Pharmacodynamic biomarkers and differential effects of TNF- and GM-CSF-targeting biologics in rheumatoid arthritis

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Abstract

Aim: The aim of our study was to identify pharmacodynamic biomarkers and assess differential effects of tumor necrosis factor (TNF)- and non-TNF-targeting agents on rheumatoid arthritis (RA) patients with an inadequate response to anti-TNF agents (anti-TNF-IR) in comparison with biologic-naïve patients.

Methods: EARTH EXPLORER 2, a phase IIb trial, evaluated golimumab, an anti-TNF antibody, and mavrilimumab, an granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor antibody, in disease-modifying antirheumatic drug (DMARD)-IR and anti-TNF-IR patients. Our current study assessed peripheral protein markers and gene expression levels in association with clinical response post-treatment in two disease strata.

Results: Serum proteomics results indicated the existence of specific pharmacodynamic markers for golimumab and mavrilimumab, regardless of prior anti-TNF treatment. In contrast, both antibodies induced early and sustained suppression of RA disease markers, including interleukin (IL)-6, C-reactive protein, IL2RA, and matrix metalloproteinase 1, in DMARD-IR patients. Golimumab-induced early changes rapidly returned toward baseline concentrations in anti-TNF-IR patients, whereas mavrilimumab-induced changes were maintained through to day 169. RNA sequencing demonstrated gene expression changes at day 169 after administration of mavrilimumab but not golimumab in anti-TNF-IR patients. Additionally, receiver operating characteristic curve and regression analysis showed the association of early IL-6 change and subsequent clinical responses to golimumab in anti-TNF-IR patients.

Conclusion: Our results revealed golimumab- and mavrilimumab-specific pharmacodynamic biomarkers, and demonstrated differential biomarker-treatment relationships in anti-TNF-IR and DMARD-IR patients, respectively. Early IL-6 change after anti-TNF antibody treatment may be a potential predictive biomarker for selection of different treatment regimens in anti-TNF-IR patients.

KEYWORDS

biological therapies, biomarkers, GM-CSF, rheumatoid arthritis, TNF
1 INTRODUCTION

Treatment of rheumatoid arthritis (RA) patients with anti-tumor necrosis factor (TNF) agents has substantially improved patient outcomes. However, many patients with RA either fail to respond adequately or lose responsiveness to treatment over time. Consortium of Rheumatology Researchers of North America registry analysis shows that around 80% of patients do not achieve remission defined by Clinical Disease Activity Index (CDAI; score ≤2.8) and Disease Activity Score of 28 joints (DAS28; score <2.6) within 1 year of anti-TNF treatment. Treatment options for patients with an inadequate response to anti-TNF agents (anti-TNF-IR) includes switching to an alternative TNF antagonist or switching to a biologic with a different mode of action, such as inhibitors of interleukin-6 (IL-6) receptor, Janus kinase (JAK), B cell-restricted surface antigen CD20, or T cell co-stimulation.

Second-line anti-TNF has been associated with lower healthcare costs and resource utilization than switching to a non-TNF agent. Observational studies and meta-analyses provided inconsistent evidence about the comparative effectiveness of different treatment strategies in anti-TNF-IR patients. Recently, a randomized controlled trial demonstrated that a non-TNF-targeted biologics had better efficacy than an alternative TNF inhibitor in anti-TNF-IR patients. However, little is known about the pathophysiologic pathways modified by different biologics in anti-TNF-IR patients compared with biologic-naïve patients, let alone identifying biomarkers to realize the promise of personalized medicine in this population of RA patients.

EARTH EXPLORER 2 (NCT01715896) was a phase IIb trial designed to compare a TNF antagonist, golimumab, with a non-TNF agent, mavrilimumab, in patients with an inadequate response to a traditional disease-modifying antirheumatic drug (DMARD–IR) and in anti-TNF-IR patients. Mavrilimumab is a fully human monoclonal antibody that inhibits the granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor-α. Several clinical trials have demonstrated its clinical efficacy and acceptable safety profile in RA patients. EARTH EXPLORER 2 indicated similar clinical benefit across measures of disease improvement at day 169 for mavrilimumab (100 mg every other week) and golimumab (50 mg every 4 weeks) in patients with a history of anti-TNF-IR. There are other GM-CSF-targeting agents, such as namilumab and GSK3196165, being developed for the treatments of RA, osteoarthritis, and plaque psoriasis.

Using samples from the head-to-head EARTH EXPLORER 2 trial, we assessed peripheral biomarkers and downstream pathways modulated by golimumab and mavrilimumab in both DMARD–IR and anti-TNF-IR patients. To our knowledge, this is the first translational study to evaluate the pharmacodynamic effects of different biologics in anti-TNF-IR patients, which reveals unique biomarker-treatment relationships and provides valuable insights into mechanisms of TNF- and non-TNF-targeting biologics in two strata of RA patients. In addition, we examine the association between early biomarker change and late clinical response to anti-TNF therapies, which may provide potential predictive utility for the selection of different treatment regimens in anti-TNF-IR patients.

2 MATERIALS AND METHODS

2.1 Clinical samples and study approval

In the phase IIb, exploratory, double-blind, randomized, parallel-group, multicenter study (EARTH EXPLORER 2), 75 DMARD–IR and 63 anti-TNF-IR patients were randomized in a 1:1 ratio to receive subcutaneous dosages of 100 mg of mavrilimumab every other week (n = 70) or 50 mg of golimumab every other week (n = 68), alternating with placebo, in combination with methotrexate (7.5-25.0 mg/ wk), for 24 weeks. Anti-TNF-IR patients were defined as those with an inadequate response, safety issue or intolerance to one or two anti-TNF agents other than golimumab, given for at least 3 months, with the last dose at least 8 weeks prior to first study dosage. The study was conducted in accordance with the principles of the Declaration of Helsinki and the International Council for Harmonisation Guidance for Good Clinical Practice and was approved by the appropriate institutional review boards or independent ethics committees at each site. All patients provided written informed consent.

2.2 Serum proteomics study

Sera from 71 DMARD–IR and 61 anti-TNF-IR RA patients at baseline and at four time points following administration of study treatment were kept frozen at −80°C before shipment to Crescendo Bioscience (San Francisco, CA, USA). An enzyme-linked immunoassorbent assay (ELISA) was used to measure chemokine (C-C motif) ligand 17 (CCL17), chemokine CXC ligand 13 (CXCL13), cluster of differentiation 163 (CD163), intercellular adhesion molecule 1 (ICAM1), and IL-2RA serum levels, and Meso Scale Discovery platform (Meso Scale Diagnostics; Rockville, MD, USA) was used to detect CCL22, IL-6, C-reactive protein (CRP), serum amyloid A (SAA), epidermal growth factor (EGF), vascular endothelial growth factor A (VEGFA), vascular adhesion molecule-1 (VCAM1), leptin, TNF receptor superfamily member 1A (TNFRSF1A), matrix metalloproteinase (MMP) 1, MMP3, resistin, and chitinase-3-like protein 1 (CHI3L1).

2.3 Transcriptome study

PAXgene whole blood samples were collected from RA patients and age-, race-, and sex-matched healthy controls to prepare sequencing libraries by total RNA extraction, ribosomal RNA (rRNA) depletion, and stranded library preparation. Whole transcriptome sequencing was then performed using the Illumina HiSeq2500 platform at GeneWiz LLC (South Plainfield, NJ, USA). Paired-end 101 base pair reads were mapped to the GRCh37/hg19 reference genome using Spliced Transcripts Alignment to a Reference (STAR). and the number of reads mapped to each Ensembl gene was calculated by HTSeq-count. For the differential gene expression analysis, we only utilized genes with more than 20 mapped reads across all samples. Normalized counts of sequence reads mapped to Ensemble genes were compared between baseline and day 169 following administration by
DESeq2. Ingenuity Pathway Analysis was used to identify enriched canonical pathways and upstream transcriptional regulators, which may explain the observed gene expression changes.

2.4 | Statistics

We analyzed serum biomarker changes from baseline using a restricted maximum likelihood-based mixed-effects model, including the fixed, categorical effects of treatment, patient subgroup, visit, and subgroup-treatment-visit interaction, as well as the continuous, fixed covariate of baseline score. Patient-level intercept and visit variables were included as random effects. An unstructured variance matrix was used to model the within-patient errors. Significant difference between treatments was tested using least-square mean changes from baseline at each visit. All analyses were conducted in R using nlme and lsmeans packages.

Spearman correlation tests were used to assess the association between biomarker and clinical score changes, and their statistical significance was determined via the asymptotic t approximation. Simple and multiple linear regression models were fitted using the least-squares approach. The area under the curve (AUC) for the separation of responders and nonresponders was tested by the receiver operating characteristic (ROC) analysis.

3 | RESULTS

3.1 | Differential regulation of serum biomarkers by anti-TNF and anti-GM-CSF receptor agents

We applied a linear mixed-effects model with baseline biomarker level adjustment to investigate post-treatment biomarker changes. Our results demonstrated a significant difference in golimumab- and mavrilimumab-induced changes of three serum proteins in both DMARD-IR and anti-TNF-IR patients (P < 0.05). The concentrations of CXCL13 were reduced by golimumab but not by mavrilimumab, whereas CCL22 was suppressed by mavrilimumab but not by golimumab in RA patients. Although both treatments reduced CCL17 concentrations, a much larger change was observed after administration of mavrilimumab (Figure 1).

Three-way interactions between treatment effects, patient subgroups, and day 85 or 169 were evident in the mixed-effects models for 6 serum proteins (P < 0.05). Both mavrilimumab and golimumab demonstrated early and sustained suppression of IL-6, CRP, CD163, IL-2RA, VEGF, and MMP1 in DMARD-IR patients. However, golimumab-induced early changes returned toward baseline concentrations, whereas mavrilimumab-elicited suppression was maintained through day 169 for anti-TNF-IR patients (Figure 2).

3.2 | Gene expression profiles of DMARD-IR and anti-TNF-IR RA patients in comparison with healthy controls

Using RNA-sequencing technology, we compared whole-transcriptome gene expressions among 20 healthy controls, 68 DMARD-IR patients, and 59 anti-TNF-IR patients at baseline. There were no significant differences in age, sex, or race between these three groups. Interestingly, anti-TNF-IR patients showed significantly greater disease activity scores, including the DAS28-CRP, Health Assessment Questionnaire Disability Index, and swollen joint count, than DMARD-IR patients, whereas concurrent medication profiles were similar between the two groups (Table 1).

The RNA-sequencing study identified 3853 (2463 up, 1390 down) genes in DMARD-IR patients and 2827 (1666 up, 1161 down) genes in anti-TNF-IR patients with dysregulated expression concentrations in comparison with healthy controls (Benjamini-Hochberg P < 0.05). Ingenuity pathway analysis demonstrated that mitochondrial dysfunction and circadian rhythm signaling were disrupted in both disease populations, consistent with their reported roles in RA pathogenesis. Interestingly, multiple immunologic pathways were enriched in dysregulated genes of anti-TNF-IR patients but not DMARD-IR patients, including signaling pathways in T cells, macrophages, and neutrophils (Figure S1).

3.3 | Differential regulation of gene expression by anti-TNF and anti-GM-CSF receptor agents in DMARD-IR and anti-TNF-IR RA patients

Post-treatment analysis demonstrated significant regulation of 1040 and 2129 transcripts in 36 and 32 DMARD-IR patients at day 169 after administration of mavrilimumab and golimumab, respectively. The upstream regulator analysis part of the ingenuity pathway analysis predicted the inhibition of granulocyte colony-stimulating factor (G-CSF), TNF, IL-1B, IL-6, and IL-17A by both mavrilimumab and golimumab in DMARD-IR patients. Strikingly, golimumab had no impact on whole-blood gene expression of 31 anti-TNF-IR patients, whereas mavrilimumab induced significant changes on 1508 transcripts in 28 anti-TNF-IR patients at day 169 after administration (Figure 3). The novel transcriptomic results may underlie the loss of efficacy of alternative TNF inhibitors in anti-TNF-IR patients. In contrast, mavrilimumab-induced suppression of GM-CSF, G-CSF, TNF, IL-6, IL-1B, IL-17A, and IL-21 indicated a sustained effect of GM-CSF blockade on myeloid and T-cell activities in anti-TNF-IR patients, who may have developed tolerance to anti-TNF agents (Figure S2).

3.4 | Predictive biomarker for anti-TNF antibody in anti-TNF-IR patients

The Spearman correlation analysis demonstrated a significant correlation between day 29 IL-6 suppression and day 169 DAS28-CRP reduction after golimumab treatment in anti-TNF-IR patients (ρ = 0.55, P < 0.01). The early IL-6 change was also correlated with later changes of other clinical scores, including Patient Global Assessment of Disease Activity (ρ = 0.56, P < 0.01) and tender joint count (ρ = 0.54, P < 0.01). In contrast, golimumab-induced early IL-6 change was not associated with clinical score improvement in DMARD-IR patients, and mavrilimumab-induced IL-6 change had no association with clinical response in either disease population. Linear
regression analysis demonstrated the significant association between early IL-6 suppression and day 169 DAS28-CRP improvement (Figure 4A), which remained true after adjusting for age, sex, and baseline DAS28-CRP and IL-6 concentrations (Table 2). The ROC curve analysis indicated the feasibility of using early IL-6 suppression to stratify American College of Rheumatology-20 response criteria (ACR20) responders from nonresponders in golimumab-treated anti-TNF-IR patients with an AUC value of 0.83. Similarly, day 29 IL-6 change has the ability to separate ACR50 or ACR70 responders from nonresponders with AUC values of 0.75 and 0.74, respectively (Figure 4B).

**FIGURE 1** Golimumab- and mavrilimumab-specific pharmacodynamic biomarkers in rheumatoid arthritis (RA). A, chemokine (C-C motif) ligand 17 (CCL17) and CCL22 concentrations were reduced by mavrilimumab, whereas B, chemokine CXC ligand 13 (CXCL13) concentrations were suppressed by golimumab in both disease populations. Shown are least-square means and associated standard errors in percentage change from baseline for each post-administration visit. Significant differences between golimumab- and mavrilimumab-induced changes were tested separately for disease-modifying antirheumatic drug inadequate response (DMARD-IR) and anti-tumor necrosis factor inadequate response (anti-TNF-IR) patients. *P < 0.05, **P < 0.01, ***P < 0.001

**4 | DISCUSSION**

Our previous study has demonstrated a suppressive effect of GM-CSF blockade on myeloid cells, including reduced macrophage-derived CCL22 levels following administration of mavrilimumab. CCL17 production was also induced by a GM-CSF-dependent pathway in monocytes/macrophages to mediate inflammation. The current results demonstrate that both CCL22 and CCL17 may serve as specific pharmacodynamic markers for GM-CSF pathway targeting therapies, while CXCL13 was specifically regulated by anti-TNF therapies in RA. CXCL13 is a marker for germinal center activity. Its suppression by golimumab is consistent with the disruptive effects of anti-TNF therapy on lymphoid germinal centers in RA patients.

Multiple GM-CSF-targeting agents are being developed for the treatments of different inflammatory disorders. GSK3196165 is a human anti-GM-CSF mAb developed by MorphoSys AG and sublicensed by GlaxoSmithKline. Phase II trials have been commenced to explore the potential of this mAb for the treatments of RA and osteoarthritis. Namilumab is a human immunoglobulin (IgG)1 anti-GM-CSF mAb developed by Takeda, which is being tested in patients with plaque psoriasis and is being compared with anti-TNF
antibody adalimumab in RA. Those anti-GM-CSF antibodies may regulate peripheral CCL22 and CCL17 levels in RA and other inflammatory disorders in a similar pattern to the effects of mavrilimumab.

Further investigation of both pharmacodynamic biomarkers may be helpful for the pharmacometric modeling and clinical development of GSK3196165 and namilumab for the treatments of inflammatory disorders.

Repeated anti-TNF treatments often cause the development of drug tolerance over time, which may be reflected by the loss of biomarker change early in the treatment regimen. Biomarker changes usually precede symptom onset or improvement after treatments. Although mavrilimumab (100 mg subcutaneously every other week) and golimumab (50 mg subcutaneously every 4 weeks) treatments induced similar clinical responses at day 169, the differential biomarker change in pattern observed at this time point suggests potentially greater long-term efficacy of mavrilimumab than golimumab in RA patients with a history of anti-TNF-IR.
Furthermore, a dosage of 150 mg mavrilimumab demonstrated greater efficacy than a dosage of 100 mg in patients with DMARD-IR. It is reasonable to expect that a higher dosage of mavrilimumab over a longer treatment period may demonstrate a better clinical response than an alternative anti-TNF agent in anti-TNF-IR patients.

The increased immune system disruption seems to be a characteristic feature of RA patients who were refractory to anti-TNF treatments. It has been suggested that higher circulating levels of Th17 cells and IL17A may be predictive biomarkers for anti-TNF-IR patients. Neutrophil granule genes exhibited higher expression in nonresponders than in responders before treatment with anti-TNF agents. Monocyte numbers have been reported to be higher in nonresponders than in responders after 3 or 6 months of anti-TNF treatment. These results suggest enhanced activity of alternative immune pathways in anti-TNF-IR patients that may be targeted for clinical improvement.

One reason for the loss of efficacy is immunogenicity, which caused changes in bioavailability and pharmacokinetics. In this case, a second anti-TNF may be as effective as a non-TNF. However, anti-drug antibodies are not detectable in most anti-TNF-IR patients, in whom alternative immune pathways may be particularly active or enhanced by prior treatment with anti-TNF agents to restore clinical activity that was originally modulated by the anti-TNF antibody.

**TABLE 2** Estimated coefficients and P values of multiple regression analysis

|                         | ∆DAS28-CRP (D169/D1) | ∆IL-6 (D29/D1) | IL-6 (D1) | DAS28-CRP (D1) | Age | Gender |
|-------------------------|----------------------|----------------|-----------|----------------|-----|--------|
| β                       | 0.26                 | 0.018          | −0.58     | 0.015          | 0.11| 0.66   |
| P value                 | 0.003                | 0.79           | 0.3       | 0.1            | 0.66|        |

Least squares multiple regression was used to assess the contribution of age, sex, baseline IL-6 concentration, baseline DAS28-CRP level, and day 29 IL-6 change to day 169 change in DAS28-CRP score after golimumab treatment in anti-tumor necrosis factor inadequate response patients. The early IL-6 change was the only significant factor for the prediction of day 169 DAS28-CRP change.

DAS28-CRP, Disease Activity Score of 28 joints using C-reactive protein.

**FIGURE 3** Differential regulation of transcript expression by golimumab and mavrilimumab in DMARD-IR and anti-TNF-IR patients. Transcriptome sequencing analysis demonstrated gene expression regulation by both treatments in DMARD-IR patients. However, significant expression changes were observed after treatment of mavrilimumab but not golimumab in anti-TNF-IR patients. Downregulated genes are shown in green and upregulated genes are shown in red post-administration of golimumab and mavrilimumab in DMARD-IR and anti-TNF-IR patients, respectively. DMARD-IR, disease-modifying antirheumatic drug inadequate response; anti-TNF-IR, anti-tumor necrosis factor inadequate response

**FIGURE 4** Early IL-6 suppression and subsequent clinical improvement after administration of golimumab in anti-TNF-IR patients. A. Regression lines and 95% confidence intervals drawn on scatter diagrams relating day 29 log2 (fold change) of IL-6 concentration and day 169 log2 (fold change) of DAS28-CRP. B. Receiver operating characteristic curves for day 29 IL-6 change to predict day 169 ACR20 (AUC = 0.83), ACR50 (AUC = 0.75), and ACR70 (AUC = 0.74) responses after golimumab treatments, respectively. ACR20/50/70, American College of Rheumatology-20/50/70 response criteria; AUC, area under the curve; CRP, C-reactive protein; DAS28, 28-joint Disease Activity Score; IL, interleukin; anti-TNF-IR, anti-tumor necrosis factor inadequate response
Redundancy in cytokine circuitry and regulation is likely to underlie the return of protein biomarker concentrations in golimumab-treated anti-TNF-IR patients. The biological effects of alternative TNF inhibitors may also be subdued in anti-TNF-IR patients. Our gene expression study has not discovered any differentially regulated transcript at day 169 after golimumab treatment in anti-TNF-IR patients, while more than 2000 transcripts were found to be regulated by golimumab in DMARD-IR patients. Switching to alternative biologics suppresses those additional mechanisms, such as the GM-CSF pathway, inducing similar or even higher biological effects in anti-TNF-IR compared to DMARD-IR patients. Our results showed differential regulation of 1040 and 1508 transcripts after mavrilimumab treatment in DMARD-IR and anti-TNF-IR patients respectively. Those molecular changes suggested a sustained level of underlying disease control maintained by mavrilimumab in both DMARD-IR and anti-TNF-IR patients. In fact, an open-label extension study has demonstrated long-term efficacy of 100 mg mavrilimumab every other week for up to >3 years across many disease activity parameters.

Taken together, our proteomics and genomics results support the potential advantage of using biologics with a different mode of action for the treatment of anti-TNF-IR patients, such as mavrilimumab, to suppress enhanced myeloid and T-cell activities. Moreover, we showed that the dynamic change in IL-6 may predict subsequent clinical benefit to an alternative anti-TNF agent in anti-TNF-IR patients. Having a blood-based biomarker early in the treatment regimen would allow timely switching to alternative biologics for patients refractory to repeated anti-TNF treatments, reducing long-term joint damage and controlling RA treatment spending. The true clinical utility remains to be confirmed in larger studies of anti-TNF-IR patient cohorts.

In summary, our study revealed golimumab- and mavrilimumab-specific pharmacodynamic biomarkers in RA patients irrespective of prior anti-TNF treatment status, which may be useful for the clinical development of anti-GM-CSF antibodies including GSK3196165 and namlumab. Sustained suppression of RA disease markers and gene expression by mavrilimumab but not by golimumab in anti-TNF-IR patients supported the potential for greater long-term disease control with anti-GM-CSF than a second anti-TNF antibody in this population of RA patients. Early change in serum IL-6 concentration may be predictive of therapeutic response to alternative anti-TNF therapies, providing guidance in the selection of treatment regimens for anti-TNF-IR patients.

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CONFLICT OF INTEREST

XG, SW, AG, DC, PR, LR, and WW are full-time employees of MedImmune and have stock in AstraZeneca.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of the article.

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