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

# Concomitant iguratimod therapy in patients with active rheumatoid arthritis despite stable doses of methotrexate: a randomized, double-blind, placebo-controlled trial

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**Abstract**
*Objectives* To investigate the efficacy and safety of iguratimod (T-614) in Japanese patients with active rheumatoid arthritis who had inadequate response to stable background methotrexate (MTX) alone.
*Methods* In this multicenter, double-blind, controlled trial, a total of 253 patients were randomized at 2:1 ratio to either the iguratimod group or the placebo group. Iguratimod was orally administered at dosages of 25 mg/day for the first 4 weeks (25 mg once daily) and 50 mg/day for the subsequent 20 weeks (25 mg twice daily). MTX at dosage of 6 or 8 mg/week was administered to patients in both groups.
*Results* The rate of 20 % improvement in American College of Rheumatology criteria (ACR20) at week 24 was 69.5 % in the iguratimod group compared with 30.7 % in the placebo group ($P < 0.001$). Significant improvements in the ACR50, ACR70, Health Assessment Questionnaire Disability Index, Disease Activity Score 28 <3.2, and rheumatoid factor were also observed. The most commonly reported adverse events (AEs) were blood iron decrease, nasopharyngitis, and lymphocyte decrease. These AEs were mild or moderate in severity. No deaths occurred.
*Conclusion* The study results suggest that iguratimod in combination with MTX was efficacious and had a manageable safety profile.

**Keywords** Disease-modifying antirheumatic drug · Iguratimod · Methotrexate · Rheumatoid arthritis · T-614

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## Introduction

Rheumatoid arthritis (RA) is a systemic inflammatory arthritis that can result in permanent joint damage, and is associated with high morbidity and mortality. Methotrexate (MTX), one of the disease-modifying antirheumatic drugs (DMARDs), has been most selected for initial RA treatment because of its efficacy, acceptable safety profile, and low cost. Recently, combination therapies of MTX with either biological agents such as infliximab [1] and adalimumab [2] or other small-molecule antirheumatic drugs such as salazosulfapyridine [3], leflunomide [4], and bucillamine [5] have been reported to have greater efficacy than MTX alone. The American College of Rheumatology (ACR) 2008 guideline recommends use of nonbiologic and biologic DMARDs based on RA disease duration, RA disease activity, prognostic factors for RA, and previous experience of DMARDs, including failure of prior MTX monotherapy [6]. Currently, treatment choices are dominated by patient and physician preferences, side-effects, and costs [7]. Because a patient's response to available medications shows variability in efficacy, toxicity, and unpredicted necessity of discontinuation, combination therapy of a new small-molecule antirheumatic drug with MTX is needed to provide more options, especially in terms of switching medications and lowering treatment costs compared with biological agents.

Iguratimod (T-614) is a small-molecule antirheumatic drug for which the rate of 20 % improvement in ACR criteria (ACR20) was not inferior to that of salazosulfapyridine in Japanese patients with active RA (63.1 % for iguratimod versus 57.7 % for salazosulfapyridine) [8]. Iguratimod suppressed tumor necrosis factor-alpha-induced production of interleukin (IL)-6, IL-8, and monocyte chemoattractant protein 1 via inhibition of nuclear factor-kappa B activation in cultured human synovial cells and human acute monocytic leukemia cells [9–11]. Iguratimod also reduced immunoglobulin (Ig) production by acting directly on human B lymphocytes without affecting B lymphocyte proliferation [12]. In a clinical trial, iguratimod significantly decreased rheumatoid factor and the production of IgG, IgM, and IgA compared with placebo in patients with active RA [8]. Thus, iguratimod has been suggested to be a clinically useful DMARD with unique mechanisms of action [8, 13, 14]. Recently, an increased release of extracellular adenosine and a decreased production of lymphotoxins such as ammonia and superoxide have been shown to be involved in the anti-inflammatory mechanisms of MTX [15–17]. Thus, the combination of MTX and iguratimod may have synergic efficacy for RA treatment, but the efficacy of the combination therapy of iguratimod with MTX in patients with RA has not been reported. This randomized, double-blind trial compared iguratimod + MTX treatment with placebo + MTX treatment in patients who had inadequate response to MTX and evaluated efficacy using the ACR20, ACR50, ACR70, Health Assessment Questionnaire Disability Index (HAQ-DI), Disease Activity Score using 28 joint counts (DAS-28), and rheumatoid factor.

## Patients and methods

### Patients

Patients who gave written informed consent were enrolled in this study. Eligible patients had a diagnosis of active RA for less than 10 years based on ACR criteria [18]. They were aged 20 to <70 years and had active RA despite MTX therapy (≥6 mg/week) for more than 12 weeks, including stable low dosages of MTX (6–8 mg/week) for at least 8 weeks before study enrollment. Eligible patients also fulfilled the following criteria: at least 6 tender joints (excluding distal interphalangeal joints), at least 4 swollen joints (excluding distal interphalangeal joints), and an erythrocyte sedimentation rate (ESR) of at least 28 mm/h or a blood C-reactive protein (CRP) concentration of at least 1.0 mg/dL. A 4-week washout period before initiation of study treatment was established for previous DMARDs (except for MTX) or immunosuppressive drugs. A 3-month washout period was established for biological antirheumatic agents and a 6-month washout period for leflunomide and other RA clinical trial drugs. Concomitant use of nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids at prednisolone-equivalent doses of 7.5 mg or less was permitted throughout the study if patients had been taking these medications at stable doses for at least 4 weeks before study drug administration.

Exclusion criteria were as follows: impaired hepatic function as shown by abnormal results on liver function tests [i.e., elevation of aspartate aminotransferase (AST) or alanine aminotransferase (ALT) levels above the upper limit of normal], known hematopoietic disorder (absolute leukocyte count <4000/μL, platelet count <100,000/μL, hemoglobin level <9.0 g/dL), positive results on serologic tests for hepatitis B or C, pregnancy or breast feeding, history of drug or alcohol abuse, persistent or severe infection, active digestive diseases, previous treatment with iguratimod, body weight <40 kg, and RA with Steinbrocker's class IV.

### Study design

The study was conducted in 99 medical institutions in Japan between August 2009 and February 2011. The study drugs were provided by the study sponsors (Toyama

Chemical and Eisai, Tokyo, Japan). An independent efficacy and safety evaluation committee was organized to discuss study protocol amendments and premature termination of the study. The study was conducted in compliance with the Declaration of Helsinki. The institutional review board at each institute approved the study protocol. This study was registered at http://clinicaltrials.gov (NCT00965757).

The study consisted of a 4-week observation period and a 24-week double-blind treatment period. Eligible patients were separated into either the iguratimod group (iguratimod + MTX) or the placebo group (placebo + MTX) in 2:1 randomization. Iguratimod was orally administered at dosages of 25 mg/day for the first 4 weeks (25 mg once daily) and 50 mg/day for the subsequent 20 weeks (25 mg twice daily). MTX at low dosages of 6 or 8 mg/week and folic acid at dosage of 5 mg/week were administered to patients in both groups for the treatment period.

### Measurement of efficacy and safety

The primary efficacy endpoint was the rate at which patients (the full analysis set) achieved ACR20 at week 24 or last observation carried forward (LOCF) [19]. Clinical improvement was assessed by 20 % improvement in 68 tender joint counts and 66 swollen joint counts, and 20 % improvement in three of the following five criteria: patient's assessment of pain intensity on a visual analog scale (VAS, 0–100 mm), patient's global assessment of disease activity on a VAS (0–100 mm), physician's global assessment of disease activity on a VAS (0–100 mm), HAQ-DI [20], and CRP, or ESR. Secondary endpoints included the ACR50, ACR70, ACR components, DAS28-CRP [21, 22], and HAQ-DI. A decrease in HAQ-DI scores shows improvement, and a decrease greater than 0.22 represents the minimum clinically important difference [23]. The state of disease activity was evaluated based on DAS28 score as remission (<2.6), low disease activity (<3.2), moderate disease activity (≥3.2 and ≤5.1), or high disease activity (>5.1) [22, 24]. These evaluations were undertaken at 8-week intervals.

Safety was evaluated by adverse event reports, laboratory assays for changes in hematologic characteristics, blood chemistry, urinalysis, and liver function, and physical examinations. These evaluations were undertaken during the observation period and at each visit in the treatment period (0, 2, 4, 6, 8, 10, 12, 16, 20, and 24 weeks after start of treatment).

### Statistical analysis

Assuming ACR20 response rates of 50 % in the iguratimod group and 25 % in the placebo group, a sample size of 128 patients and 64 patients (randomization ratio of 2:1), respectively, was estimated to be necessary to demonstrate a 25 % difference in ACR20 response rates with 90 % power for Fisher's exact test and an alpha of 0.05. Taking potential dropouts into consideration, a total of 240 subjects (160 patients in the iguratimod group and 80 patients in the placebo group) was estimated to be necessary.

Demographic and baseline characteristics were compared between the groups using the *t* test for continuous variables and Fisher's exact test for categorical variables.

All efficacy analyses were primarily performed on the full analysis set, defined as all randomized patients who received at least one dose of study drug and from whom at least one assessment of efficacy under double-blind medication was available. The primary efficacy endpoint of ACR20 at week 24 (LOCF) was compared between the iguratimod group and the placebo group using the Fisher's exact test. For ACR20, results on the per protocol set were also presented. Changes from baseline in individual ACR core components, immunological test values, HAQ-DI, and DAS28 were presented as summary statistics for each group, and intragroup comparison was made using the paired *t* test and intergroup comparison was made using the *t* test.

All safety analyses were performed on the safety analysis set, defined as all randomized patients who received at least one dose of study drug and from whom at least one assessment of safety under double-blind medication was available. The incidence of adverse events was calculated, and the two groups were compared using Fisher's exact test.

Significance levels in the tests were as follows: two-sided 15 % for uniformity between groups and two-sided 5 % for intergroup and intragroup comparisons.

## Results

### Patient characteristics

A total of 389 patients were assessed for eligibility. Among these patients, 253 eligible patients were randomly assigned to the iguratimod group ($n = 165$) and the placebo group ($n = 88$) in 2:1 ratio (Fig. 1). One patient in the iguratimod group was excluded from the safety analysis set and full analysis set because data on efficacy and safety were not available. A total of 34 patients (20 patients in the iguratimod group and 14 patients in the placebo group) were excluded from the per protocol set due to protocol violation, eligibility violation, or/and early discontinuation of medication (less than 16 weeks or less than 8 weeks due to aggravation of symptoms) (Fig. 1).

The percentage of patients who did not complete the 24-week treatment was 10.3 % in the iguratimod group and

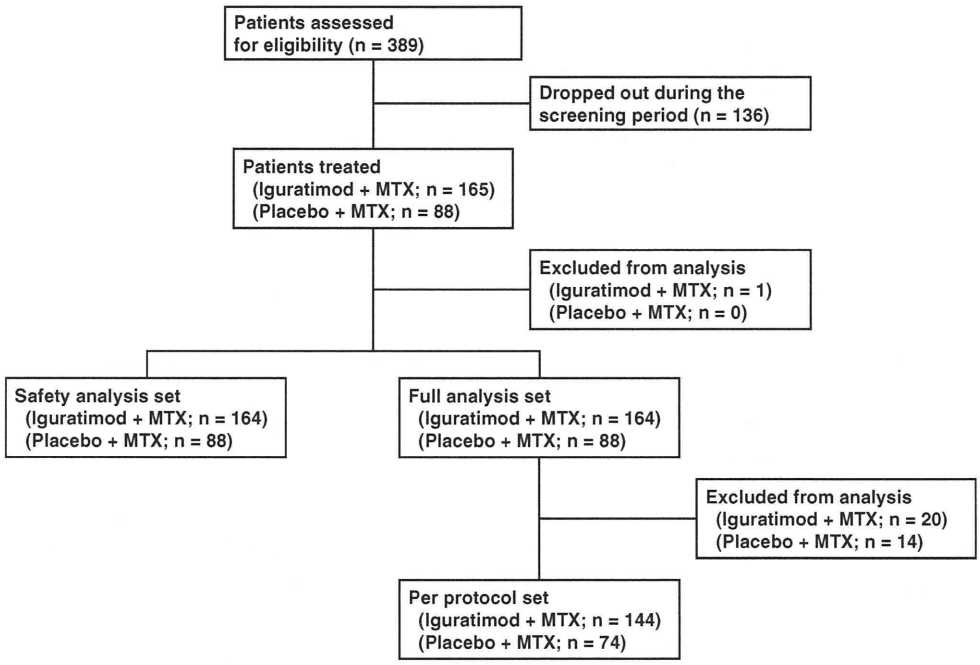


**Fig. 1** Randomization protocol and patient disposition. Eligible patients were allocated to either the iguratimod group (iguratimod + MTX) or the placebo group (placebo + MTX) in 2:1 ratio. *MTX* methotrexate

**Table 1** Demographics at baseline of patients with active rheumatoid arthritis (full analysis set)

| | Iguratimod + MTX ($n$ = 164) | Placebo + MTX ($n$ = 88) |
|---|---|---|
| Female, $n$ (%) | 134 (81.7) | 70 (79.5) |
| Age (SD), years | 54.8 ± 9.9 | 53.5 ± 10.0 |
| Age ≥65 years, $n$ (%) | 32 (19.5) | 16 (18.2) |
| Duration of RA (SD), months | 53.8 ± 35.0 | 50.3 ± 34.0 |
| Positive for rheumatoid factor, $n$ (%) | 128 (78.0) | 67 (76.1) |
| Positive for anti-CCP antibodies, $n$ (%) | 144 (87.8) | 78 (88.6) |
| Previous therapy with DMARDs except for MTX, $n$ (%) | 61 (37.2) | 28 (31.8) |
| Concomitant medication, $n$ (%) | | |
| NSAIDs | 151 (92.1) | 81 (92.0) |
| Corticosteroids | 86 (52.4) | 48 (54.5) |
| Folic acid | 164 (100) | 88 (100) |
| MTX at baseline, $n$ (%) | | |
| 6 mg/week | 51 (31.1) | 27 (30.7) |
| 8 mg/week | 113 (68.9) | 61 (69.3) |

Values are number of patients (%) or mean ± SD

*RA* rheumatoid arthritis, *CCP* cyclic citrullinated peptides, *DMARDs* disease-modifying antirheumatic drugs, *NSAIDs* nonsteroidal anti-inflammatory drugs, *MTX* methotrexate

20.5 % in the placebo group; 4.2 % of patients in the iguratimod group and 12.5 % in the placebo group discontinued due to aggravation of symptoms, and 4.2 % in the iguratimod group and 3.4 % in the placebo group discontinued due to adverse events.

Table 1 presents baseline demographics, and Table 2 presents baseline clinical characteristics of patients in the safety analysis set and the full analysis set. There were no statistically significant differences between groups at baseline ($P < 0.15$). In each group, 92 % of patients were treated with NSAIDs. Only one patient in each group had been previously treated with biologic DMARDs. A total of 37.2 and 31.8 % of patients in the iguratimod and placebo groups, respectively, had history of treatment with nonbiologic DMARDs (except for MTX) and/or immunosuppressants.

## ACR response rates

The ACR20 response rate, which was the primary efficacy endpoint in this study, was significantly improved by 24-week (LOCF) treatment with iguratimod compared with placebo: 69.5 % (iguratimod) versus 30.7 % (placebo) in the full analysis set ($P < 0.001$). Similarly, the secondary efficacy endpoints, ACR50 and ACR70, at week 24 in the iguratimod group were significantly greater than those of the placebo group: 38.4 versus 15.9 % for ACR50 ($P < 0.001$) and 17.1 versus 5.7 % for ACR70 ($P = 0.010$), respectively. Figure 2 shows the ACR20 response rate as a function of treatment period, indicating that a significant improvement in ACR20 was also achieved by 8- and 16-week treatments with iguratimod compared with placebo. The data in Fig. 2 were based on observed cases. When ACR20 was analyzed based on the per protocol set,

**Table 2** Mean changes in secondary variables from baseline to week 24 (LOCF) (full analysis set)

| | Iguratimod + MTX ($n = 164$) | Placebo + MTX ($n = 88$) | $P$ value[a] |
|---|---|---|---|
| Tender joint count ($n$) | | | |
| Baseline | 12.5 ± 6.5 | 13.3 ± 8.1 | |
| Change from baseline | −7.4 ± 6.0 | −4.6 ± 7.8 | 0.001 |
| Swollen joint count ($n$) | | | |
| Baseline | 11.5 ± 6.3 | 11.1 ± 5.7 | |
| Change from baseline | −6.5 ± 5.9 | −2.9 ± 6.7 | <0.001 |
| Patient's assessment of pain (mm) | | | |
| Baseline | 47.5 ± 22.2 | 46.4 ± 23.1 | |
| Change from baseline | −22.0 ± 23.8 | −2.5 ± 27.0 | <0.001 |
| Patient's global assessment of disease activity (mm) | | | |
| Baseline | 47.7 ± 24.3 | 50.1 ± 23.5 | |
| Change from baseline | −21.2 ± 26.4 | −5.0 ± 27.1 | <0.001 |
| Physician's global assessment of disease activity (mm) | | | |
| Baseline | 52.6 ± 18.3 | 53.2 ± 19.0 | |
| Change from baseline | −27.1 ± 19.3 | −10.4 ± 26.6 | <0.001 |
| HAQ-DI | | | |
| Baseline | 0.82 ± 0.55 | 0.73 ± 0.51 | |
| Change from baseline | −0.35 ± 0.45 | 0.03 ± 0.55 | <0.001 |
| C-reactive protein level (mg/dL) | | | |
| Baseline | 1.84 ± 1.94 | 1.71 ± 1.58 | |
| Change from baseline | −0.53 ± 2.07 | 0.47 ± 2.03 | <0.001 |
| Erythrocyte sedimentation rate (mm/h) | | | |
| Baseline | 45.6 ± 21.0[b] | 41.8 ± 22.5 | |
| Change from baseline | −9.3 ± 20.8[b] | 2.6 ± 19.7 | <0.001 |
| Rheumatoid factor (U/mL) | | | |
| Baseline | 117.1 ± 181.9 | 147.9 ± 279.1 | |
| Change from baseline | −37.4 ± 63.0 | 31.7 ± 190.9 | <0.001[c] |
| IgG (mg/dL) | | | |
| Baseline | 1535 ± 377 | 1517 ± 350 | |
| Change from baseline | −152 ± 190 | 15 ± 151 | <0.001[c] |
| IgM (mg/dL) | | | |
| Baseline | 129 ± 154 | 124 ± 63 | |
| Change from baseline | −15 ± 25 | 5 ± 22 | <0.001[c] |
| IgA (mg/dL) | | | |
| Baseline | 311 ± 123 | 307 ± 109 | |
| Change from baseline | −42 ± 41 | −2.3 ± 38 | <0.001[c] |
| DAS28-CRP | | | |
| Baseline | 4.87 ± 0.89 | 4.97 ± 0.86 | |
| Change from baseline | −1.51 ± 1.22 | −0.66 ± 1.28 | <0.001 |
| HAQ-DI responders (>0.22), $n$ (%) | 104 (63.4) | 32 (36.4) | <0.001 |

Values are the mean ± SD

[a] Intergroup comparisons between the changes were made by $t$ test unless indicated

[b] $n = 163$

[c] These intergroup comparisons were made by Wilcoxon rank-sum test. *HAQ-DI* Health Assessment Questionnaire Disability Index, *DAS28* Disease Activity Score using 28 joint counts, *CRP* C-reactive protein level

ACR20 in the iguratimod group (71.5 %) was significantly greater than that in the placebo group (35.1 %) ($P < 0.001$).

The ACR20 response rate in the full analysis set at week 24 in the iguratimod group did not depend on the duration of RA disease; 71.1 % (32 of 45 patients) for <2 years, 64.0 % (32/50) for 2–5 years, and 72.5 % (50/69) for 5–10 years showed an ACR20 response. Corresponding values in the placebo group were 29.2 % (7/24), 34.5 % (10/29), and 28.6 % (10/35), respectively. Furthermore, ACR20 at week 24 in the iguratimod group was not significantly affected by the presence or absence of history of treatment with DMARDs (except for MTX) and/or immunosuppressants; 65.6 and 71.8 % showed an ACR20 response in the presence and absence of this previous history, respectively ($P = 0.483$). Corresponding values in the placebo group were 21.4 and 35.0 %, respectively.

### Changes from baseline in individual ACR core components, immunological test values, HAQ-DI, and DAS28

Changes from baseline in secondary variables at week 24 (LOCF) are presented in Table 2. In the iguratimod group, tender joint count, swollen joint count, patient's assessment of pain, patient's global assessment of disease activity, physician's global assessment of disease activity, CRP level, and ESR rate at week 24 significantly improved compared with baseline (all values of $P \leq 0.001$; intragroup paired $t$ test comparisons). In the placebo group, tender joint count, swollen joint count, and physician's global assessment of disease activity at week 24 significantly improved compared with baseline, but no significant

improvements were found in patient's assessment of pain, patient's global assessment of disease activity, and ESR rate. Furthermore, in the placebo group, a significant worsening in CRP level was found ($P = 0.032$; intragroup comparison).

Changes at week 24 from baseline in tender joint count, swollen joint count, patient's assessment of pain, patient's global assessment of disease activity, physician's global assessment of disease activity, CRP level, and ESR in the iguratimod group were significantly greater than those in the placebo group (all values of $P \leq 0.001$).

The rheumatoid factor at week 24 in the iguratimod group significantly improved compared with baseline ($P < 0.001$; intragroup comparison), but that in the placebo group worsened, although not significantly ($P = 0.652$; intragroup comparison). Total amounts of serum IgG, IgM, and IgA at week 24 in the iguratimod group significantly decreased compared with baseline (all values of $P < 0.001$; intragroup comparison), but those at week 24 in the placebo group did not significantly change compared with baseline. The change of rheumatoid factor at week 24 from baseline in the iguratimod group was significantly greater than that in the placebo group ($P < 0.001$) (Table 2). Similarly, changes in the total amounts of IgG, IgM, and IgA in the iguratimod group were also significantly greater than those in the placebo group, respectively (all values of $P < 0.001$) (Table 2).

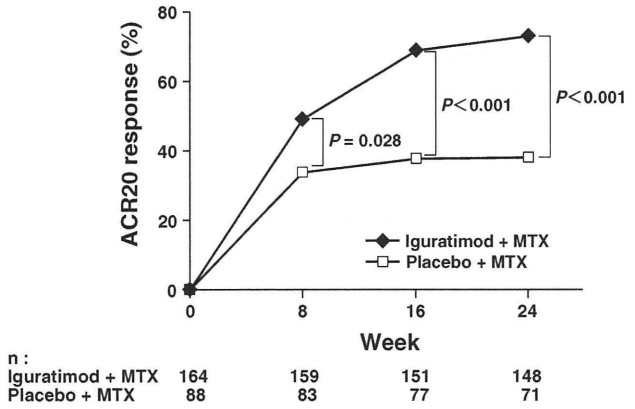


**Fig. 2** Rate of response for patients who achieved 20 % improvement in American College of Rheumatology rheumatoid arthritis criteria (ACR20) as a function of treatment period in iguratimod and placebo groups. Data are based on observed cases

Physical function as measured by HAQ-DI at week 24 significantly improved compared with baseline in the iguratimod group ($P < 0.001$; intragroup comparison), but almost no change was found in the placebo group. The mean change in HAQ-DI of −0.35 in the iguratimod group at week 24 from baseline was significantly different from that of 0.03 in the placebo group ($P < 0.001$). Significantly more patients in the iguratimod group achieved a minimum clinically important difference (−0.22) in HAQ-DI at week 24 (LOCF) compared with patients in the placebo group (63.4 versus 36.4 %, respectively; $P < 0.001$).

Mean disease activity score (DAS28-CRP) in the iguratimod group in the full analysis set at week 24 was 3.37 ± 1.18, which was significantly lower than the score of 4.31 ± 1.31 in the placebo group ($P < 0.001$). Significantly more patients in the iguratimod group showed remission (DAS28-CRP <2.6) at week 24 (LOCF) compared with the placebo group (27.4 versus 9.1 %, respectively; $P < 0.001$). Significantly more patients in the iguratimod group showed low disease activity (DAS28-CRP <3.2) compared with patients in the placebo group (47.6 versus 20.5 %, respectively; $P < 0.001$) (Fig. 3).

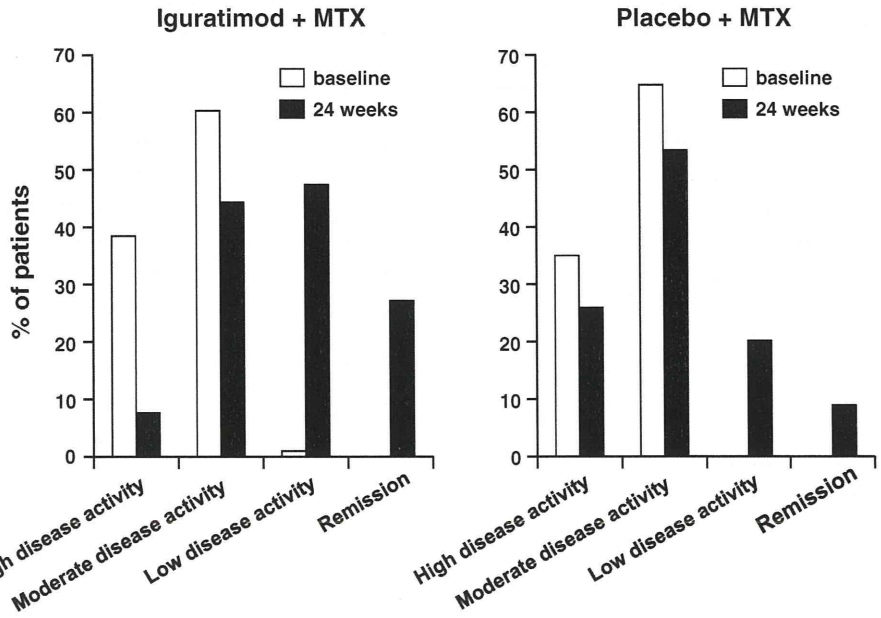


**Fig. 3** Percentage of patients in remission with low, moderate, and high RA disease activities at baseline and at the end of 24-week treatment (LOCF) (full analysis set). The measure of the disease activity is DAS28-CRP; remission (DAS28 <2.6), low disease activity (<3.2), moderate disease activity (≥3.2 and ≤5.1), and high disease activity (>5.1). *DAS28* Disease Activity Score using 28 joint counts, *CRP* C-reactive protein

### Safety

Adverse events (AEs) were reported in 80.5 % of patients in the iguratimod group and 75.0 % in the placebo group, with no significant differences between groups. AEs in older patients (≥65 years) occurred in 96.9 % of patients in the iguratimod group and 81.3 % of patients in the placebo group, and AEs in younger patients (<65 years) occurred in 76.5 and 73.6 % of patients, respectively. Table 3 presents the AEs occurring in ≥5 % of patients. No statistically significant difference was seen in incidence for any of the AEs listed in Table 3 between groups. The most commonly reported AEs coded by the Medical Dictionary for Regulatory Activities-preferred term were blood iron decreased, nasopharyngitis, and lymphocyte count decreased in both the iguratimod and placebo groups. These AEs were mild or moderate in severity. Seven patients in the iguratimod group discontinued due to AEs: cell marker (KL-6) increased, interstitial lung disease, stomatitis, white blood cell count decreased, back pain, diarrhea/flank pain, and anemia/white blood cell count decreased/red blood cell count decreased/hemoglobin decreased/hematocrit decreased. Three patients in the placebo group discontinued due to AEs: synovial rupture, cardiac failure, and joint sprain. Serious AEs were reported by 5 patients in the iguratimod group (gastroduodenal ulcer, tendon rupture, carbon monoxide poisoning, interstitial lung disease, and retinal hemorrhage) and 3 patients in the placebo group (synovial rupture, fallopian tube cancer, and cardiac failure). No deaths were reported.

Increases in ALT and AST levels were reported in both the iguratimod and placebo groups (Table 3). ALT or AST levels more than 100 U/L were observed in 3 patients in the iguratimod group (1.8 %) and in 2 patients in the placebo group (2.3 %). No patients discontinued study treatment due to increases in ALT and AST levels. One patient each in the iguratimod group showed a notable decrease in leukocyte (<2.0 × $10^3$/μL) and erythrocyte (<2.5 × $10^6$/μL) counts; these abnormal laboratory findings resolved after the patients stopped study treatment. No notable trends in blood pressure compared with baseline were observed in any group.

**Table 3** Adverse events occurring in ≥5 % of patients

| | Iguratimod + MTX ($n$ = 164) | Placebo + MTX ($n$ = 88) |
|---|---|---|
| Nasopharyngitis | 28 (17.1) | 14 (15.9) |
| Pharyngitis | 7 (4.3) | 6 (6.8) |
| Upper respiratory tract inflammation | 9 (5.5) | 2 (2.3) |
| Stomatitis | 11 (6.7) | 2 (2.3) |
| Lymphocyte count decreased | 23 (14.0) | 8 (9.1) |
| AST increased | 16 (9.8) | 5 (5.7) |
| ALT increased | 9 (5.5) | 7 (8.0) |
| β2-Microglobulin increased | 13 (7.9) | 2 (2.3) |
| β2-Microglobulin urine increased | 11 (6.7) | 1 (1.1) |
| Blood iron decreased | 35 (21.3) | 16 (18.2) |

Values are the number of patients (%)

*AST* aspartate aminotransferase, *ALT* alanine aminotransferase

## Discussion

This study is the first to demonstrate that the combination of two small-molecule RA agents, iguratimod and MTX, is associated with statistically and clinically meaningful improvements in patients with active RA with inadequate response to MTX compared with the combination of placebo and MTX. The primary endpoint of ACR20 response rate at week 24 was 69.5 % in the iguratimod group compared with 30.7 % in the placebo group ($P < 0.001$). The ACR20 rate with iguratimod was significantly improved compared with placebo at week 8 and week 16 (Fig. 2). Treatment with iguratimod for 24 weeks was consistently superior to placebo for the ACR50, ACR70, HAQ-DI, and DAS28-CRP.

Recently, guidance for treatment to target was proposed to improve the management of RA in clinical practice [25], in which low disease activity was set as an acceptable therapeutic goal, particularly in patients with long-standing disease, considerable joint damage, and several prior treatment failures. In the present study, the mean duration of RA was more than 4 years in both groups and the numbers of tender joints and swollen joints were ≥6 and ≥4, respectively, at baseline. Thus, we consider that the therapeutic goal for patients enrolled in the present study is "low disease activity" (DAS28-CRP <3.2). After the 24-week treatment, this goal was achieved in 47.6 % of patients in the iguratimod group and 20.5 % in the placebo group. At baseline, patients with low disease activity were 1.2 % in the iguratimod group and 0 % in the placebo group. Furthermore, clinical remission (DAS28-CRP <2.6) was achieved in 27.4 % of patients in the iguratimod group and 9.1 % in the placebo group.

B cells can produce autoantibodies against antigens such as the Fc region of IgG, the target of rheumatoid factor. The sensitivity of rheumatoid factor in the diagnosis of RA is about 75 % in most cross-sectional studies, and about 25 % of patients with RA have no detectable serum rheumatoid factor [26]. Rheumatoid factor-positive RA patients had more severe disease, both functionally and radiographically, than rheumatoid factor-negative patients [27].

Recently, the presence of rheumatoid factor or anti-cyclic citrullinated peptide (anti-CCP) antibodies and elevated IgG levels have been shown to be two simple biomarkers that can be used routinely before therapy to predict response to rituximab, a B cell-depleting monoclonal antibody, in patients with refractory RA [28]. In the present study, rheumatoid factor after 24-week treatment with iguratimod + MTX significantly decreased from baseline by 33 % ($P < 0.001$), whereas with placebo + MTX it increased from baseline by 14 % (not significant). Furthermore, IgG, IgM, and IgA levels at week 24 in the iguratimod group significantly decreased from baseline (all values of $P < 0.001$), whereas these levels in the placebo group did not significantly change (slight increases for IgG and IgM and a slight decrease for IgA) (Table 2). These results indicate that iguratimod and/or iguratimod-induced synergic effects have immunological actions, but MTX alone did not.

Previously, ALT and AST levels of more than 100 U/L were observed in 9.8 and 6.9 %, respectively, of RA patients who were treated with iguratimod (without MTX) for 52 weeks [13]. These ALT and AST increases were mostly evident between week 4 and week 8 and resolved spontaneously during treatment or upon discontinuation of treatment [13]. Thus, one of the safety concerns when combining MTX with iguratimod was potential hepatotoxicity. However, in the present study, ALT and AST levels of more than 100 U/L were found only in 1.2 and 0.6 %, respectively, of RA patients treated with iguratimod + MTX; similar increases were seen in 1.1 and 1.1 %, respectively, of patients treated with placebo + MTX. These results indicate that the combination of iguratimod with MTX did not increase the risk of hepatotoxicity. Because this study selected patients who had been treated with MTX for more than 12 weeks and had AST or ALT levels less than the upper limit of normal range, a possibility is considered that the hepatic function in the patients previously treated with MTX had a relatively good safety profile for use of iguratimod + MTX. One patient each in the iguratimod group showed a notable decrease in leukocyte ($<2.0 \times 10^3/\mu L$) and erythrocyte ($<2.5 \times 10^6/\mu L$) counts, and these abnormal laboratory findings resolved after patients stopped study treatment. These results suggest that the combination therapy of iguratimod with MTX can be used safely with hepatic enzyme and hematologic monitoring.

In this study, lower dosages of MTX (6 or 8 mg/week) were used, compared with dosages used in Europe and the USA, because the approved maximum dosage in Japan was 8 mg/week at the beginning of this study (higher dosages of MTX were approved in February 2011). A future study is necessary to confirm whether the greater efficacy of combination therapy with MTX and iguratimod is achieved when a higher dosage of MTX is used instead of the present low dosages.

Because the mode of action of iguratimod is different from that of MTX and the present efficacy of the combination therapy of MTX + iguratimod is greater than that of MTX + placebo, the present combination therapy is a good treatment option for patients who have inadequate response to MTX or for patients who cannot afford expensive biological agents.

In conclusion, the present new combination of iguratimod with MTX is efficacious and tolerated over 24 weeks in patients with active RA with inadequate response to MTX.

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