Original Article

Title:

Add-on effectiveness of methotrexate or iguratimod in patients with rheumatoid arthritis exhibiting an inadequate response to Janus kinase inhibitors: The ANSWER cohort study

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Abstract

Objectives

This multicenter, retrospective study evaluated the effectiveness of add-on methotrexate (MTX) or iguratimod (IGU) in patients with rheumatoid arthritis exhibiting an inadequate response to Janus kinase inhibitors (JAKi).

Methods

Forty-five patients were treated with new additional MTX (n = 22) or IGU (n = 23) and followed for 6 months. Patients' background: age, 59.2 years; Disease activity score of 28 joints with C-reactive protein (DAS28-CRP), 3.4; clinical disease activity index (CDAI), 15.7; biological disease-modifying antirheumatic drug (DMARD)-switched cases, 77.8%; first JAKi cases, 95.6%; JAKi treatment: tofacitinib (n = 25), baricitinib (n = 17), upadacitinib (n = 2), and peficitinib (n = 1) for 9.6 months.

Results

Thirty-five patients continued the combination therapy for 6 months without significant change of concomitant glucocorticoid or other conventional synthetic DMARDs. DAS28-CRP (MTX, 3.6 to 2.6, $P < 0.05$; IGU, 3.3 to 2.1, $P < 0.001$) and CDAI (MTX, 16.7 to 8.8, $P < 0.05$; IGU, 14.6 to 6.5, $P < 0.01$) improved significantly from baseline. Using the EULAR criteria, 45.4% (MTX) and 39.1% (IGU) achieved moderate or good response, and 40.9% (MTX) and 39.1%

(IGU) achieved ACR20 criteria.

Conclusions

Adding MTX or IGU to inadequate responders of JAKi can be considered as a complementary treatment.

Keywords

Iguratimod, Inadequate response, Janus kinase inhibitor, Methotrexate, Rheumatoid arthritis

Introduction

Janus kinase inhibitors (JAKi) suppress the JAK-signal transducer and activator of transcription (STAT) pathways, leading to inhibition of interleukin (IL)-6, granulocyte macrophage colony-stimulating factor (GM-CSF), interferon (IFN)-α/β/γ, and other cytokines associated with the pathology of rheumatoid arthritis (RA) [1]. According to the recommendations of the 2019 European League Against Rheumatism (EULAR), JAKi are equivalent to other biological disease-modifying antirheumatic drugs (bDMARDs) [2]. However, in patients who cannot use conventional synthetic (cs) DMARDs as a comedication, anti-IL-6 receptor antibody (aIL-6R) and targeted synthetic (ts) DMARDs, such as JAKi, may have some advantages compared with other bDMARDs [2]. In addition, combination therapy with csDMARDs is more effective than

monotherapy for all bDMARDs and tsDMARDs. When methotrexate (MTX) is part of combination therapy, high MTX doses may not be necessary to increase the efficacy (10 mg/week may be sufficient to increase the efficacy) [2].

If treatment with tsDMARD fails, treatment with other bDMARDs or tsDMARDs may be considered, although their efficacy and safety still remain unknown [2]. Recent cohort-based studies have demonstrated that JAKi showed better retention due to effectiveness compared to tumor necrosis factor inhibitors (TNFi) and equivalent retention compared to other non-TNFi, such as aIL-6R [3-5]. Thus, JAKi may have some advantages compared to TNFi when treatment does not include csDMARDs. However, in rare cases, patients exhibit an inadequate response to JAKi (JAKi-IR). If JAKi-IR occurs, no reliable evidence supports the use of bDMARDs or JAKi or adding on specific csDMARDs, may be due to the difficulty in recruiting patients. To avoid multiple JAKi failures, adding on specific csDMARDs to improve JAK-IR may be considered at first.

MTX inhibits not only IL-6 but also IL-1 and IL-8 from various cell types [6]. On the other hand, iguratimod (IGU), a novel csDMARD introduced clinically in 2012 in Japan (also known as T-614), inhibits TNF-α, IL-6, IL-1, and IL-8 from various cell types [7]. TNF-α, IL-1, and IL-8 play important roles in the pathology of RA, although they are not directly involved in the JAK

pathway [8-13]. We hypothesized that in patients with JAKi-IR, new administration of MTX or IGU may improve the efficacy of JAKi, by inhibiting key cytokines that are not directly involved in JAK pathways. Japan is the only country to approve five JAKi, including tofacitinib (TOF; 2013), baricitinib (BAR; 2017), peficitinib (PEF; 2019), upadacitinib (UPA; 2020), and filgotinib (FIL; 2020). In addition, a multicenter cohort study may have some advantages in the recruitment of rare cases such as JAKi-IR.

Materials and Methods

Patients

The Kansai Consortium for Well-being of Rheumatic Disease Patients (ANSWER) cohort is an observational, multicenter registry, which collects data from every out-patient visit of RA patients in the Kansai district of Japan [5, 14-19]. Data were retrospectively collected from patients who were examined at seven major university-related hospitals (Kyoto University, Osaka University, Osaka Medical and Pharmaceutical University, Kansai Medical University, Kobe University, Nara Medical University, and Osaka Red Cross Hospital). RA was diagnosed based on the 1987 RA classification criteria of the American College of Rheumatology (ACR) [20] or the 2010 ACR/EULAR RA classification criteria [21]. In Japan, public national health

insurance covers 70%–90% of medical expenses, and csDMARDs, bDMARDs, or JAKi can be administered at the discretion of attending rheumatologists, in accordance with the Japan College of Rheumatology guidelines [22]. The dose of each agent was based on manufacturers' recommendations. The oral glucocorticoid dose was calculated as the prednisolone equivalent. The inclusion criteria for this study were as follows: 1) inadequate response to JAKi followed by new additional administration of MTX (the MTX group) or IGU (the IGU group) from 2014 to 2021; 2) follow-up of at least 6 months after MTX or IGU administration, and 3) the combined prednisolone (PSL) or other csDMARDs were at least not increased during the 6 months of the study. An inadequate response to JAKi was defined based on previous reports [23, 24], and included all of the following: 1) JAKi was used at least 1 month before additional MTX or IGU administration; 2) the clinical disease activity index (CDAI) score > 2.8 (more than low disease activity) [21] at the time of MTX or IGU administration; and 3) either tender joint count (TJC), swollen joint count (SJC), patient global assessment of disease activity (Pt-GA), or physician global assessment of disease activity (Ph-GA) were the same or increased compared to the disease activity 1–3 months before MTX or IGU administration.

Primary nonresponders were defined as patients who exhibited an inadequate response to JAKi within 3 months after JAKi initiation, and secondary nonresponders were defined as patients who exhibited an inadequate response to JAKi more than 3 months after JAKi initiation [23]. In

addition to the JAKi, patients were treated with MTX 2–8 mg/week or IGU 25 mg/day at baseline, and the MTX or IGU were increased to 16 mg/week or 50 mg/day, respectively, at the discretion of the physician in accordance with the Japan College of Rheumatology guidelines for the use of methotrexate and the manufacturers' recommendations [25]. Effectiveness and safety were evaluated at 1, 3, and 6 months after MTX or IGU administration.

Outcome variables

Disease activity was assessed by serum C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), serum matrix metalloproteinase-3 (MMP-3), and rheumatoid factor (RF). For composite measures, the TJC of 28 joints, SJC of 28 joints, Pt-GA (100 mm), Ph-GA (100 mm), disease activity score of 28 joints (DAS28) with CRP (DAS28-CRP) [26], and the CDAI score were evaluated. The DAS28-CRP was divided into four categories: remission (≤ 2.3), low disease activity (2.3–2.7), moderate disease activity (2.7–4.1), and high disease activity (> 4.1). The CDAI was divided into four categories: remission (≤ 2.8), low disease activity (2.8–10), moderate disease activity (10–22), and high disease activity (> 22) [27]. Observations points made at the following times: 1–3 months before the start of MTX or IGU (before IR), at the start of MTX or IGU (baseline), and 1, 3, and 6 months after the administration of MTX or

IGU. Clinical responses were defined by the ACR as 20% improvement criteria [28] and EULAR response criteria [26].

Statistical analysis

Longitudinal changes of each parameter before and after MTX or IGU administration were examined using the Wilcoxon signed-rank test or chi-squared test. The data of patients who dropped out of the combination therapy were calculated as a missing value. Statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R software (R Foundation for Statistical Computing, Vienna, Austria) [29]. A two-sided *P*-value of <0.05 was considered statistically significant.

Results

Demographic data and concomitant medications

The clinical characteristics at baseline and 6 months for patients in the MTX group (n = 22) are shown in Table 1. Eighteen patients (81.8%) continued the combination therapy for 6 months. Two patients discontinued treatment due to ineffectiveness, and two patients discontinued

treatment due to changing hospitals. No serious adverse events led to treatment discontinuation. Twenty patients in the MTX group (90.9%) were treated with the first JAKi. JAKi treatment was TOF (n = 14), BAR (n = 7), and PEF (n = 1), for an average of 8.7 months. Seven patients were primary nonresponders, and 15 patients were secondary nonresponders. The add-on MTX mean dose was 6.0 mg/week at baseline and 7.5 mg/week at 6 months. No significant changes were observed in the mean doses and PSL. The prescription rates for other csDMARDs, including leflunomide (LEF), iguratimod (IGU), bucillamine (BUC), salazosulfapyridine (SASP), and tacrolimus (TAC), did not significantly change throughout the study.

The clinical characteristics at baseline and 6 months of patients in the IGU group (n = 23) are shown in Table 2. Seventeen patients (73.9%) continued the combination therapy for 6 months; six patients discontinued treatment due to ineffectiveness. No serious adverse events led to treatment discontinuation. All patients in the IGU group were treated with the first JAKi. JAKi treatment was TOF (n = 11), BAR (n = 10), and UPA (n = 2), for an average of 10.4 months. Four patients were primary nonresponders, and 19 patients were secondary nonresponders. The add-on IGU dose was 25.0 mg/day at baseline and 37.0 mg/day at 6 months (IGU were increased to 50. 0 mg/day in 11 patients). Twelve patients (52.2%) in the IGU group were treated with MTX at a mean dose of 8.2 mg/week at baseline. No significant changes were observed in the mean doses and prescription rates of MTX or PSL, and the prescription rate of

other csDMARDs did not significantly change throughout the study.

Patients were older, disease duration was longer, eGFR was lower, and disease activity was lower in the IGU group compared with these parameters in the MTX group. The attending physicians may have treated elderly patients with IGU rather than MTX due to lower renal function and lower disease activity.

Effectiveness

Fig. 1 shows the longitudinal changes in laboratory parameters, including serum CRP, ESR, MMP-3, and RF. CRP levels decreased significantly in the IGU group at 6 months ($P = 0.039$) compared to the levels at baseline. MMP-3 levels decreased from 1 month ($P = 0.011$) to 6 months ($P = 0.016$) compared with levels at baseline in the IGU group. RF decreased significantly from 3 months ($P = 0.0086$) to 6 months ($P = 0.013$) compared with levels at baseline in the MTX group.

Fig. 2 shows longitudinal changes in clinical variables associated with disease activity, including TJC, SJC, Pt-GA, and Ph-GA. In the MTX group, SJC significantly decreased from 3 months ($P = 0.005$) to 6 months ($P = 0.012$), Pt-GA significantly decreased from 3 months ($P =$

0.0021) to 6 months (P = 0.018), and Ph-GA significantly decreased from 3 months (P = 0.0020) to 6 months (P = 0.0041) compared with these parameters at baseline. In the IGU group, TJC significantly decreased at 6 months (P = 0.0079), Pt-GA significantly decreased from 3 months (P = 0.041) to 6 months (P = 0.041), and Ph-GA significantly decreased at 6 months (P = 0.0053) compared with these parameters at baseline.

Fig. 3 a–b shows longitudinal changes in composite measures of disease activity, including DAS28-CRP and CDAI. In the MTX group, DAS28-CRP significantly decreased from 3 months (P = 0.025) to 6 months (P = 0.036) compared with levels at baseline. In the IGU group, DAS28-CRP significantly decreased from 3 months (P < 0.001) to 6 months (P < 0.001) compared with levels at baseline. In the MTX group, CDAI significantly decreased from 3 months (P = 0.0016) to 6 months (P = 0.014) compared with levels at baseline. In the IGU group, CDAI significantly decreased at 6 months (P = 0.0024) compared with levels at baseline.

Fig. 3 c–e shows treatment responses. The percentages of patients who achieved ACR 20 in the MTX group were 27.3%, 45.5%, and 40.9% at 1, 3, and 6 months, respectively. The percentages of patients who achieved ACR 20 in the IGU group were 21.7%, 26.1%, and 39.1% at 1, 3, and 6 months, respectively (Fig. 3c). Based on the EULAR treatment response, 22.7% of patients showed a moderate response and 22.7% showed a good response at 3 to 6 months in the

MTX group (Fig. 3d). In the IGU group, 17.4% of patients showed a moderate response and 21.7% of patients showed a good response at 6 months (Fig. 3e).

Fig. 4 shows longitudinal changes in disease activity distribution and treatment response. Based on the DAS28-CRP, in the MTX group, 77.3% of patients had moderate or high disease activity at baseline, which decreased to 27.3% at 6 months (Fig. 4a). In the IGU group, 65.2% of patients had moderate or high disease activity at baseline, which decreased to 21.7% at 6 months (Fig. 4b). Based on CDAI, in the MTX group, 59.1% of patients had moderate or high disease activity at baseline, which decreased to 27.3% at 6 months (Fig. 4c). In the IGU group, 56.5% of patients had moderate or high disease activity at baseline, which decreased to 21.7% at 6 months (Fig. 4d).

Factors associated with treatment responses

At 6 months in the MTX group, no significant differences were observed between EULAR moderate or good responders (n = 10) and nonresponders (n = 10) (excluding the 2 patients who changed hospitals) in baseline age, disease duration, RF and anti-cyclic citrullinated peptide antibody (ACPA) positivity, DAS28-CRP, CDAI, the ratio of primary or secondary nonresponders, and combined JAKi, PSL, or other csDMARDs. However, the number of

previously treated JAKi or bDMARDs (1.1 ± 1.2) in the responder group was lower compared to that of the nonresponder group (4.0 ± 2.7) (P = 0.0089). In the responder group, 50% of the cases were JAKi or bDMARDs naïve; in the nonresponder group, 80% of the patients had been treated with more than three JAKi or bDMARDs. In addition, the responder group tended to have a lower rate of previous aIL-6R treatment (30%) compared to the rate in the nonresponder group (80%) (P = 0.070) (Supplementary Table 1). Moreover, 50% (n = 11/22) of patients were previously treated by MTX, and the ratio of the EULAR moderate or good responders was 63.6% (n = 7/11) in the MTX-naïve group and 33.3% (n = 3/9) in the MTX-experienced group (P = 0.37). Considering CRP as an alternative marker of IL-6, 50% (n = 10/20) of patients showed CRP > 0.30 mg/dl at baseline. Finally, the ratio of the EULAR moderate or good responders was 60.0% (n = 6/10) in the low-CRP group and 40.0% (n = 4/10) in the high-CRP group (P = 0.66).

At 6 months in the IGU group, no significant differences were observed between EULAR moderate or good responders (n = 9) and nonresponders (n = 14) in baseline age, RF and ACPA positivity, DAS28-CRP, CDAI, the ratio of primary or secondary nonresponders, and combined JAKi, PSL, or MTX and other csDMARDs. However, the disease duration was longer in the responder group (21.3 ± 9.4 years) compared to the disease duration in the nonresponder group (10.5 ± 7.9 years) (P = 0.0098) (Supplementary Table 2). In the IGU group, 47.8% (n = 11/23)

of patients showed CRP > 0.30mg/dl at baseline. Finally, the ratio of the EULAR moderate or good responders was 33.3% (n = 4/12) in the low-CRP group and 45.5% (n = 5/11) in the high-CRP group (P = 0.68).

Discussion

To the best of our knowledge, this is the first study to investigate the effectiveness of adding MTX or IGU to the treatment regime in patients with JAKi-IR. To date, little is known about the detailed mechanisms of JAKi-IR. Regarding predictors of JAKi treatment response, seropositive (ACPA positive) RA patients are more likely to achieve ACR20/50/70 than seronegative patients when treated with TOF [30]. In addition to seropositivity, patients with RA-associated interstitial lung disease (RA-ILD) tend to show higher treatment responses to JAKi [31]. The ACPA titer is associated with the presence of RA-ILD [32], which are both related to the JAK-STAT pathway [33, 34]. However, in this study, ACPA positivity in JAKi-IR patients was similar to our previous reports, including most of the JAKi treated patients [3, 5, 14]. In addition, we failed to obtain enough data to determine the association with RA-ILD.

IL-2, IL-4, IL-6, IL-23, GM-CSF, and IFN are directly involved in the JAK-STAT pathway, while TNF-α, IL-1, and IL-17 are not [35]. A recent in vitro report demonstrated that JAKi,

such as TOF, BAR, FIL, and UPA, may inhibit 43%–55% of IL-6-induced phosphorylation of STAT1 in monocytes when used at the standard dose [36]. On the other hand, aIL-6R may occupy more than 95% of the IL-6R when used at a clinically high dose, according to an in vitro simulation [37]. Taken together, JAKi-IR may occur in 1) patients that is dominated by cytokines, such as TNF-α, IL-1, and IL-17, which are not directly involved in the JAK-STAT pathway or 2) patients dominated by very high levels of IL-6, which cannot be sufficiently suppressed by JAKi. To rescue these respective patients, 1) adding csDMARDs that can inhibit TNF-α, IL-1, and IL-17 may be hopeful, and 2) adding csDMARDs that can further inhibit IL-6 by pathways other than the JAK-STAT pathway may be hopeful.

MTX is a folic acid antagonist, which inhibits aminoimidazole-4-carboxamide ribonucleotide transformylase, leading to increased adenosine release and activation of adenosine receptor A2a and inhibition of nuclear factor-kappa B (NF-κB) activation [38]. Consequently, MTX inhibits the activity or production of not only IL-6 but also IL-1 and IL-8, which are important in RA pathology but not directly involved in the JAK-STAT pathway [6]. In addition, MTX increases gene expression of anti-inflammatory cytokines, such as IL-4 and IL-10, which inhibit arthritis progression but are inhibited by JAKi [39, 40]. MTX also inhibits angiogenesis, neutrophil chemotaxis, and expression of metalloproteinase and adhesion molecules in synovial fibroblast, which may lead to further inhibition of synovitis [6]. Indeed, the BAR + MTX combination was

more effective compared to BAR monotherapy, especially in radiographic progression [41].

IGU inhibits NF-κB activation by interfering with NF-κB translocation from the cytoplasm to the nucleus without affecting the degradation of IκB-α [7]. Consequently, IGU inhibits not only IL-6 and GM-CSF but also TNF-α, IL-1β, and IL-8 from synovial cells and monocytic cells [8-13]. Moreover, a recent report showed that IGU markedly decreased IL-6-induced IL-17 and MMP-3 levels in synovial fibroblasts from RA patients [42]. These pro-inflammatory cytokines play important roles in the pathology of RA, although they are not directly involved in the JAK pathway. Taken together, these unique modes of action of MTX and IGU that are not directly involved in the JAK pathway may play complementary roles in patients with JAKi-IR.

Determining which patients will respond to each add-on therapy is important. MTX-responders, based on the EULAR criteria, were comprised of a lower number of patients with previous JAKi or bDMARDs treatments and tended to have lower rates of previous aIL-6R treatment compared with the nonresponder group. Of note, only 4.5% of patients in the MTX group also received IGU. On the other hand, IGU-responders had a longer disease duration compared to nonresponders but showed no apparent tendency for other clinical backgrounds. In the IGU group, 52.2% of patients were also treated with MTX. Adding on MTX may be more effective in patients without previous aIL-6R treatment because aIL-6R-IR patients may have RA

strongly dominated by other cytokines rather than IL-6, and MTX mainly inhibits IL-6 [6]. IGU inhibits both JAK-related (IL-6 and GM-CSF) and non-JAK-related (TNF-α, IL-1β, and IL-8) pro-inflammatory cytokines [7]. Adding IGU to JAKi-IR patients who are intolerant to MTX, patients who are already added MTX but showed poor response, or with multi-bDMARDs-IR (including aIL-6R) may be a viable strategy.

The effectiveness of low-dose MTX in Japanese populations should be mentioned. Intra-erythrocyte MTX-polyglutamate concentration, which is a useful biomarker of MTX efficacy, was 65 nmol/L with 13.4 mg/week MTX treatment in patients from the United States but reached 94 nmol/L with 10.3 mg/week MTX treatment in Japanese patients [43].

There are several limitations to this study. This was a retrospective, cohort-based study; therefore, patients were not randomized and the effectiveness of MTX and IGU was not compared. Because JAKi-IR is a rare condition, the number of patients who met the inclusion criteria was relatively small. Most patients were treated by either TOF or BAR, and the effectiveness in other JAKi should be investigated in future studies. Comorbidities like RA-ILD, which could potentially affect drug selection and retention, were not evaluated. Most of the patients were treated with the first JAKi, and the effectiveness in multi-JAKi-IR patients remains unclear. In the MTX group, 50% (n = 11/22) of patients were previously treated by

MTX, but the reasons of MTX discontinuation remained unclear. In the IGU group, 52.2% (n = 12/23) of patients were combined with MTX. The adverse effects might have been underestimated due to the small number of patients and the short duration of follow-up. Whether this combination therapy protects the joints from radiographic damage should be evaluated in prospective, randomized, and longer-duration studies.

In conclusion, the results of this retrospective study demonstrated that new add-on use of MTX or IGU is an effective complementary therapy for JAKi-refractory RA patients, especially those who are treated by the first JAKi.

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companies had no roles in the study design, data collection, data analysis, data interpretation, or writing of the report.

Conflict of interests

KE is affiliated with the Department of Musculoskeletal Regenerative Medicine, Osaka University, Graduate School of Medicine, which is supported by Taisho. KE has received research grants from AbbVie, Amgen, Asahi-Kasei, Astellas, Chugai, Eisai, Mitsubishi-Tanabe, Ono Pharmaceutical, Teijin Pharma, and UCB Japan. KE has received payments for lectures from AbbVie, Amgen, Asahi-Kasei, Astellas, Ayumi, Bristol-Myers Squibb, Chugai, Eisai, Eli Lilly, Janssen, Mitsubishi-Tanabe, Ono Pharmaceutical, Pfizer, Sanofi, and UCB Japan. TH received a research grant and/or speaker fee from Astellas, Chugai, GlaxoSmithKline, Nippon Shinyaku, and Eisai. YM received a research grant and/or speaker fee from Eli Lilly, Chugai, Pfizer, Bristol-Myers Squibb, and Mitsubishi-Tanabe. MHirao received a speaker fee from Astellas, Ono Pharmaceutical, Eli Lilly, Mitsubishi-Tanabe, Pfizer, Ayumi, and Takeda. MHashimoto received a research grant and/or speaker fee from Mitsubishi-Tanabe, Eisai, Eli Lilly, Bristol-Myers Squibb, and Novartis Pharma. KM is affiliated with a department that is financially supported by fiver pharmaceutical companies (Asahi-Kasei, Mitsubishi-Tanabe, Chugai, Ayumi, and UCB Japan) and the city governments (Nagahama City and Toyooka City).

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Ethical approval

The representative facility of this registry was Kyoto University, and this observational study was conducted in accordance with the Declaration of Helsinki, with the approval of the ethics committees of the following seven institutes: Kyoto University (2016-03-24/approval No. R053), Osaka University (2015-11-04/approval No. 15300), Osaka Medical and Pharmaceutical University (2014-07-14/approval No. 1529), Kansai Medical University (2017-11-21/approval No. 2014625), Kobe University (2015-03-20/approval No. 1738), Nara Medial University (2018-01-23/approval No. 1692), and Osaka Red Cross Hospital (2015-09-01/approval No. 644). The board of the Osaka University Hospital Ethics Committee waived the requirement for patient informed consent because of the anonymous nature of the data. Written informed consent was obtained from the participants in other institutes.

Authors' contributions

KE was responsible for conception and design. KE, TH, YM, YO, MHashimoto, KM, AO, SJ, RH, TK, HS, YS, HA, MK, and EY contributed to data extraction and interpretation. KE, WY, and KY contributed to the design and conduction of statistical analysis. KE prepared the manuscript. AK, MHirao, SO, and KN supervised the manuscript. All the authors read and approved the final manuscript.

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Figure legends

Figure 1. Changes in clinical laboratory variables before and after new methotrexate or iguratimod administration. Mean values of (a) CRP, (b) ESR, (c) MMP-3, and (d) RF are shown. Bars indicate standard error. * $P < 0.05$, ** $P < 0.01$ from baseline. MTX, methotrexate; IGU, iguratimod; IR, inadequate response; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; MMP-3, matrix metalloproteinase-3; RF, rheumatoid factor.

Figure 2. Changes in clinical variables before and after new methotrexate or iguratimod

administration. Mean values of (a) tender joint count, (b) swollen joint count, (c) Pt-GA, and (d) Ph-GA are shown. Bars indicate standard error. * $P < 0.05$, ** $P < 0.01$ from baseline. MTX, methotrexate; IGU, iguratimod; IR, inadequate response; Pt-GA, patient's global assessment of disease activity; Ph-GA, physician's global assessment of disease activity.

Figure 3. Changes in composite measures of disease activity and clinical response before and after new methotrexate or iguratimod administration. Mean values of (a) DAS28-CRP and (b) CDAI, and response to each treatment according to (c) the ACR 20% criteria and (d) the EULAR criteria. Bars indicate standard error. * $P < 0.05$, ** $P < 0.01$, ** $P < 0.001$ from baseline. MTX, methotrexate; IGU, iguratimod; IR, inadequate response; DAS28-CRP, disease activity score assessing 28 joints with C-reactive protein; CDAI, clinical disease activity index; ACR20, American College of Rheumatology 20% improvement criteria; EULAR, European League against Rheumatic Diseases.

Figure 4. Changes in the distribution of disease activity before and after new methotrexate or iguratimod administration. Distribution of DAS28-CRP in (a) the MTX group and (b) the IGU group. Disease activity was defined by DAS28-CRP as follows: remission (≤2.3), low disease activity (2.3–2.7), moderate disease activity (2.7–4.1), and high disease activity (>4.1). The

distribution of CDAI in (c) the MTX group and (d) the IGU group. Disease activity was defined by CDAI as follows: remission (≤2.8), low disease activity (2.8–10), moderate disease activity (10–22), and high disease activity (>22). MTX, methotrexate; IGU, iguratimod; IR, inadequate response; DAS28-CRP, disease activity score assessing 28 joints with C-reactive protein; CDAI, clinical disease activity index.

Table 1. Clinical characteristics at baseline and 6 months for rheumatoid arthritis patients who were treated with additional methotrexate (n = 22)

Variable	Baseline	6 months
Sex	15 females, 7 males	
Age (years)	55.9 ± 14.8	
Body mass index (kg/m^2)	22.1 ± 3.0	
Duration of disease (years)	10.4 ± 7.8	
RF positivity (%)	72.7% (16/22)	
ACPA positivity (%)	77.3% (17/22)	
Number of previously treated Bio or JAKi	Naïve (7), 2nd (0), 3rd or more (15)	
Previously treated Bio (n)	TNFi (15), aIL-6Ra (11), CTLA4-Ig (9)	
Order of treated JAKi (n)	first JAKi (20), switched JAKi (2)	
Combined JAKi (n)	TOF (14), BAR (7), PEF (1)	
Treatment duration of JAKi (months)	8.7 ± 9.2	
Type of JAKi failure (n)	primary (7), secondary (15)	
MTX dose (mg/week), usage (%)	6.0 ± 2.0, 100% (22/22)	7.5 ± 2.8**, 100% (22/22)
PSL dose (mg/day), usage (%)	5.9 ± 3.2, 59.1% (13/22)	5.6 ± 3.1, 59.1% (13/22)
LEF usage (%)	0%	0%
IGU usage (%)	4.5% (1/22)	4.5% (1/22)
BUC usage (%)	9.1% (2/22)	9.1% (2/22)
SASP usage (%)	18.2% (4/22)	13.6% (3/22)
TAC usage (%)	13.6% (3/22)	9.1% (2/22)
CRP (mg/dL)	0.8 ± 1.7	0.6 ± 1.1
MMP-3 (ng/mL)	191.5 ± 182.9	131.5 ± 109.4
RF (IU/mL)	145.4 ± 176.9	85.0 ± 99.9*
eGFR (ml/min/1.73 m^2)	83.7 ± 17.6	78.3 ± 18.7
Swollen joint count, 0–28	4.8 ± 4.4	1.7 ± 2.6*
Tender joint count, 0–28	3.6 ± 4.1	1.9 ± 2.8
Pt-GA (0–100 mm)	51.4 ± 25.4	33.6 ± 20.2*
Ph-GA (0–100 mm)	32.4 ± 22.0	15.2 ± 13.8**

HAQ-DI	0.8 ± 0.8	0.8 ± 0.7
DAS28-CRP	3.6 ± 1.3	2.6 ± 1.1*
CDAI	16.7 ± 10.7	8.8 ± 6.6*

Data are expressed as mean ± standard deviation. n/N (%) = number of patients with measurements/total number of patients (%).

* $P < 0.05$, ** $P < 0.01$ compared to baseline.

RF, rheumatoid factor; ACPA, anti-cyclic citrullinated peptide antibody; Bio, biologics; JAKi, janus kinase inhibitor; TNFi, tumor necrosis factor inhibitors; aIL-6R, anti-interleukin-6 receptor; CTLA4-Ig, cytotoxic T lymphocyte-associated antigen-4-Ig; TOF, tofacitinib; BAR, baricitinib; PEF, peficitinib; MTX, methotrexate; PSL, prednisolone; LEF, leflunomide; IGU, iguratimod; BUC, bucillamine; SASP, salazosulfapyridine; TAC, tacrolimus; CRP, C-reactive protein; MMP-3, matrix metalloproteinase-3; eGFR, estimated glomerular filtration rate; Pt-GA, patient's global assessment of disease activity; Ph-GA, physician's global assessment of disease activity; HAQ-DI, Health Assessment Questionnaire disability index; DAS28-CRP, disease activity score assessing 28 joints with CRP; CDAI, clinical disease activity index.

Table 2. Clinical characteristics at baseline and 6 months of patients who were treated with additional iguratimod (n = 23)

Variable	Baseline	6 months
Sex	20 females, 3 males	
Age (years)	62.4 ± 11.9	
Body mass index (kg/m^2)	21.2 ± 3.9	
Duration of disease (years)	15.1 ± 10.0	
RF positivity (%)	82.6% (19/23)	
ACPA positivity (%)	87.0% (20/23)	
Number of previously treated Bio	Naïve (2), 2nd (6), 3rd or more (15)	
Previously treated Bio (n)	TNFi (14), aIL-6Ra (14), CTLA4-Ig (11)	
Order of treated JAKi (n)	first JAKi (23)	
Combined JAKi (n)	TOF (11), BAR (10), UPA (2)	
Treatment duration of JAKi (months)	10.4 ± 8.6	
Type of JAKi failure (n)	primary (4), secondary (19)	
MTX dose (mg/week), usage (%)	8.2 ± 4.4, 52.2% (12/23)	8.2 ± 4.5, 52.2% (12/23)
PSL dose (mg/day), usage (%)	5.1 ± 3.9, 47.8% (11/23)	4.3 ± 3.0, 47.8% (11/23)
LEF usage (%)	0%	0%
IGU dose (mg/day), usage (%)	25.0 ± 0.0, 100.0% (23/23)	37.0 ± 0.0**, 82.6% (19/23)
BUC usage (%)	8.7% (2/23)	4.3% (1/23)
SASP usage (%)	30.4% (7/23)	26.1% (6/23)
TAC usage (%)	13.0% (3/23)	13.0% (3/23)
CRP (mg/dL)	0.9 ± 1.7	0.1 ± 0.1*
MMP-3 (ng/mL)	131.1 ± 64.0	88.5 ± 54.0*
RF (IU/mL)	590.3 ± 935.2	429.0 ± 631.2
eGFR (ml/min/1.73 m^2)	70.5 ± 17.2	71.3 ± 21.4
Swollen joint count, 0–28	4.1 ± 6.7	1.0 ± 1.8
Tender joint count, 0–28	3.1 ± 5.0	0.5 ± 0.8**
Pt-GA (0–100 mm)	49.5 ± 23.9	36.9 ± 22.2*

Ph-GA (0–100 mm)	28.1 ± 18.0	13.0 ± 13.2**
HAQ-DI	1.4 ± 1.0	0.9 ± 1.0
DAS28-CRP	3.3 ± 1.4	2.1 ± 0.7***
CDAI	14.6 ± 12.3	6.5 ± 4.1**

Data are expressed as mean ± standard deviation. n/N (%) = number of patients with measurements/total number of patients (%).

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to baseline.

RF, rheumatoid factor; ACPA, anti-cyclic citrullinated peptide antibody; Bio, biologics; JAKi, janus kinase inhibitor; TNFi, tumor necrosis factor inhibitors; aIL-6R, anti-interleukin-6 receptor; CTLA4-Ig, cytotoxic T lymphocyte-associated antigen-4-Ig; TOF, tofacitinib; BAR, baricitinib; UPA, upadacitinib; MTX, methotrexate; PSL, prednisolone; LEF, leflunomide; IGU, iguratimod; BUC, bucillamine; SASP, salazosulfapyridine; TAC, tacrolimus; CRP, C-reactive protein; MMP-3, matrix metalloproteinase-3; eGFR, estimated glomerular filtration rate; Pt-GA, patient's global assessment of disease activity; Ph-GA, physician's global assessment of disease activity; HAQ-DI, Health Assessment Questionnaire disability index; DAS28-CRP, disease activity score assessing 28 joints with CRP; CDAI, clinical disease activity index.

Figure 1

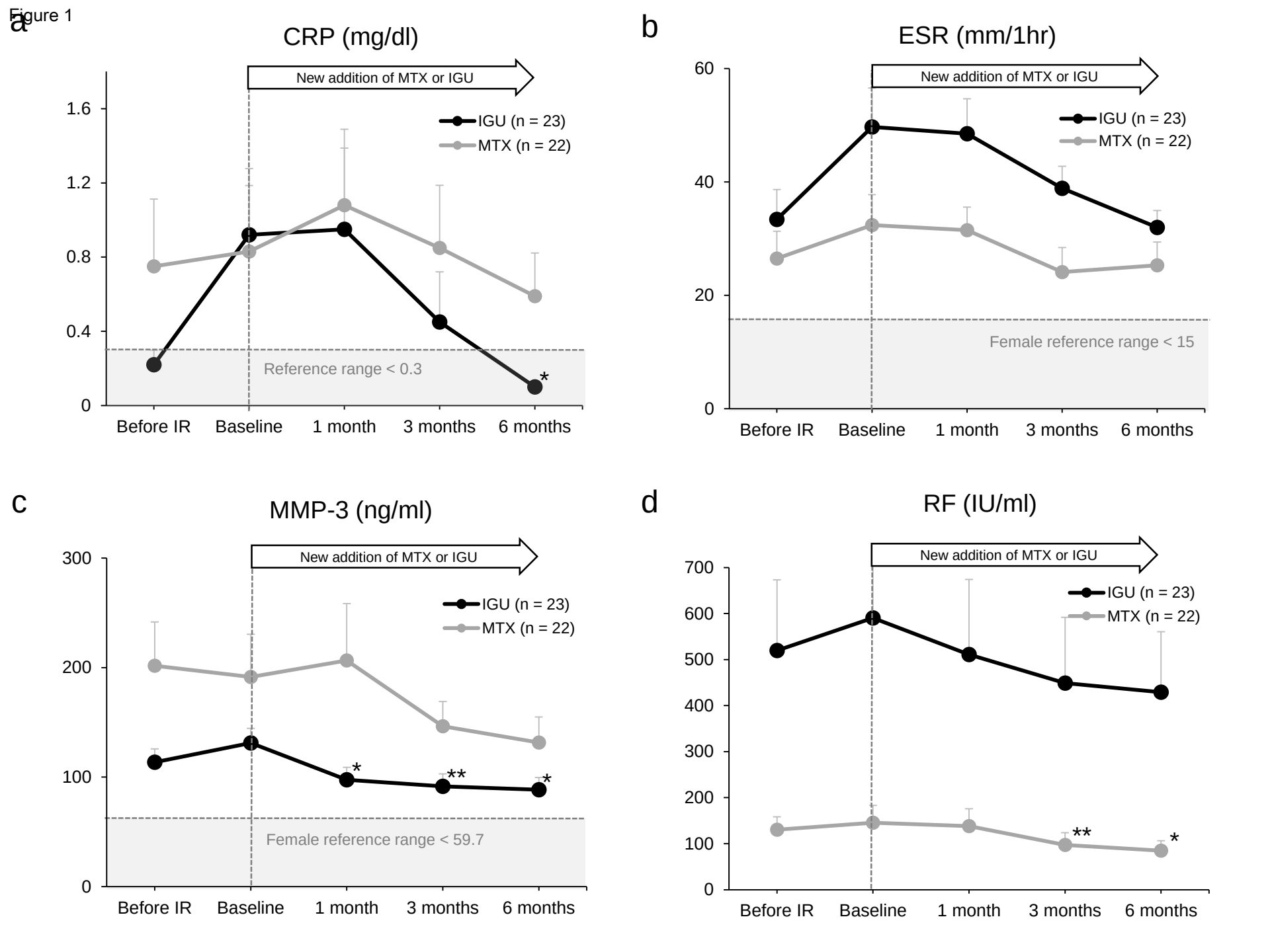

a
CRP (mg/dl)
New addition of MTX or IGU
IGU (n = 23)
MTX (n = 22)
1.6
1.2
0.8
0.4
0
Reference range < 0.3
*
Before IR
Baseline
1 month
3 months
6 months
b
ESR (mm/1hr)
New addition of MTX or IGU
IGU (n = 23)
MTX (n = 22)
60
40
20
0
Female reference range < 15
Before IR
Baseline
1 month
3 months
6 months
c
MMP-3 (ng/ml)
New addition of MTX or IGU
IGU (n = 23)
MTX (n = 22)
300
200
100
0
*
**
*
Female reference range < 59.7
Before IR
Baseline
1 month
3 months
6 months
d
RF (IU/ml)
New addition of MTX or IGU
IGU (n = 23)
MTX (n = 22)
700
600
500
400
300
200
100
0
**
*
Before IR
Baseline
1 month
3 months
6 months

Figure 2

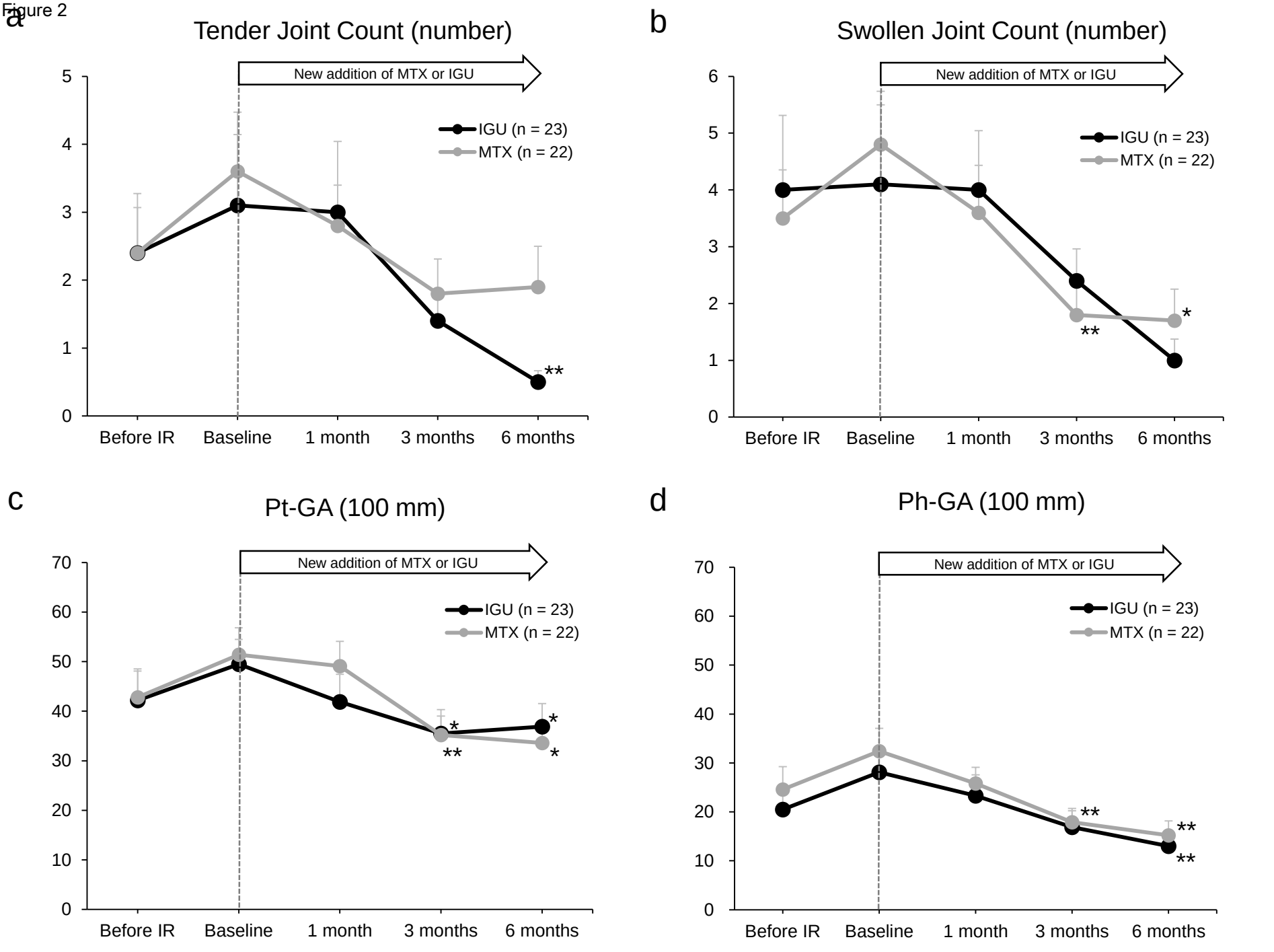

a
Tender Joint Count (number)
New addition of MTX or IGU
IGU (n = 23)
MTX (n = 22)
**
Before IR
Baseline
1 month
3 months
6 months
b
Swollen Joint Count (number)
New addition of MTX or IGU
IGU (n = 23)
MTX (n = 22)
**
*
c
Pt-GA (100 mm)
New addition of MTX or IGU
IGU (n = 23)
MTX (n = 22)
*
**
*
*
d
Ph-GA (100 mm)
New addition of MTX or IGU
IGU (n = 23)
MTX (n = 22)
**
**
**

Figure 3

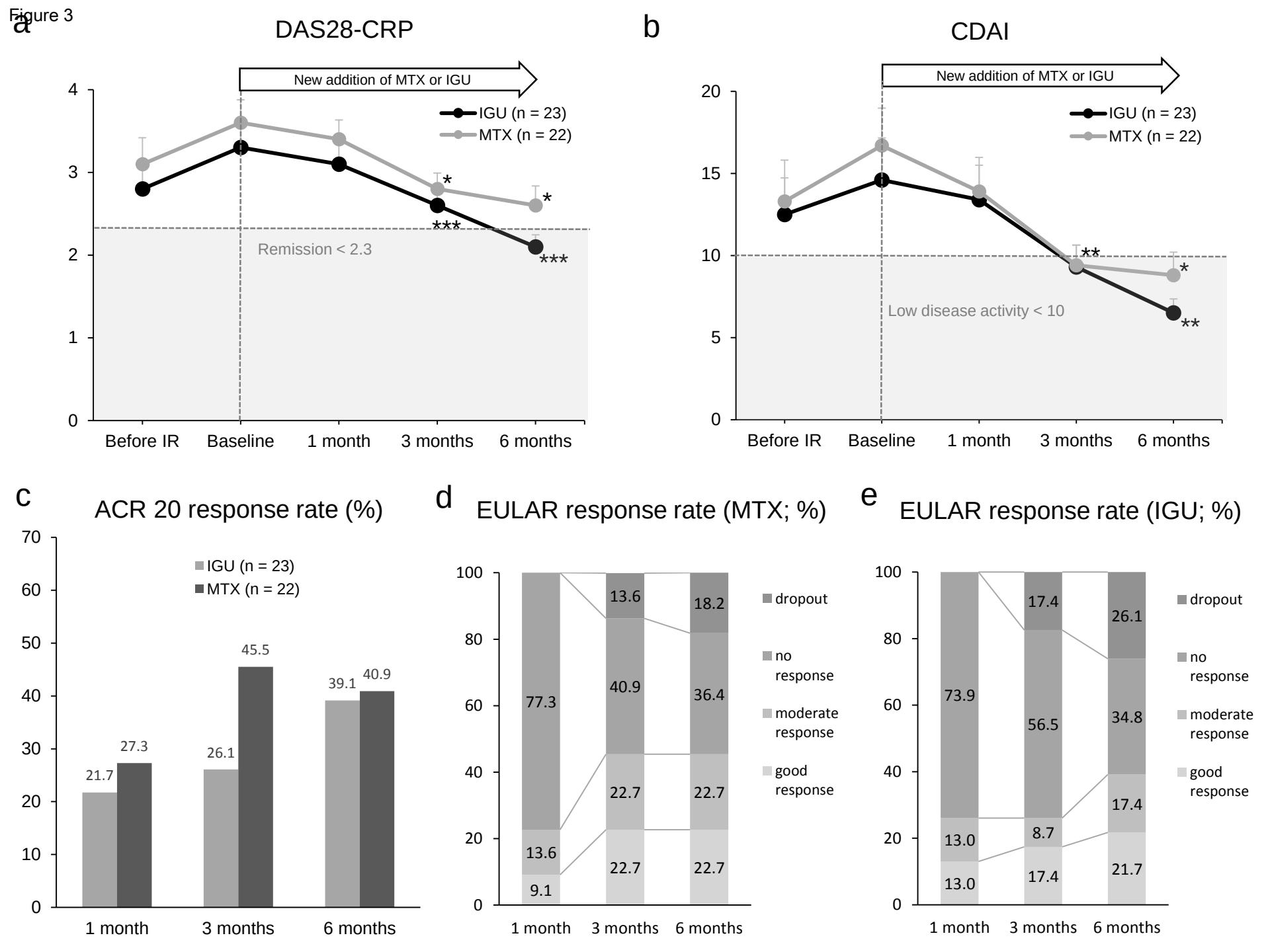
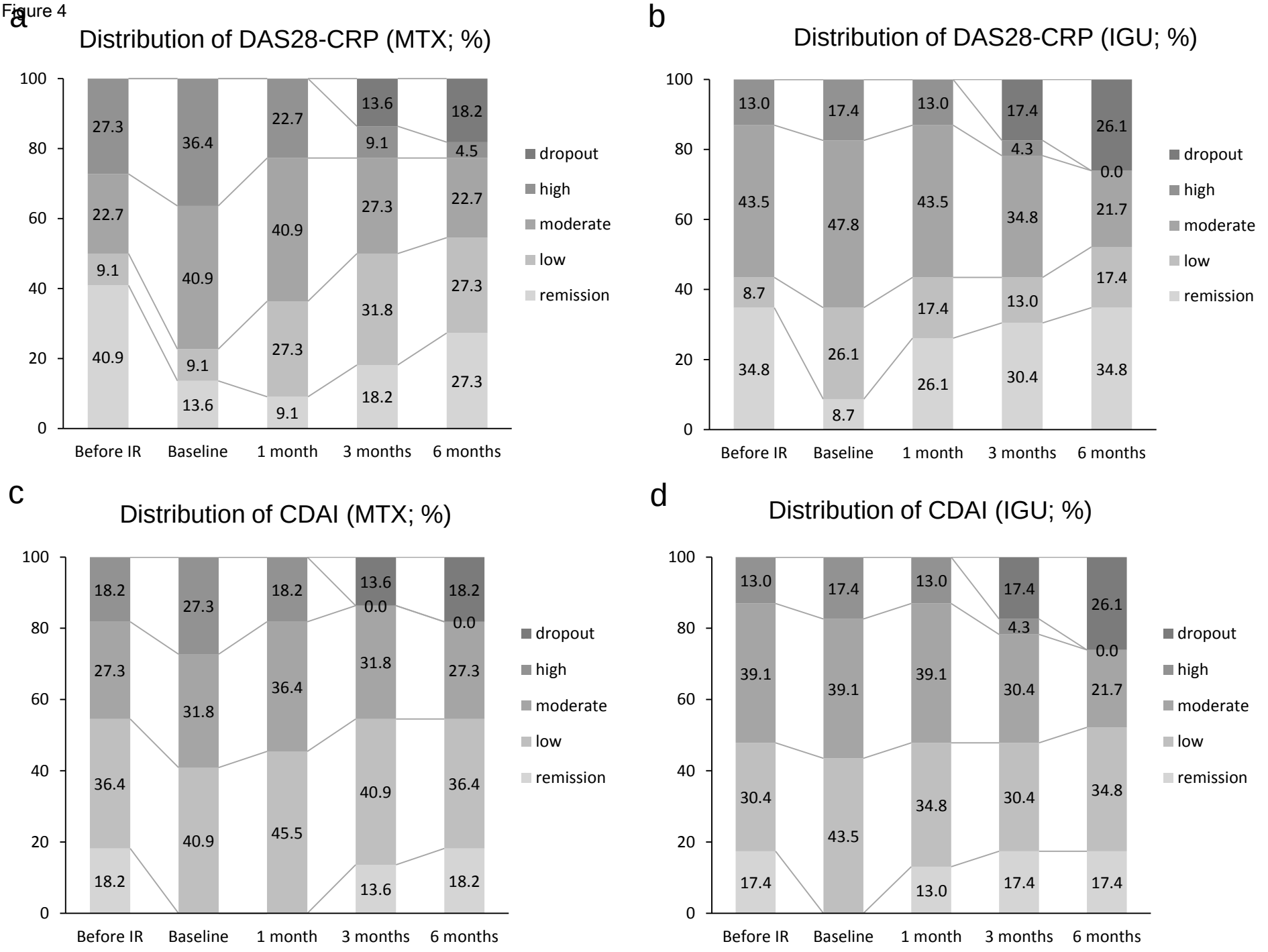

a
DAS28-CRP
New addition of MTX or IGU
IGU (n = 23)
MTX (n = 22)
Remission < 2.3
Before IR
Baseline
1 month
3 months
6 months
b
CDAI
New addition of MTX or IGU
IGU (n = 23)
MTX (n = 22)
Low disease activity < 10
c
ACR 20 response rate (%)
IGU (n = 23)
MTX (n = 22)
21.7
27.3
26.1
45.5
39.1
40.9
d
EULAR response rate (MTX; %)
77.3
13.6
9.1
13.6
40.9
22.7
22.7
18.2
36.4
22.7
22.7
dropout
no response
moderate response
good response
e
EULAR response rate (IGU; %)
73.9
13.0
13.0
17.4
56.5
8.7
17.4
26.1
34.8
17.4
21.7

Figure 4


a
Distribution of DAS28-CRP (MTX; %)
100
80
60
40
20
0
27.3
22.7
9.1
40.9
36.4
40.9
9.1
13.6
22.7
40.9
27.3
9.1
13.6
9.1
27.3
31.8
18.2
18.2
4.5
22.7
27.3
27.3
Before IR
Baseline
1 month
3 months
6 months
dropout
high
moderate
low
remission
b
Distribution of DAS28-CRP (IGU; %)
13.0
43.5
8.7
34.8
17.4
47.8
26.1
8.7
13.0
43.5
17.4
26.1
17.4
4.3
34.8
13.0
30.4
26.1
0.0
21.7
17.4
34.8
c
Distribution of CDAI (MTX; %)
18.2
27.3
36.4
18.2
27.3
31.8
40.9
18.2
36.4
45.5
13.6
0.0
31.8
40.9
13.6
18.2
0.0
27.3
36.4
18.2
d
Distribution of CDAI (IGU; %)
13.0
39.1
30.4
17.4
17.4
39.1
43.5
13.0
13.0
39.1
34.8
13.0
17.4
4.3
30.4
30.4
17.4
26.1
0.0
21.7
34.8
17.4