Abstract

Objective. Treatment with CTLA-4Ig blocks T-cell activation and is clinically effective in RA. However, it is unknown if specific CD4⁺ T-cell subsets in blood at baseline predict remission after CTLA-4Ig, or other biological treatments with different modes of action, and how treatment affects CD4⁺ T cells in patients with untreated early RA (eRA).

Methods. This study included 60 patients with untreated eRA from a larger randomized trial. They were treated with methotrexate combined with CTLA-4Ig (abatacept, n = 17), anti-IL6 receptor (tocilizumab, n = 21) or anti-TNF (certolizumab-pegol, n = 22). Disease activity was assessed by clinical disease activity index (CDAI), DAS28, swollen joint counts, tender joint counts, CRP and ESR. The primary outcome was CDAI remission (CDAI ≤2.8) at week 24. Proportions of 12 CD4⁺ T-cell subsets were measured by flow cytometry at baseline and after 4, 12 and 24 weeks of treatment.

Results. In patients treated with CTLA-4Ig, the proportions of PD-1⁺ TFh and CTLA-4⁺ conventional CD4⁺ T cells at baseline predicted CDAI remission at week 24. CD4⁺ T-cell subset proportions could not predict remission after treatment with anti-IL6R or anti-TNF. The percentage of regulatory T cells (Tregs) expressing CTLA-4 decreased in all treatment arms by 24 weeks, but only CTLA-4Ig treatment significantly reduced the proportions of Tregs and PD-1⁺ T follicular helper (TFh) cells.

Conclusion. These findings indicate that circulating proportions PD-1⁺ TFh and CTLA-4⁺ conventional CD4⁺ T cells at baseline may serve as predictive biomarkers for remission in early RA after CTLA-4Ig treatment.

Key words: Rheumatoid arthritis, CD4⁺ T cells, remission, CTLA-4Ig, anti-IL6R, anti-TNF

Introduction

RA is a chronic autoimmune disease that is characterized by synovial inflammation and subsequent joint destruction if not treated early and effectively [1]. In RA, CD4⁺ T cells have been identified as key promoters of disease pathology through genetic association studies as well as functional in vivo and in vitro studies (reviewed in [2, 3]). However, the contribution of specific CD4⁺ T-cell subsets in RA inflammation is not fully understood. In patients with untreated early RA (eRA) (a subset of patients included in the present study), we have previously shown that circulating proportions of T helper (Th) 2 and Th17 cells as well as CTLA-4⁺CD4⁺ T cells are higher in patients compared with healthy controls [4]. We also showed that baseline proportions of Th2, Th17 and Th1Th17 as well as CTLA-4⁺CD4⁺ T cells...
correlate with disease activity in male but not female patients [5].

The introduction of biological DMARD (bDMARD) has resulted in improved clinical outcomes for individuals diagnosed with RA. In patients with established RA, successful treatment with bDMARDs has been shown to alter the proportions of specific T-cell subsets in the circulation. Treatment with CTLA-4Ig (abatacept), an inhibitor of T-cell activation that blocks co-stimulatory receptors CD80/86 on antigen presenting cells [6], has been shown to reduce the proportions of both activated Tfh, Th17 and regulatory T cells (Treg) [7, 8]. Anti-IL6 receptor (tocilizumab) or soluble TNF receptor (etanercept) treatment were found to increase the proportion of Tregs and reduce the proportion of Th17 cells [9, 10]. However, the findings are not consistent as others have shown that anti-IL6R does not affect proportions of Th17 cells [11], and that TNF blocking therapy does not alter proportions of Tregs [12]. The effects of bDMARDs on CD4+ T-cell subset proportions in patients with established RA are, however, difficult to compare due to differences in disease duration and treatment history, as well as inconsistent markers used to define the T-cell subsets. Moreover, most studies have not compared the effects of different bDMARDs head-to-head. In untreated eRA patients, the comparative effects of different bDMARDs on circulating proportions of specific CD4+ T-cell subsets have never been studied.

Despite the introduction of bDMARDs, <50% of eRA patients achieve strict remission (CDAI < 2.8) within one year of treatment [13, 14]. Thus far, no useful biomarkers for selection of targeted bDMARDs or prediction of remission have been discovered [15]. Because bDMARDs have distinct modes of action, different biomarkers may be required to predict treatment outcome for each type of bDMARD. As CTLA-4Ig functions as an inhibitor of T-cell activation, circulating proportions of certain CD4+ T-cell subsets might serve as biomarkers for remission after CTLA-4Ig treatment.

In a cohort of 60 untreated eRA patients treated with CTLA-4Ig, anti-IL6R or anti-TNF, we examined whether CD4+ T-cell subsets at baseline could predict remission after 24 weeks of treatment, and we also compared the effects of the three therapies head-to-head on proportions of circulating CD4+ T-cell subsets.

**Methods**

**Patients**

This study was done on a subset of patients from three of the four randomized arms in the NORD-STAR trial, a phase four investigator-initiated, randomized, observer-blinded clinical trial [16], and followed 60 individuals with untreated eRA during their first 24 weeks of treatment. Patients' baseline characteristics are shown in Table 1. Patients were diagnosed according to the ACR/EULAR 2010 criteria. No patients had received prior DMARD or corticosteroid treatment. The inclusion criteria were:

- ≥18 years of age,
- ≥2 swollen joints (66 joint count),
- ≥2 tender joints (68 joint count),
- DAS28-CRP of ≥3.2 and a symptom duration <24 months (retrospective patient-reported symptoms).

Patients also had to be RF-positive or ACPA-positive or have a CRP level ≥10 mg/l. All patients were recruited at the rheumatology clinics at Sahlgrenska University Hospital (n = 46) or at Skåne University Hospital (n = 14), Sweden. All study participants gave informed consent and the regional ethics committees of Stockholm (Dnr. 2011/2069–31/4) and Gothenburg (Dnr. 691–12 and amendment T270-13) approved the study protocol. The study was conducted in compliance with the Helsinki Declaration and all patients gave written informed consent.

**Study format and clinical evaluation**

The complete treatment protocol and results from the primary clinical outcome at week 24 in the full NORD-STAR cohort have been published elsewhere [16]. In brief, patients who fulfilled the inclusion criteria were randomized to four different treatment arms. As the focus of this study was to investigate the effect of targeted treatments, only patients treated with biological DMARDs were analysed. All patients received MTX escalated to 25 mg/week within the first 4 weeks. In addition to MTX, patients received CTLA-4Ig (abatacept; Bristol Myers Squibb, New York City, NY, USA), anti-IL6R (tocilizumab; Hoffmann-La Roche, Basel, Switzerland) or anti-TNF (certolizumab-pegol; UCB, Brussels, Belgium). Oral steroids were not allowed. The primary clinical outcome was the achievement of remission according to the Clinical Disease Activity Index (CDAI) at week 24 [17]. Disease activity was assessed at baseline (before the start of treatment), and after 4, 12 and 24-weeks of treatment by the following parameters: swollen joint counts in 28 and 66 joints (SJC28 and SJC66, respectively), tender joint counts in 28 and 68 joints (TJC28 and TJC68, respectively), CRP, ESR, CDAI and DAS28 [18]. ACPA and RF positivity was determined at the start of the study by multiplexed anti-CCP test (BioPlex from BioRad, Hercules, CA, USA) and nephelometry (Beckman Coulter, Brea, CA, USA), respectively, and positivity was determined according to cut-off levels in the local clinical immunology laboratories. Blood samples were drawn at baseline (within 1–2 weeks of RA diagnosis) and after 4, 12 and 24 weeks of treatment. T-cell subset proportions were analysed by flow cytometry.

**Flow cytometry**

To reduce variability, all flow cytometry analyses were performed by the same staff at the Clinical Immunology Laboratory and Transfusion Medicine at the Sahlgrenska University Hospital in Gothenburg. Peripheral blood mononuclear cells (PBMCs) were separated from whole blood by the use of Lymphoprep (Axis-Shield, Oslo, Norway). In fresh PBMC, T-cell subsets were defined and characterized by the use of flow cytometry, as previously
Baseline characteristics of untreated early RA patients in each respective treatment arm

|                         | MTX + CTLA-4Ig (n = 17) | MTX + anti-IL6R (n = 21) | MTX + anti-TNF (n = 22) | P-value |
|-------------------------|--------------------------|--------------------------|--------------------------|---------|
| Age, years\(^a\)        | 61 (21-77)               | 51 (25-72)               | 60.5 (21-71)             | 0.22\(^d\) |
| Symptom duration, months\(^a,b\) | 6 (2-23)                | 6 (1.5-21)               | 4.5 (1-18)               | 0.62\(^d\) |
| Female sex, n (%)        | 12 (71)                  | 14 (67)                  | 13 (59)                  | 0.79\(^d\) |
| Smoker (%)\(^a\)         | 2 (12)                   | 4 (19)                   | 4 (18)                   | 0.83\(^d\) |
| CRP, mg/L\(^a\)          | 10 (2-92)                | 6 (0.3-25)               | 18.5 (2-180)             | 0.04\(^c\) |
| ESR, mm/h\(^a\)          | 28 (8-101)               | 19 (5-37)                | 32.5 (7-98)              | 0.03\(^c\) |
| SJC66\(^a\)              | 8 (3-19)                 | 12 (3-17)                | 11.5 (3-28)              | 0.23\(^c\) |
| TJC68\(^a\)              | 13 (3-25)                | 12 (2-47)                | 14.5 (2-35)              | 0.60\(^c\) |
| SJC28\(^a\)              | 6 (3-14)                 | 9 (2-13)                 | 7.5 (3-24)               | 0.51\(^c\) |
| TJC28\(^a\)              | 9 (0-13)                 | 8 (0-24)                 | 7.5 (1-27)               | 0.86\(^c\) |
| DAS28-CRP\(^a\)          | 4.9 (3.8-6.5)            | 4.7 (2.7-6.9)            | 5.4 (3.2-8.3)            | 0.14\(^c\) |
| DAS28-ESR\(^b\)          | 5.2 (4.2-7.2)            | 5.2 (2.6-7.1)            | 5.8 (3.6-8.7)            | 0.17\(^c\) |
| CDAI\(^a\)               | 26.0 (14.3-41.7)         | 27.8 (10.5-62.1)         | 28.1 (10.1-68.7)         | 0.77\(^c\) |
| ACPA\(^-\), n (%)        | 15 (88)                  | 18 (86)                  | 17 (77)                  | 0.67\(^c\) |
| RF\(^-\), n (%)           | 12 (71)                  | 18 (86)                  | 13 (59)                  | 0.17\(^c\) |
| ACPA\(^+\) and RF\(^\text{N}\), n (%) | 11 (65)              | 15 (71)                  | 13 (59)                  | 0.70\(^c\) |
| ACPA\(^\text{N\#}\) and RF\(^\text{N\#}\), n (%) | 1 (6)                 | 0 0                      | 5 (23)                  | 0.03\(^c\) |

Abatacept (CTLA-4Ig), tocilizumab (anti-IL6R), certolizumab-pegol (anti-TNF). \(^a\)Median and range. \(^b\)Retrospective patient-reported pain in the joints before RA diagnosis. \(^c\)Difference between treatment arms, Kruskal–Wallis test followed by Dunn’s multiple comparisons. \(^d\)Difference between treatment arms, Fishers exact test. \(^e\)Current daily smoker.

Multivariate factor analysis (SIMCA-P+ software; Umetrics, Umeå, Sweden) by orthogonal projection to latent structures-discriminant analysis (OPLS-DA) was used to investigate the association between circulating proportions of CD4\(^+\) T-cells subsets and whether patients did, or did not, achieve remission (CDAI \(\leq 2.8\)) at week 24. Log transformation was applied to all variables in multivariate factor analysis to normalize the data, and each variable was scaled by dividing it by one standard deviation (unit variance scaling) to ensure that all variables were given equal weight in the models. The quality of each of the OPLS-DA models was measured by the parameters R2 (i.e. how well the variance of the data is explained by the model) and Q2 (i.e. how well the model predicts the variable data). To avoid the problem of multiple testing, univariate statistical analyses were only applied to the variables that contributed most to each of the respective OPLS models. The univariate analyses performed were Mann–Whitney U test, paired Wilcoxon matched-pairs signed rank test, Spearman rank correlation test, Kruskal–Walls test followed by Dunn’s multiple comparison test (GraphPad Prism Software, La Jolla, CA, USA) and Fisher’s exact test (IBM SPSS Statistics, Armonk, NY, USA) as described in the respective figure legends. Logistic regression,
Results

Biological treatments display distinct effects on circulating proportions of T-cell subsets in patients with untreated eRA

Baseline characteristics for untreated eRA patients in each treatment arm are shown in Table 1. There were no significant differences in median age or in symptom duration between treatment arms. Composite measures of disease activity DAS28 and CDAI, as well as TJC, SJC and number of ACPA+ and/or RF+ patients did not differ significantly between treatment arms. However, baseline CRP, ESR and number of ACPA neg and RF neg patients were higher in the anti-TNF arm than in the MTX arm (Fig. 2A–B). The proportions of memory CD4+ T cells increased after anti-TNF treatment (P=0.02, data not shown), but resulted in lower proportions of Th1 and CXCR3+Th17 cells at week 24 compared with baseline (Fig. 2A). Treatment with anti-TNF resulted in decreased proportions of Th2 and Th17 cells at week 24 compared with baseline (Fig. 2A). The proportions of memory CD4+ T cells increased after anti-TNF treatment (P<0.0001), but resulted in lower proportions of Th17 and CXCR3+Th17 cells at week 24 compared with baseline (Fig. 2B). Treatment with anti-TNF resulted in decreased proportions of Th2 and Th17 cells at week 24 compared with baseline (Fig. 2B). The proportions of memory CD4+ T cells increased after anti-TNF treatment (P<0.0001), but resulted in lower proportions of Th17 and CXCR3+Th17 cells at week 24 compared with baseline (Fig. 2B). Th17 cells at week 24 compared with baseline, while proportions of Th2, Th1Th17 and CXCR3+Th17 cells (Fig. 2A–B). Thus, despite a significant reduction in disease activity in all treatment arms, the change in circulating proportions of memory CD4+ T cells and several subpopulations thereof differed between treatments.

With regard to follicular T helper and regulatory T cells, CTLA-4lg treatment resulted in reduced proportions of PD1+TFh, Tregs and Tregs expressing CTLA-4 at week 24 compared with baseline, while proportions of TFh increased (Fig. 3A–B). Anti-IL6R treatment decreased the proportions of Tregs expressing CTLA-4 but increased the proportions of TRegs at week 24 compared with baseline (Fig. 3A–B). Treatment with anti-TNF resulted in decreased proportions of Tregs expressing CTLA-4 at week 24 compared with baseline, while proportions of TFh, PD-1+TFh and TFregs increased (Fig. 3A). Thus, the change in

Fig. 1 Difference in disease activity at baseline and after 24 weeks

RA disease activity as measured by the Clinical Disease Activity Index (CDAI) at baseline (0w) and at 24 weeks (24w) in patients treated with (A) methotrexate (MTX) + CTLA-4lg (abatacept, n=17), (B) MTX + anti-IL6 receptor (tocilizumab, n=21) and (C) MTX + anti-TNF (certolizumab-pegol, n=22). Dotted line indicates limit for remission (CDAI≤2.8). Paired Wilcoxon matched-pairs signed rank test *P ≤0.05, **P ≤0.01, ***P ≤0.001 and ****P ≤0.0001.
circulating T-cell subset proportions markedly differed between treatment arms, although the fraction of Tregs that express CTLA-4 decreased from baseline to week 24 in all treatment arms.

Circulating proportions of specific CD4⁺ T-cell subsets at baseline predict remission in patients treated with CTLA-4Ig

We subsequently examined whether proportions of CD4⁺ T-cell subsets at baseline could predict remission (CDAI ≤ 2.8) at week 24 in each treatment arm, respectively. OPLS-DA was used to identify T-cell subset proportions that associated with remission at week 24. T-cell subsets that showed the strongest relation to remission status in OPLS-DA were also evaluated by univariate analysis and logistic regression analysis, presented as ROC curves, to assess their diagnostic value. OPLS-DA showed that multiple T-cell subsets associated with remission in the CTLA-4Ig treatment arm (Fig. 4A). In an OPLS-DA loading plot, the X-variables (T-cell subset proportions at baseline) extend in either a positive or negative direction to indicate their association with remission (binary Y-variable) at week 24. Univariate analyses showed that proportions of PD-1⁺TFh and NonTregs that express CTLA-4 were significantly higher at baseline in patients who achieved CDAI remission at week 24 than those who did not (Fig. 4B). In ROC analysis, the AUC for the proportions of PD-1⁺TFh and NonTregs that express CTLA-4 were in both analyses 0.8 (CI 0.57–1.0 and 0.59–1.0, respectively) (Fig. 4C). When both subsets PD-1⁺TFh and NonTregs expressing CTLA-4 were combined into a single model, AUC increased to 0.89 (CI 0.72–1.0) and sensitivity increased to 89% with no reduction in specificity (Fig. 4C). Therefore, higher proportions of these subsets in combination at baseline would indicate a higher probability of remission in patients who are treated with CTLA-4Ig. OPLS-DA models for the anti-IL6R and anti-TNF arms showed poor association to CDAI remission (Fig. 5A–B).
However, in the anti-IL6R treatment arm the proportion of Tregs that express CTLA-4 was significantly higher in patients who achieved remission than in those who did not (Fig. 5C). The ROC curve for the proportion of Tregs expressing CTLA-4 at baseline had an AUC of 0.81 (CI 0.62–1.0), but the specificity was only 60% (Fig. 5D). The anti-TNF arm contained the majority of the double seronegative (ACPAneg and RF neg) patients. When these patients were removed from the OPLS-DA model, a Q2 value of –0.29 was obtained, and when seronegativity was added as a parameter in the original model, Q2 decreased to –0.23 (data not shown). A negative Q2 value indicates that a model is not predictive and, therefore, unreliable. Thus, the anti-TNF OPLS-DA model was not improved by either removing or adding seronegativity as a component in the analysis. In conclusion, our findings show that circulating proportions of specific T-cell subsets may predict remission in untreated eRA patients treated with MTX combined with CTLA-4Ig, but not with anti-IL6R or anti-TNF.

**Discussion**

The use of bDMARDs has improved clinical outcomes for individuals diagnosed with RA, but less than half of eRA patients achieve remission within one year of treatment [13, 14]. Quantitative biomarkers for the optimal selection of targeted treatment are needed to further improve outcomes. Circulating proportions of specific T-cell subsets were predictive of clinical remission in eRA patients treated with MTX plus CTLA-4Ig, but not in those treated with anti-IL6R or anti-TNF.
CD4⁺ T-cell subsets may represent usable biomarkers for CTLA-4Ig selection in untreated eRA. For the first time, we here investigated the effect of three different bDMARDs head-to-head on circulating proportions of CD4⁺ T-cell subsets in patients with untreated eRA and if specific CD4⁺ T-cell subsets could predict remission in patients treated with CTLA-4Ig and compared with two other biological treatment modalities. We report that CTLA-4Ig, anti-IL6R and anti-TNF treatment display distinct effects on multiple CD4⁺ T-cell subsets but all decreased the proportions of CTLA-4⁺ Tregs. We also demonstrate that the proportions of PD-1⁺TFh cells and NonTregs expressing CTLA-4 at baseline may predict remission in untreated eRA patients treated with CTLA-4Ig, but not anti-IL6R or anti-TNF.

In the present study, the disease activity decreased in all three treatment arms. In line with the reduced level of inflammation in the patients, we found significantly reduced proportions of CTLA-4⁺ Tregs across all treatment arms. This is likely a result of a less need for T-cell regulation when the inflammatory activity is lower. CTLA-4 is expressed by activated Tregs and it is a crucial part of Treg suppressor function [19], and animal studies have shown that Tregs require IL-2 to maintain suppressor function [20]. Indeed, patients with eRA have increased plasma concentration of IL-2 compared with healthy controls [21]. Therefore, decreased inflammation and plasma levels of IL-2 may contribute to the reduced proportions of CTLA-4⁺ Tregs after treatment with CTLA-4Ig, anti-IL6R and anti-TNF.

CTLA-4Ig treatment resulted in reduced proportions of total memory CD4⁺ T cells and several of the analysed T helper subsets. Indeed, CTLA-4Ig inhibits T-cell activation and subsequent differentiation into effector phenotypes by blocking the interaction between CD28 on T cells and co-stimulatory receptors CD80/86 on antigen presenting cells [6]. Specifically, we found that proportions of Th17, CXCR3⁺Th2, CXCR3⁺Th17 and PD-1⁺TFh cells decreased significantly, while the proportions of TFh increased. Note that PD-1 expression by TFh indicate recent antigen exposure and that PD-1⁺TFh is the activated population of the TFh subset [22].

(A) OPLS-DA column loading plot showing the association between remission (binary Y-variable) and T-cell subset proportions (X-variables) in patients treated with methotrexate (MTX) + CTLA-4Ig. (B) Comparison of circulating proportions of PD-1⁺TFh and conventional CD4⁺ T cells (NonTregs) expressing CTLA-4 at baseline in patients who did or did not achieve remission (CDAI≤2.8) at week 24. Mann–Whitney U-test, *P ≤0.05. Bars indicate median. (C) ROC curves of the proportions of PD-1⁺TFh and NonTregs expressing CTLA-4 at baseline, in individual regression models (dotted and dashed lines, respectively) or combined in one multiple regression model (black line). AUC: area under the curve; CI: 95 percent confidence interval; PPV: positive predictive value (PPV); NPV: negative predictive value.
In line with our findings, CTLA-4Ig treatment reduced the proportions of Th17 cells and activated TFh cells in patients with established RA [7, 23], and decreased the proportions of PD-1+TFh and CCR7negPD-1+TFh also decreased after 1 year of CTLA-4Ig treatment in patients with recent onset type 1 diabetes [25]. However, these studies did not measure proportions of Th2, CXCR3+Th2 or CXCR3+Th17 cells. We found that CTLA-4Ig treatment also reduced the proportions of Tregs. Similarly, CTLA-4Ig treatment in patients with established RA results in a decrease in proportions of Tregs [26].

The only T helper subset proportions that were significantly reduced by IL-6 blocking therapy were the Th17 and CXCR3+Th17 subsets. Indeed, IL-6 is a primary inducer of Th17 differentiation [27], and eRA patients have increased proportions of Th17 cells in circulation and elevated plasma concentration of IL-6 compared with healthy controls [4, 21]. Thus, decreased proportions of Th17 and CXCR3+Th17 cells in eRA patients treated with anti-IL6R is likely a result of suppressed Th17 differentiation. Treatment with anti-TNF resulted in increased proportions of CD4+ memory T cells and several T helper subsets. In concordance with our findings, one study showed increased proportions of CD4+ memory T cells and TFh cells after anti-TNF treatment, but opposite to our study they also found increased Th17 proportions [7]. The latter may be due to differences in response to anti-TNF therapy, as proportions of Th17 cells decrease in patients who respond to anti-TNF therapy, while Th17 proportions increase in non-responders [28]. Reduced migration into inflamed joints may contribute to the increased proportions of CD4+ memory T cells after anti-TNF therapy, as transendothelial migration is mediated in part by endothelial expression of the adhesion molecule VCAM-1 that is induced by TNF [29].

We hypothesized that circulating proportions of CD4+ T-cell subsets might be used as biomarkers for selection of CTLA-4Ig treatment. We here show that baseline proportions of PD-1+TFh cells and NonTregs expressing CTLA-4 predicted remission in untreated eRA patients who were treated with CTLA-4Ig. Furthermore, we found that the PPV and NPV improved when proportions of PD-1+TFh cells and NonTregs expressing CTLA-4 were combined into a single prediction model. In contrast, baseline CD4+ T-cell subset proportions could not predict remission after anti-IL6R or anti-TNF treatment. In established RA, a previous study that compared the effect of CTLA-4Ig, anti-IL6R and anti-TNF on T-cell proportions showed that baseline proportions of TFh correlate with the reduction in disease activity only in patients treated with CTLA-4Ig [7]. CTLA-4Ig also appears more effective in seropositive RA patients [30, 31], which can be explained by the fact that TFh are specialized B-cell supporting cells that mediate B-cell activation and antibody production. Indeed, CTLA-4Ig treatment reduces the proportion of switched memory B cells as well as autoantibody levels in RA patients [32].

FIG. 5 The relationship between circulating proportions of T-cell subsets at baseline and remission at week 24

OPLS-DA column loading plots showing the association between remission (binary Y-variable) and T-cell subset proportions (X-variables) in patients treated with (A) MTX + anti-IL6 receptor (tocilizumab) or (B) MTX + anti-TNF (certolizumab-pegol). (C) Comparison of circulating proportions of regulatory T cells (Tregs) expressing CTLA-4 in patients who did or did not achieve remission by week 24 (anti-IL6 receptor arm). Two tailed Mann–Whitney U-test, *P <0.05. Bars indicate median. (D) ROC curve of the proportion of regulatory T cells (Tregs) expressing CTLA-4 at baseline for prediction of remission by week 24. AUC: area under the curve; CI: 95 percent confidence interval; PPV: positive predictive value; NPV: negative predictive value.
The use of a clearly defined cohort of treatment-naive eRA patients should be considered the first strength of this study. Secondly, for the first time we compare the effects of three different bDMARDs on circulating proportions of CD4⁺ T-cell subsets head-to-head in a randomized clinical trial. Lastly, by analysing fresh PBMC, we avoid the effect of freezing and/or ex vivo stimulation that may have contributed to inconsistent T-cell data in prior studies. However, analysis of peripheral blood may not be fully representative of immunological changes in the joints. The prediction analyses also did not account for other possibly predictive factors, such as age, sex, autoantibodies or smoking status. Finally, the use of a binary outcome variable, i.e. remission, prevents measurement of a possible dose response relation between the predictor and the outcome.

Conclusion

While CTLA-4Ig, anti-IL6R and anti-TNF therapy all result in a significant reduction in disease activity in untreated eRA patients, they have distinct effects on circulating proportions of CD4⁺ T-cell subsets. Proportions of PD-1⁺TFh cells and of NonTregs expressing CTLA-4 combined may serve as prognostic markers in untreated eRA patients treated with CTLA-4Ig. If these findings can be replicated in a larger cohort, patients more likely to benefit from CTLA-4Ig treatment could be identified earlier and more accurately, potentially reducing treatment costs and improving quality of life.

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Data availability statement

The data underlying this article will be shared on reasonable request to the corresponding author J.A. or A.R.

Supplementary data

Supplementary data are available at Rheumatology online.

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