
Respiratory, thoracic, and mediastinal disorders	7 (11.5)	8 (11.9)	8 (12.1)
Upper respiratory tract inflammation	5 (8.2)	3 (4.5)	3 (4.5)

in physical function over the course of 12 months in patients with active RA despite MTX treatment [17]. The efficacy and dose response, based on ACR20 response rates, and the safety of abatacept in the present study were similar to those reported in Western patients [10], suggesting that the results of global Phase III studies of abatacept [14, 18, 21] can be extrapolated to Japanese patients.

This study showed that the efficacy of 10 mg/kg abatacept was significantly greater than that of placebo in Japanese patients with active RA despite MTX therapy, based on the differences in ACR20, ACR50, and ACR70 response rates. These results in Japanese patients differ from those of the global Phase II study [10]. At week 24, the ACR20 response rates in the global Phase II study were 60.0, 41.9, and 35.3 % in the 10 mg/kg abatacept, 2 mg/kg abatacept, and placebo groups, respectively [10], compared to 77.0, 62.7, and 21.2 %, respectively, in the present study.

The high rate of response to 2 mg/kg abatacept among Japanese patients may be due to differences in baseline characteristics between patients in the global Phase II study [10] and the Japanese patients in our study. The Japanese patients enrolled in our study had a shorter duration of disease compared to those in the global study (mean duration 7.3–8.5 vs. 8.9–9.7 years, respectively), and fewer tender and swollen joints (mean number of tender joints 21.0–21.8 vs. 28.2–30.8, respectively; mean number of swollen joints 16.6–17.6 vs. 20.2–21.8, respectively). In addition, the patients in our study were treated with a lower dose of MTX than were patients in the global study (mean dose 7.1–7.3 mg/week vs. 15.0–15.8 mg/week, respectively) but had a higher mean CRP concentration (mean concentration 3.0–3.4 vs. 2.9–3.2 mg/dL, respectively).

Although the 2 mg/kg abatacept dose achieved high ACR response rates, 10 mg/kg abatacept had more rapid effects, with significant improvements in ACR20 and ACR50 response rates compared with placebo at week 4 in the 10 mg/kg group versus weeks 8 and 12, respectively, in the 2 mg/kg abatacept group. Based on these data, the 10 mg/kg dose was identified as the optimal dosage to rapidly achieve remission in Japanese patients.

Changes in disease activity were also assessed using the DAS28-CRP, which has been used in several pivotal studies [14, 18]. Generally, the European League Against Rheumatism (EULAR) response rates were greater when assessed using the DAS28-CRP than with the DAS28-ESR. A retrospective clinical study of infliximab identified a new

threshold for the definition of high and low disease activity states [27]. Both the DAS28-CRP and DAS28-ESR were shown to be valid and comparable measures of disease activity in patients with RA treated with abatacept [28]. In the present study, 24.6 % of patients treated with 10 mg/kg abatacept achieved remission, defined as DAS28-CRP <2.6, by week 24.

Abatacept demonstrated a good risk-to-benefit profile in the present Japanese patients with active RA; it was generally well tolerated, and the most common AEs, such as nasopharyngitis and upper respiratory tract inflammation, were similar to those reported with other biological agents [29–32]. Of note, no tuberculosis or infusion reactions were observed in this study. These findings are supported by the results of other studies in different patient populations, which have also shown abatacept to be well tolerated and to have a well-characterized safety profile [10, 13, 19]. The lack of immunogenicity observed in patients treated with abatacept in this study suggests that the development of resistance to this treatment is unlikely. Further studies, including post-marketing surveillance studies, are required to further evaluate the safety of abatacept.

The findings of this Phase II bridging study, and those of previous studies, support the role of T cell activation in RA and confirm the validity of inhibiting T cell activation as a therapeutic target in this disease.

RA is a major cause of chronic inflammation in patients worldwide and has a complex etiology, which includes both environmental and genetic factors. Several genes that confer susceptibility for the development of RA have been identified; some of these interact with environmental factors, while others are restricted to particular populations. Furthermore, some of the genes present in particular ethnic groups are present in Asian and European populations [33, 34]. Here, we demonstrated that abatacept was effective in Japanese patients, with outcomes equivalent to those seen in global studies, which included European patients.

In conclusion, abatacept demonstrated good efficacy at the 10 mg/kg dose compared with placebo, and was well tolerated with a good benefit-to-risk profile in Japanese patients with active RA despite MTX therapy. These findings indicate that 10 mg/kg is an appropriate clinical dose and is expected to be clinically useful in Japanese patients with active RA. Taken together, abatacept is suitable for the treatment of patients with active RA despite MTX therapy, regardless of ethnicity.

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Conflict of interest TT has received lecture fees from Abbott, Astellas Pharma, Brystol-Meyers, Chugai Pharma, Eisai Pharma, Mitsubishi-Tanabe Pharma, Pfizer, Takeda Pharmaceutical. AY is employee of Bristol-Myers K.K. NM has received research grants, consultant fees, and/or speakers' bureau honoraria from Chugai Pharmaceutical Co., Tanabe-Mitsubishi Pharmaceutical Co., Takeda Pharmaceutical Co., Pfizer Japan, Abbott Japan, Eisai Pharmaceutical Co., Astellas Pharmaceutical Co., and Bristol-Myers Squibb.

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