EXTENDED REPORT

An expanded population of pathogenic regulatory T cells in giant cell arteritis is abrogated by IL-6 blockade therapy

Chie Miyabe, Yoshishige Miyabe, Klemen Strle, Nancy D Kim, John H Stone, Andrew D Luster, Sebastian Unizony

ABSTRACT

Objectives Randomised-controlled trials have recently proven the efficacy of the interleukin (IL)-6 receptor antagonist tocilizumab (TCZ) in giant cell arteritis (GCA). However, the mechanism of action of IL-6 blockade in this disease is unknown. Moreover, the role of regulatory T (Treg) cells in the pathogenesis of GCA remains underexplored. Given the plasticity of Tregs and the importance of IL-6 in their biology, we hypothesised that TCZ might modulate the Treg response in GCA. We therefore characterised the Treg compartment of patients with GCA treated with TCZ.

Methods We classified 41 patients with GCA into three groups: active disease (aGCA, n=11), disease remission on corticosteroids (rGCA-CS, n=19) and disease remission on TCZ (rGCA-TCZ, n=11). Healthy controls (HCs) were included for comparison. We determined the frequency, phenotype and function of peripheral blood Tregs.

Results Patients with aGCA demonstrated a hypoproliferating Treg compartment enriched in IL-17-secreting Tregs (IL-17+Tregs). Tregs in patients with aGCA disproportionally expressed a hypofunctional isoform of Foxp3 that lacks exon 2 (Foxp3Δ2). Foxp3Δ2-expressing Tregs coexpressed CD161, a marker commonly associated with the Th17 lineage, significantly more often than full-length Foxp3-expressing Tregs. Compared with those of HCs, GCA-derived Tregs demonstrated impaired suppressor capacity. Treatment with TCZ, in contrast to CS therapy, corrected the Treg abnormalities observed in aGCA. In addition, TCZ treatment increased the numbers of activated Tregs (CD45RA⁻Foxp3-high) and the Treg expression of markers of trafficking (CCR4) and terminal differentiation (CTLA-4).

Conclusions TCZ may exert its therapeutic effects in GCA by increasing the proliferation and activation of Tregs, and by reverting the pathogenic Treg phenotype seen during active disease.

INTRODUCTION

Giant cell arteritis (GCA) is the most frequent primary vasculitis in Western countries. The main histopathological feature of the disease comprises a granulomatous inflammatory process rich in CD4⁺T cells and macrophages that involves large-sized and medium-sized arteries. Most patients develop relapsing courses despite prolonged treatments with corticosteroid (CS), which invariably lead to drug-related toxicity. Agents that maintain disease remission and spare the use of CS are therefore the greatest unmet need for this patient population.

An imbalance among CD4⁺T helper (Th)1, Th17 and regulatory T (Treg) cells is thought to contribute to the pathogenesis of GCA. Patients with new-onset disease demonstrate Th1 and Th17 cell infiltrates in their arteries and an expansion of these cell subsets in peripheral blood. Conversely, decreased numbers of Tregs in the peripheral circulation are found in patients with GCA, regardless of the state of disease activity. Although the Th17 axis is sensitive to CS treatment, some reports suggest that the abnormalities described in both the Th1 and Treg subsets are resistant to CS therapy, thereby accounting for the high relapse rate in GCA following CS tapering.

The interleukin (IL)-6 pathway is a novel target in GCA. Patients with GCA demonstrate increased IL-6 RNA expression within inflamed arteries and elevated IL-6 protein levels in the peripheral blood during active disease. Recently, two randomised controlled trials showed that tocilizumab (TCZ), a monoclonal antibody against the IL-6 receptor (IL-6R), is effective in maintaining disease remission in absence of CS. However, the mechanism of action of IL-6 signalling blockade in GCA remains unknown.

Considerable phenotypical and functional plasticity exists within the Treg and the Th17 cell subsets. Th17 cells and Tregs develop from a common naïve CD4⁺T cell precursor under the influence of transforming growth factor-β (TGF-β). In the presence of proinflammatory mediators (eg, IL-6 or IL-21), TGF-β-stimulated CD4⁺T cells differentiate into Th17 cells, whereas in the absence of an inflammatory microenvironment these TGF-β-stimulated precursors are induced to become Tregs. Furthermore, under specific circumstances, fully differentiated Tregs may lose their suppressive function and become IL-17-producing cells (eg, pathogenic Tregs and exFoxp3 Th17 cells).

One mechanism regulating the divergent fates between Tregs and Th17 cells involves the molecular antagonism of RAR-related orphan receptor (ROR) γt (RORC in humans) by Foxp3 through the domain encoded by the exon 2 of the FOXP3 gene. Tregs that express a spliced variant of Foxp3 lacking exon 2 (Foxp3Δ2) are less suppressive, and more likely to become IL-17 producing Tregs.
Increased numbers of Foxp3Δ2+Tregs have been reported in anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis.24 It is not known, however, whether this abnormality is also present in patients with GCA. In addition, cells that express both Foxp3 and IL-17 have been detected in inflamed GCA arteries,19 but whether this cell population is present in peripheral circulation, and most importantly, whether disease treatment reverts the pathogenic phenotype of these Tregs has been insufficiently studied.

We aimed to characterise the regulatory CD4+ T cell compartment in peripheral blood of patients with GCA and to investigate the effects of IL-6R blockade therapy with TCZ on the frequency, phenotype and function of those cells.

### MATERIALS AND METHODS

#### Study population

We evaluated 41 patients with GCA in a cross-sectional study. Patients with GCA were classified into one of three categories based on disease activity and treatment: active disease (aGCA, n=11), disease remission on CS monotherapy (rGCA-CS, n=19) and disease remission on TCZ therapy (rGCA-TCZ, n=11). Among the subjects with aGCA, three had new-onset disease and eight were sampled during a disease relapse. We also evaluated samples from 10 healthy controls (HCs). Upon diagnosis, all patients had been treated with CS according to the standard of care for GCA.1 Patients in the TCZ group (rGCA-TCZ group) received their IL-6R blockade therapy because of relapsing disease or prohibitive CS-related toxicity during previous treatment courses. Once on TCZ, patients underwent a prednisone taper of variable rate, but generally faster than the standard of care in order to ameliorate or prevent CS-related toxicity. Other clinical information is provided in the online supplementary text.

#### Cell isolation, culture and flow cytometry

CD4+ T cells were purified (>90% purity) from whole blood using RosetteSep CD4 enrichment antibody cocktail (StemCell Technologies) according to manufacturer’s instructions. Cells were labelled with Pacific Blue-conjugated anti-CD4, fluorescein isothiocyanate-conjugated anti-CD45RA, phycoerythrin (PE)-Cy7-conjugated anti-CCR4, PE-conjugated anti-CTLA4, allophycocyanin (APC)-Cy7-conjugated anti-IL-17A, PE-Cy7-conjugated anti-CD25, PE-conjugated anti-Ki67, APC-conjugated anti-CD161 (BioLegend); Alexa Fluor 488-conjugated anti-Foxp3 and Alexa Fluor 700-conjugated anti-Foxp3. Foxp3Δ2 was detected using clone PCH101 (eBiocience) that recognises the Δ2 isoform of the protein and clone 150D (BioLegend) that recognises exon 2.24 25 Data were acquired on a LSRFortessa cell analyser (BD biosciences) and analysed with FlowJo software.

#### Treg suppression assays

CD4+CD25+ Tregs were isolated from a pool of CD4+ T cells using CD25 MicroBeads (Miltenyi Biotec). CD4+CD25+ conventional T cells were incubated for 10 min at 37°C in 10 μM carboxyfluorescein N-hydroxysuccinimidy1 ester (CFSE) (Invitrogen), washed with phosphate buffered saline containing 2% fetal calf serum (FCS), and resuspended in complete Roswell Park Memorial Institute (RPMI) medium. CFSE-labelled CD4+CD25+ cells (1×10⁴) were co-incubated with varying concentrations of autologous CD4+CD25+ Tregs to create conventional T cell to T reg ratios of 8:1, 4:1, 2:1 and 1:1. Cultures were stimulated for 4 days with Treg Suppression Inspectior (Miltenyi Biotec), or left unstimulated. Proliferation of conventional CD4+T cells was measured by assessing CFSE dilution by flow cytometry.

#### Statistical analysis

Categorical variables were compared between groups using Fisher’s exact test. Continuous variables were compared between groups using paired and unpaired Student’s t-test, Mann-Whitney test, analysis of variance or Kruskal-Wallis test as appropriate. In order to account for confounders on the number of specific CD4+ T cell subsets (eg, CS dose) we used linear regression. Statistical significance cut-off was 0.05. p Values were two-sided. Stata V13 (StataCorp LP) was used for all analyses.

### RESULTS

#### Baseline characteristics of patients with GCA and HCs

We aimed to characterise the regulatory CD4+ T cell compartment, and impaired T reg proliferation has been implicated in the pathogenesis of autoimmunity.31 Thus, we investigated the proliferative capacity of Tregs in patients with GCA and HCs by measuring the expression of Ki67, a marker of the most terminally differentiated activated effector T cells (see online supplementary figure S1A, B). We then classified Tregs into three functionally distinct subpopulations based on the level of expression of Foxp3 and CD45RA.26 (1) CD45RA+Foxp3high (activated Treg, aTreg), (2) CD45RA−Foxp3low (resting Treg, rTreg) and (3) CD45RA−Foxp3low (non-suppressive Foxp3low cells) cells (figure 1A). We observed that the mean per cent of aTregs was significantly greater in patients in the rGCA/TCZ group (1.3% (SD 0.9)) compared with patients in the rGCA-CS group (0.6% (SD 0.4)) (p=0.01) (figure 1B). There were no significant differences among groups in terms of rTregs and non-suppressive Foxp3low cells (see online supplementary figure S2). The phenotype of cellular activation observed in Tregs derived from patients in the rGCA-TCZ group was then confirmed by measuring on all CD4+Foxp3+ cells the expression of CCR4 and CTLA-4, markers of the most terminally differentiated activated effector Tregs.26–30 (figure 1B). The differences between patients in the rGCA-TCZ group and patients in the rGCA-CS group in terms of the numbers of aTregs, CD4+Foxp3+CCR4+ cells and CD4+Foxp3+CTLA-4+ cells remained statistically significant after analyses adjusted for age and CS dose. As expected, rTregs demonstrated higher expression of CD25, CCR4 and CTLA-4 when compared with rTregs and non-suppressive Foxp3low cells (figure 1C). These findings demonstrate that in patients with GCA, remission maintenance with IL-6 blockade therapy is associated with increased Treg activation.

#### TCZ increases the frequency of activated Tregs

We first measured the population of Tregs defined as CD4+ T cells expressing Foxp3 and found no significant differences among groups (see online supplementary figure S1A, B). We then classified Tregs into three functionally distinct subpopulations based on the level of expression of Foxp3 and CD45RA.26 (1) CD45RA+Foxp3high (activated Treg, aTreg), (2) CD45RA−Foxp3low (resting Treg, rTreg) and (3) CD45RA−Foxp3low (non-suppressive Foxp3low cells) cells (figure 1A). W e observed that the mean per cent of aTregs was signifi- cantly greater in patients in the rGCA/TCZ group (1.3% (SD 0.9)) compared with patients in the rGCA-CS group (0.6% (SD 0.4)) (p=0.01) (figure 1B). There were no significant differences among groups in terms of rTregs and non-suppressive Foxp3low cells (see online supplementary figure S2). The phenotype of cellular activation observed in Tregs derived from patients in the rGCA-TCZ group was then confirmed by measuring on all CD4+Foxp3+ cells the expression of CCR4 and CTLA-4, markers of the most terminally differentiated activated effector Tregs.26–30 (figure 1B). The differences between patients in the rGCA-TCZ group and patients in the rGCA-CS group in terms of the numbers of aTregs, CD4+Foxp3+CCR4+ cells and CD4+Foxp3+CTLA-4+ cells remained statistically significant after analyses adjusted for age and CS dose. As expected, rTregs demonstrated higher expression of CD25, CCR4 and CTLA-4 when compared with rTregs and non-suppressive Foxp3low cells (figure 1C). These findings demonstrate that in patients with GCA, remission maintenance with IL-6 blockade therapy is associated with increased Treg activation.

#### TCZ restores the impaired proliferative capacity of Tregs

Tregs are among the most actively replicating cells within the CD4+T cell compartment, and impaired Treg proliferation has been implicated in the pathogenesis of autoimmunity.31 Thus, we investigated the proliferative capacity of Tregs in patients with GCA and HCs by measuring the expression of Ki67, a...
Table 1  Characteristics of the patients with GCA and the healthy individuals at baseline

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<th>aGCA (n=11)</th>
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Analysis: Analysis of variance, Kruskal-Wallis, Student’s t-test and Fisher’s exact test.
*Controls versus all patients with GCA.
†Indicates MR angiography, CT angiography or positron emission tomography.
‡rGCA-CS versus rGCA-TCZ.

dGCA, active GCA; CS, corticosteroids (prednisone); GCA, giant cell arteritis; MTX, methotrexate; rGCA-CS, GCA in remission on CS; rGCA-TCZ, GCA in remission on TCZ without or without CS; TCZ, tocilizumab.

Figure 1  TCZ therapy increases the numbers of activated regulatory T (Treg) cells. CD4+ T cells were purified from peripheral blood of patients with GCA and healthy controls (HCs) by negative selection. (A) Representative flow cytometry plots of CD4+ T cells classified according to the expression of CD45RA and Foxp3 in (1) resting Tregs (rTregs, subset I), (2) activated Tregs (aTregs, subset II) and (3) non-suppressive Foxp3low cells (subset III). (B) Frequencies of aTregs, CD4+Foxp3CCR4+ cells and CD4+Foxp3CTLA-4+ cells in patients with GCA (rGCA-TCZ, n=9; rGCA-CS, n=18; aGCA, n=11) and HCs (n=10). (C) Representative histograms showing the expression of CD25, CCR4 and CTLA-4 in rTregs (I), aTregs (II) and non-suppressive Foxp3low cells (III). Analysis: Student’s t-test. Error bars represent means and SD. aGCA, active GCA; CS, corticosteroids; GCA, giant cell arteritis; rGCA-CS, GCA in remission on CS; rGCA-TCZ, GCA in remission on TCZ without or without CS; TCZ, tocilizumab.

TCZ decreases the numbers of Foxp3Δ2+ Tregs

During Treg ontogeny, the exon 2 of Foxp3 directly inhibits key transcription factors that drive the Th17 cell differentiation programme. Recently, less suppressive Tregs that disproportionately express Foxp3Δ2 have been reported in human autoimmune disease. We therefore analysed the expression of full-length Foxp3 and Foxp3Δ2 in proliferating Treg cells in patients with GCA and HCs (figure 3A). We observed that the mean per cent of Ki67+Foxp3Δ2+Tregs in patients with active disease (15.4% (SD 6.4); p=0.02) and patients in the aGCA group (16.8% (SD 3.5); p=0.04) (figure 3A, B). These differences persisted in CS dose- and age-adjusted analyses. The expression of Ki67 in CD4+Foxp3+ T cells, however, did not differ significantly among groups (see online supplementary figure S3). These results suggest that Treg proliferation is impaired in GCA and that TCZ, in contrast to CS, selectively restores the Treg replicative potential without influencing the proliferation of non- regulatory CD4+ T cells.

**Figure 2** TCZ therapy restores impaired regulatory T (Treg) cell proliferation. (A) Representative flow cytometry plots of Ki67+ T cells in patients with GCA and healthy controls (HCs). (B) Frequencies of Ki67+ T cells within CD4+Foxp3+ T cells in patients with GCA (rGCA-TZC, n=5; rGCA-CS, n=7; aGCA, n=5) and HC (n=5). Analysis: Student’s t-test. Error bars represent means and SD. aGCA, active GCA; GCA, giant cell arteritis; rGCA-CS, GCA in remission on CS; rGCA-TZC, GCA in remission on TCZ without or without CS; TCZ, tocilizumab.

**Figure 3** TCZ reduces the numbers of Foxp3Δ2+ Tregs

Because our data showed that proliferating Tregs derived from patients with active disease preferentially expressed Foxp3Δ2, and these cells were also characterised by CD161 co-staining, we examined the Treg production of IL-17 (figure 4A). We found that the mean per cent (SD) of IL-17+Tregs in aGCA, rGCA-CS, rGCA-TZC and HC was 4.40% (1.29), 2.68% (1.36), 1.29% (1.69) and 1.94% (1.14), respectively (figure 4B). Whereas no significant differences in the numbers of IL-17+Tregs existed between HCs and patients in the rGCA-TZC group (p=0.39), patients in the rGCA-TZC group demonstrated significantly lower numbers of IL-17+Tregs than patients in the aGCA group (p<0.01) and a trend towards fewer of these cells compared with patients in the rGCA-CS group (p=0.06). The differences among GCA groups persisted in CS dose- and age-adjusted analyses. In concordance with prior reports, the main source of IL-17 within the Treg population of patients with active disease resided in the CD45RA Foxp3low non-suppressive cell subset (figure 4C, D). These results demonstrate that the IL-17-producing Treg

IL-17-producing Tregs may also express other Th17-related markers such as CD161. For this reason, we further characterised the phenotype of proliferating Tregs that either expressed full-length Foxp3 or Foxp3Δ2 by measuring the coexpression of CD25 and CD161 (figure 3C). We found that Tregs that expressed full-length Foxp3 coexpressed high amounts of CD25 (ie, CD25high) significantly more often than TH2 Tregs expressing Foxp3Δ2 (mean 74.88% (SD 13.26) vs 39.63% (SD 18.39); p<0.01). In contrast, Foxp3Δ2-expressing Tregs coexpressed CD161 significantly more often than did full-length Foxp3-expressing Tregs (mean 7.54% (SD 7.00) vs 0.66% (1.17); p<0.01) (figure 3D). The correlation between full-length Foxp3 and CD25 and between Foxp3Δ2 and CD161 was equivalent across all groups (aGCA, rGCA-CS, rGCA-TZC and HC) (see online supplementary figure S4). In summary, Foxp3Δ2-expressing Tregs demonstrated decreased coexpression of CD25 and increased coexpression of CD161, a phenotype that suggests the potential for IL-17 production.
population is expanded in peripheral blood during periods of GCA activity, and that TCZ abrogates this abnormality more efficiently than do CS.

Treg function is impaired in GCA

Previous research has shown that IL-6 may decrease T reg function. In patients with new-onset GCA, however, T regs have been reported to be competent regardless of disease activity or CS treatment. To investigate whether patients with GCA in our cohort had normal or impaired T reg function and to examine whether IL-6R blockade influenced this function, we performed suppression assays coculturing CD4+CD25− conventional T cells and CD4+CD25+ T regs. The results showed no significant differences in T reg function among the GCA groups (figure 5A, B).

However, T regs derived from patients with GCA taken together demonstrated significantly impaired suppressive ability when compared with Tregs derived from HCs (figure 5A, B). To assess for the potential confounding effect of proinflammatory CD25+ effector cells that could have been included in the population of CD4+CD25+ cells used for functional assays, we analysed the number of non-T regs (CD4+CD25+CD45RA− cells) and non-suppressing Foxp3low cells (CD4+CD25+CD45RA− cells) in comparison to the number of aT regs (CD4+CD25++CD45RA− cells) and rT regs (CD4+CD25++CD45RA+ cells) within the CD4+CD25+ pool and found no significant differences among groups (see online supplementary figure S5).

**DISCUSSION**

We sought to characterise the peripheral Treg compartment in GCA and to evaluate whether IL-6R blockade was associated with modulation of the Treg response. Our results showed that patients with active disease have a defective and likely pathogenic T reg population that demonstrates decreased proliferation, overexpression of Foxp3Δ2 and increased IL-17 production. In addition, our study revealed a mechanism by which IL-6 signaling inhibition may exert its therapeutic effects in GCA. Unlike therapy with low to moderate doses of CS, treatment with TCZ restored the Treg proliferative capacity, reverted the pathogenic T reg phenotype (Foxp3Δ2 and IL-17 expression) and increased the expression of markers of T reg activation, trafficking and terminal differentiation (Foxp3high, CD25high, CCR4 and CTLA-4).

Foxp3 largely controls the phenotype and function of Tregs. Three variants of Foxp3 have been described, a full-length and two spliced forms (Foxp3Δ2 and Foxp3 lacking exon 2 and 7 (Foxp3Δ2Δ7)). Although the regulation and function of Foxp3Δ2 and Foxp3Δ2Δ7 are poorly understood, exon 2 is known to encode a repression domain that blocks the
activity of the transcription factors RORγt (RORC in humans) and RORα, which are involved in the differentiation of CD4+ cells towards the Th17 phenotype.22 32 37 Foxp3Δ2 is regarded as a hypofunctional isoform of Foxp323 32 and increased expression of this spliced variant has been reported as a mechanism of immune dysregulation in ANCA-associated vasculitis.24 Herein, we demonstrate for the first time that Tregs in patients with GCA preferentially express Foxp3Δ2 over full-length Foxp3. Moreover, we show that Foxp3Δ2 Tregs are often CD161hiCD25low which suggests potential for IL-17 production.20 We therefore predict that Foxp3Δ2 Tregs in GCA lose their suppressive function, and themselves become pathogenic as a source of IL-17.

The functional plasticity of Tregs is highly dependent on the surrounding microenvironment,17 18 20 and the stability of Tregs is thought to play a role in the pathogenesis of inflammatory disorders.20 21 38 39 IL-17-producing Tregs have been detected in inflamed tissues of patients with autoimmune conditions such as rheumatoid arthritis, in which IL-6 may induce Tregs to become IL-17+ cells.20 In some cases, IL-17-producing Tregs seem to ‘relax’ their suppressive function,20 but in others, they retain full regulatory capacity.40 41 Of note, IL-17Foxp3Δ2 cells have also been found infiltrating arteries of patients with GCA.10 However, their functional characterisation, contribution to disease pathogenesis and response to treatment have not been fully elucidated. Here we show that IL-17-producing Tregs are also present in peripheral blood of patients with GCA during periods of disease activity, and that their expansion normalises following IL-6R blockade therapy. In accordance to prior reports, we found that IL-17-producing Tregs in GCA also express other markers commonly associated with the Th17 lineage (eg, CD161)20 and reside within the CD45RA−CD161hiCD25low cell subset.20 26 Because we observed that TCZ led to pronounced reduction of both, Foxp3Δ2 and IL-17 expression, we speculate that by a yet undefined mechanism, IL-6 promotes the transcription of Foxp3Δ2 in Tregs with subsequent polarisation of these cells towards the Th17 phenotype. The function of IL-17-producing Tregs and Foxp3Δ2 Tregs in GCA remains to be determined.

The reasons why Samson et al8 found reduced frequencies of functional Tregs in patients with GCA and we did not are not apparent. Possible explanations include differences in the methods used to isolate Tregs as well as differences in the characteristics of the populations analysed. Our cohort was composed of patients with GCA with long disease duration and prolonged CS exposure (mean 27 months). In contrast, the cohort studied by Samson et al was comprised of newly diagnosed patients, whose CS treatment was relatively short (mean 3.4 months). It is possible that early phases of the disease are characterised by decreased numbers of competent Tregs, which tend to normalise in number over time, but become functionally deficient under the influence of chronic inflammatory stimuli or prolonged CS exposures.

Tregs from patients with GCA undergoing TCZ therapy did not demonstrate enhanced ability to suppress the proliferation of conventional T cells despite the increased expression of effector molecules (eg, CTLA-4). Although this apparent discrepancy could represent a type II error, other possibilities also exist. First, an augmented regulatory response can be achieved not only by increasing Treg function, but also by increasing the trafficking of Tregs to the sites of inflammation. CCR4 is
Figure 5 Regulatory T (Treg) cell function in patients with GCA and healthy controls (HCs). 10^6 CFSE-labelled CD4^+CD25^− conventional T cells stimulated with anti-CD3/CD28 antibodies were incubated for 4 days with varying concentrations of autologous CD4^+CD25^− Tregs (HC: HCs; GCA: GCA) to create ratios of 8:1, 4:1, 2:1 and 1:1. Proliferation of conventional T cells was measured by determination of CFSE dilution by flow cytometry. (A) Conventional T cell proliferation plots from representative patients with GCA and HCs (conventional T cell to Treg ratio 1:1). (B) Suppression assays in patients with GCA (rGCA-TCZ, n=4; rGCA-CS, n=9; sGCA, n=4) and HCs (n=5). Analysis: Student’s t-test. Error bars represent means and SD. Asterisks denote statistically significant differences compared with HCs. sGCA, active GCA; GCA, giant cell arteritis; rGCA-CS, GCA in remission on CS; rGCA-TCZ, GCA in remission on TCZ without or without CS; TCZ, tocilizumab; T eff = conventional T cells.

Correction notice This article has been corrected since it published Epub ahead of print. The fifth sentence of the introduction has been amended and co-author details have been updated.

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Basic and translational research


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