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ORIGINAL RESEARCH

# Filgotinib Modulates Inflammation-Associated Peripheral Blood Protein Biomarkers in Adults with Active Rheumatoid Arthritis and Prior Inadequate Response to Methotrexate

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## ABSTRACT

***Introduction***: Our aim was to evaluate protein biomarker changes related to the administration of filgotinib, a Janus kinase (JAK) 1 preferential inhibitor, in patients with moderately to severely active rheumatoid arthritis (RA) with inadequate response to methotrexate.

***Methods***: Plasma and serum samples were collected from patients enrolled in FINCH 1 (NCT02889796), a Phase 3 trial. Patients with stable backgrounds of methotrexate were randomly assigned once-daily oral filgotinib 200 or 100 mg, subcutaneous adalimumab 40 mg every 2 weeks (W), or placebo. Up to 35 biomarkers were analyzed at baseline, W4, and W12 with enzyme-linked immunosorbent assays and chemiluminescence and electrochemiluminescence assays.

***Results***: At baseline, four distinct biomarker clusters were identified. The strongest intragroup correlations were in bone-cartilage resorption/inflammation and JAK/signal transducer and activator of transcription (STAT) signaling activity. At baseline, significant positive correlations were identified for cytokines with patient-reported pain and with patient mea-

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sures of fatigue. Filgotinib reduced levels of cytokines associated with inflammation and cell migration as early as W4 and through W12. Compared to adalimumab, filgotinib induced significant reductions in bone-related turnover biomarkers, N-telopeptide of type 1 collagen and C-telopeptide 1, as well as biomarkers associated with baseline disease activity. No baseline predictors of therapeutic response to filgotinib were identified.

***Conclusions***: Filgotinib reduced peripheral protein biomarkers associated with JAK/STAT signaling, inflammatory signaling, immune cell migration, and bone resorption as soon as W4 in FINCH 1. Effects were dose-dependent and consistent with the clinical efficacy of filgotinib observed in FINCH 1. The changes in peripheral biomarkers associated with filgotinib treatment in methotrexate-experienced patients are consistent with changes observed in both methotrexate-naïve and biologic disease-modifying antirheumatic drug-experienced RA populations. These data demonstrate dose-dependent effects of preferential JAK1 inhibition by filgotinib on peripheral blood protein biomarkers in methotrexate-experienced patients with RA.

***Trial Registration***: ClinicalTrials.gov, NCT02889796.

**Keywords:** Cytokines; Rheumatoid factor; Methotrexate; Biological therapy; Inflammation; Biomarker

**Key Summary Points**

The oral Janus kinase (JAK) 1 preferential inhibitor filgotinib reduced rheumatoid arthritis (RA) signs and symptoms, improved physical function, inhibited radiographic progression, and was well tolerated up to 52 weeks in patients in the FINCH 1 (NCT02889796) study who had moderately to severely active RA and an inadequate response to methotrexate.

This study profiles the changes in peripheral blood protein biomarkers and their relationship with RA disease activity in the FINCH 1 patient population.

The evaluation of peripheral biomarkers in this population helps improve the understanding of multiple effects of preferential JAK1 inhibition by filgotinib on systemic inflammatory processes.

While filgotinib induced significant reductions in N-telopeptide of type 1 collagen and C-telopeptide 1 when compared with adalimumab, the findings overall suggest that the baseline biomarker clusters identified here will not have utility in differentiating responsiveness to JAK inhibition vs. tumor necrosis factor blockade

## INTRODUCTION

The primary goal when treating rheumatoid arthritis (RA) is to establish an absence of disease activity, or remission. Standard therapies for RA include conventional synthetic disease-modifying antirheumatic drugs (csDMARDs), such as methotrexate, which is considered the gold standard for the treatment of RA. In fact, both the European Alliance of Associations for Rheumatology (EULAR) and the American College of Rheumatology guidelines recommend inclusion of methotrexate in first-line therapies for patients with RA [1, 2]. Despite recent advances in RA treatment and management, disease activity tends to persist in many patients even with the use of current therapies. In a registry study of patients with RA of varying duration, fewer than 25% of patients receiving csDMARDs or tumor necrosis factor alpha (TNFα) inhibitors achieved remission after 12 months [3]. Another study in a similar population showed that 25% of patients achieved clinical remission when treated with methotrexate monotherapy over 2 years [4]. Given that csDMARDs may be ineffective for some patients with RA, additional therapies for patients who do not respond adequately to standard-of-care treatments are needed.

Recent research interrogating synovial tissue samples from RA joints by means of single-cell transcriptomics and mass cytometry has

provided further evidence of the critically important role of activated immune cells and fibroblasts, increased cytokine expression, and associated provocative pathways, especially the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway [5]. JAK/STAT signaling is involved in many biologic processes, including those related to immune regulation [6]. Furthermore, several combinations of JAKs and STATs and peripheral cytokines may lead to many downstream biologic effects, highlighting the versatile nature of this pathway [7].

Currently approved JAK inhibitors comprise small-molecular-weight, orally available, targeted synthetic disease-modifying antirheumatic drugs that target members of the JAK family of kinases [7, 8]. EULAR guidelines recommend considering treatment with JAK inhibitors if the treatment target is not achieved with a first-line csDMARD therapy [1]. Baricitinib (a selective JAK1 and JAK2 inhibitor) and tofacitinib (a preferential JAK1 and JAK3 inhibitor) were the first JAK inhibitors to be introduced for the treatment of RA and, when administered with concomitant methotrexate, have demonstrated efficacy comparable to that of biologic anti-TNF and methotrexate. However, subsequent safety studies in patients with RA aged $\geq$ 50 years with at least one cardiovascular risk factor have identified potential differences between biologic anti-TNF and tofacitinib in relative risk for certain adverse event outcomes [9].

Filgotinib, a JAK1 preferential inhibitor approved in Europe and Japan for the treatment of moderately to severely active RA [10], has demonstrated tolerability, safety, and superior efficacy compared with placebo in patients with active RA who had inadequate response to methotrexate (FINCH 1) [11], inadequate response to biologic disease-modifying antirheumatic drugs (bDMARDs; FINCH 2) [12], or limited or no prior exposure to methotrexate (FINCH 3) [13]. Here, we report findings from a longitudinal, hypothesis-generating study to identify biomarkers associated with RA disease activity that were altered by treatment with filgotinib plus methotrexate vs. methotrexate monotherapy and vs. adalimumab plus methotrexate using samples collected from patients enrolled in FINCH 1. This work aimed to further clarify the molecular impact of filgotinib on biomarkers associated with RA pathobiology and to identify any relationships between baseline biomarkers and therapeutic response.

## METHODS

### Study Design

Plasma and serum samples were obtained from patients with active RA who had inadequate response to methotrexate (FINCH 1; NCT02889796) [11]. Patient characteristics, study design, and interventions have all been described previously. Briefly, eligible patients with RA with a stable background of methotrexate were randomly assigned once-daily oral filgotinib 200 or 100 mg, subcutaneous adalimumab 40 mg every 2 weeks, or placebo. The FINCH 1 trial was conducted in accordance with the Declaration of Helsinki and International Council for Harmonisation Good Clinical Practice guidelines, and it was approved by the Advarra Central Institutional Review Board (Reference # 00000971). The study protocol was approved by the international review board or ethics committee at each study site, and all patients provided written informed consent. Plasma samples were obtained at baseline and weeks 4 and 12 and were stored at −80 °C until analyzed. The FINCH 1 study design is shown in Supplemental Figure S1.

### Biomarker Analysis

Biomarkers were evaluated using an enzyme-linked immunosorbent assay and chemiluminescence and electrochemiluminescence assays. Samples were analyzed for biomarkers using validated, commercially available immunoassays from either Meso Scale Discovery (Rockville, MD, USA) or Pacific Biosciences (Menlo

Park, CA, USA). A detailed list of the platforms and analytes used in this experiment are detailed in Supplementary Table S1.

### Data Analysis

Multiscale bootstrap resampling was applied to samples to identify highly correlated biomarker clusters at baseline. Mean changes in biomarker levels from baseline to weeks 4 and 12 were compared between treatment arms using placebo-adjusted estimates from a linear mixed-effects model. Baseline correlations between biomarkers were assessed using a nominal *P* value cutoff of 0.05. Baseline correlations were also made between biomarkers and the following clinical response measures: Disease Activity Score in 28 Joints with C-reactive protein (DAS28[CRP]), swollen joint count of 28 joints (SJC28), tender joint count of 28 joints (TJC28), Clinical Disease Activity Index (CDAI), patient assessment of pain, Physician's Global Assessment of Disease Activity (PHGADA); Patient's Global Assessment of Disease Activity (PTGADA) and Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F). All correlations were determined using Spearman's rank correlation coefficient.

For the active-drug treatment arms, associations between change in biomarker level and change in clinical scores were evaluated using the following linear mixed-effects model, adjusting for potentially confounding baseline covariates (age, sex, and race):

$$\Delta \text{ Disease Activity} \sim \Delta \text{ Protein Biomarker} + \text{Baseline Covariates}$$

Prognostic and predictive biomarkers were assessed using the following linear model for change in disease activity or logistic regression model for binary outcomes, respectively:

$$\text{Disease Activity} \sim \text{Baseline Biomarker} + \text{Baseline Covariates} + \text{Baseline Biomarker} \times \text{Treatment}$$

Biomarker cluster score was calculated using either the geometric mean or first component of principle component analysis for protein levels within each cluster; a similar regression analysis was performed using cluster score as the main effect term. Statistical significance of the coefficient for the "Baseline Biomarker" main effect term was interpreted as evidence of a prognostic biomarker, while a statistically significant coefficient for the "Cluster × Treatment" interaction term suggested the presence of a predictive cluster. All analyses of treatment effects were corrected for multiple testing using a false discovery rate of 0.05. We considered clinical responses at weeks 12 and 24 as dependent variables. Multiple testing correction using a false discovery rate (FDR) was applied within each clinical response and each time point with a threshold of 0.05.

## RESULTS

### Baseline Patient Characteristics

Baseline demographics and disease characteristics of patients from FINCH 1 were balanced across treatment groups and described in detail previously [11]. Overall, 1755 patients received study drug. Most patients were White (67.5%) and female (81.8%), and patients had a mean age of 53 years. Across treatment groups at baseline, mean DAS28(CRP), Simple Disease Activity Index, CDAI, swollen joint count of 66 joints, tender joint count of 68 joints, and FACIT-F scores were 5.7, 40.8, 39.2, 16.0, 24.0, and 27.4, respectively (Supplementary Table S2) [11]. In total, up to 35 biomarkers were analyzed at baseline, week 4, and week 12 in 564 patients ($n$ = 139, filgotinib, 100 mg; $n$ = 149, filgotinib, 200 mg; $n$ = 140, adalimumab; $n$ = 136, placebo).

### Baseline Biomarker Associations

At baseline, four biomarker clusters were identified and annotated based on the known function of the biomarkers investigated: (1) lymphocyte differentiation, (2) lymphocyte migration-related, (3) bone erosion, and (4) cartilage damage and synovial proliferation.

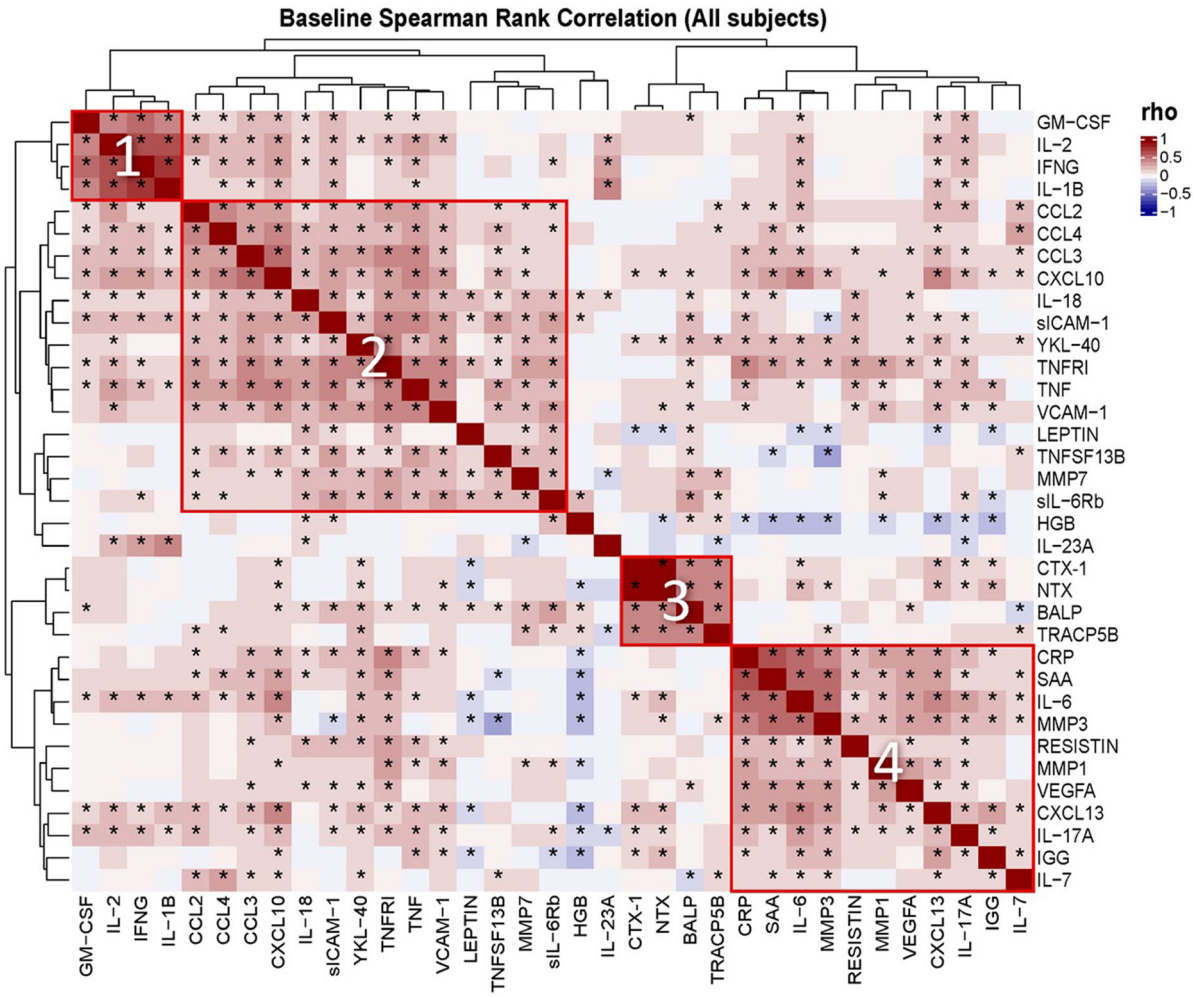
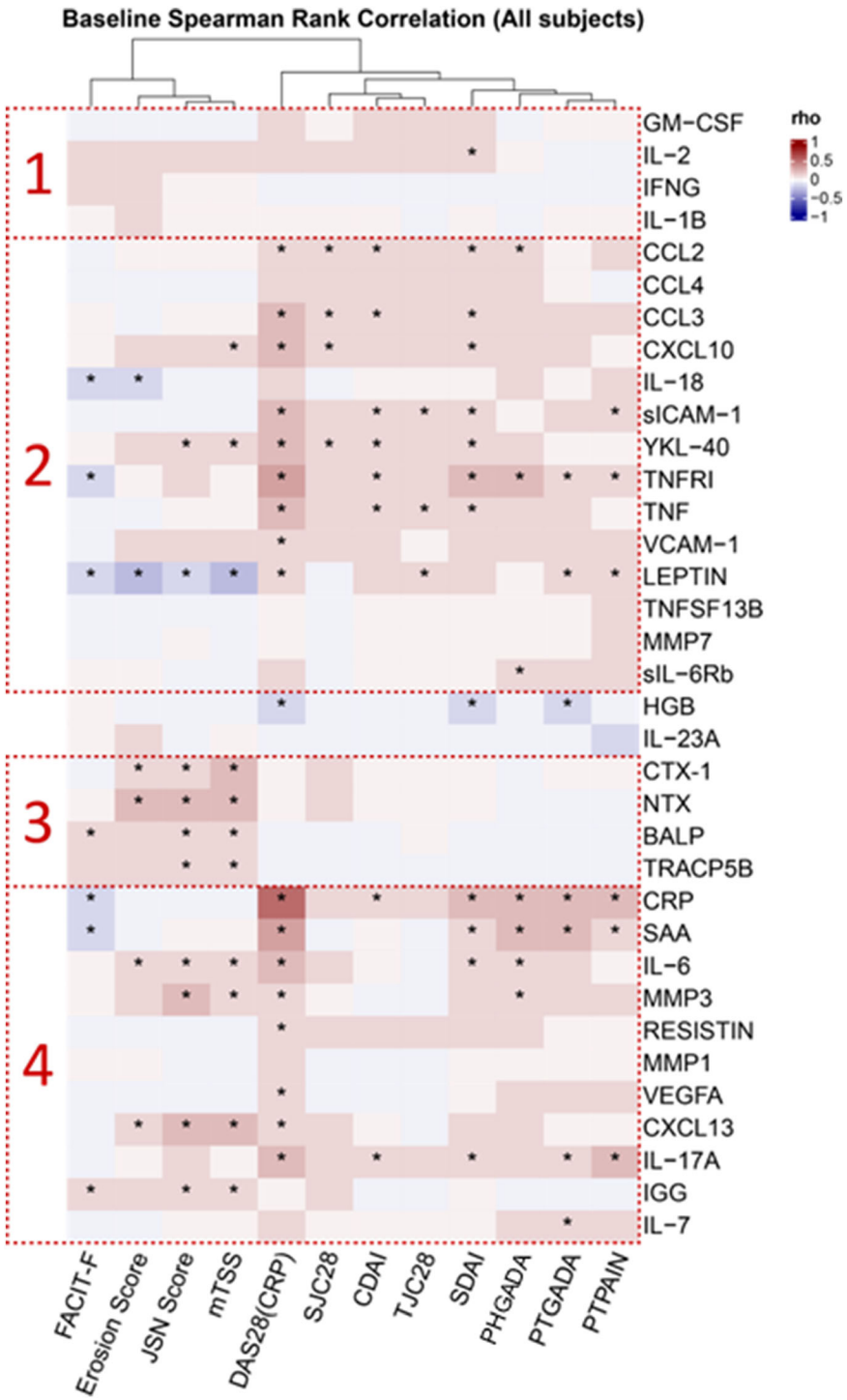



**Fig. 1** Baseline biomarker associations, Spearman rank correlation, all participants. Hierarchical clustering of baseline biomarkers using Ward's minimum variance method identified four statistical clusters: (1) lymphocyte differentiation, (2) lymphocyte migration-related, (3) bone erosion, and (4) cartilage damage and synovial proliferation. *Heatmap color* denotes pairwise Spearman rank correlation coefficients (rho) of baseline biomarkers; *red* and *blue* indicate positive and negative correlations, respectively. * indicates a nominal $P$ value $< 0.05$. *BALP* bone alkaline phosphatase, *CCL* chemokine ligand, *CRP* C-reactive protein, *CTX* collagen cross-linked C-telopeptide, *CXCL* CXC motif chemokine ligand, *GM-CSF* granulocyte–macrophage colony-stimulating factor, *HGB* hemoglobin, *IFNG* interferon gamma, *IGG* immunoglobulin G, *IL* interleukin, *MMP* matrix metalloproteinase, *NTX* cross-linked N-telopeptide of type 1 collagen, *SAA* serum amyloid A, *sICAM* soluble intercellular adhesion molecule, *TNF* tumor necrosis factor, *TNFR1* TNF receptor 1, *TNFSF13B* TNF superfamily member 13b, *TRACP* tartrate-resistant acid phosphatase, *VCAM* vascular cell adhesion protein, *VEGFA* vascular endothelial growth factor A

The strongest intragroup correlations between clusters were found in biomarkers known to be associated with bone-cartilage resorption, inflammation, or signaling via JAK/STAT receptors (Fig. 1). Statistically significant correlations (FDR $P < 0.05$) were identified between 16 non-CRP biomarkers and baseline DAS28(CRP). DAS28(CRP) was positively correlated with the greatest number of biomarkers at baseline. Significant positive correlations were identified between the non-CRP-based disease measure of CDAI and chemokines, including


Baseline Spearman Rank Correlation (All subjects)
rho
1
0.5
0
−0.5
−1
1
2
3
4
GM−CSF
IL−2
IFNG
IL−1B
CCL2
CCL4
CCL3
CXCL10
IL−18
sICAM−1
YKL−40
TNFRI
TNF
VCAM−1
LEPTIN
TNFSF13B
MMP7
sIL−6Rb
HGB
IL−23A
CTX−1
NTX
BALP
TRACP5B
CRP
SAA
IL−6
MMP3
RESISTIN
MMP1
VEGFA
CXCL13
IL−17A
IGG
IL−7
FACIT−F
Erosion Score
JSN Score
mTSS
DAS28(CRP)
SJC28
CDAI
TJC28
SDAI
PHGADA
PTGADA
PTPAIN

◀**Fig. 2** Baseline biomarkers associated with mTSS. Heatmap showing the Spearman rank correlation between baseline biomarkers (*in rows*) and baseline clinical response measures (*in columns*). Tiles in the heatmap are colored based on Spearman rank correlation coefficients; *red* and *blue* indicate positive and negative correlations, respectively. * indicates a nominal $P$ value $< 0.05$. Hierarchical clustering of baseline clinical measures was based on Ward's minimum variance method. Baseline biomarker clustering was kept in the same order as in Fig. 1. Erosion score is a subcomponent of mTSS. *BALP* bone alkaline phosphatase, *CCL* chemokine ligand, *CDAI* Clinical Disease Activity Index, *CRP* C-reactive protein, *CTX* collagen cross-linked C-telopeptide, *CXCL* CXC motif chemokine ligand, *DAS28(CRP)* Disease Activity Score in 28 Joints with CRP, *Erosion Score* subcomponent of mTSS, *FACIT-F* Functional Assessment of Chronic Illness Therapy-Fatigue, *GM-CSF* granulocyte–macrophage colony-stimulating factor, *HGB* hemoglobin, *IFNG* interferon gamma, *IGG* immunoglobulin G, *IL* interleukin, *JSN Score* joint space narrowing (subcomponent of mTSS), *MMP* matrix metalloproteinase, *mTSS* modified total Sharp score, *NTX* cross-linked N-telopeptide of type 1 collagen, *PHGADA* Physician's Global Assessment of Disease Activity, *PTGADA* Patient's Global Assessment of Disease Activity, *PTPAIN* patient assessment of pain, *SAA* serum amyloid A, *SDAI* Simple Disease Activity Index, *SICAM* soluble intercellular adhesion molecule, *SJC28* swollen joint count of 28 joints, *TJC28* tender joint count of 28 joints, *TNF* tumor necrosis factor, *TNFR1* TNF receptor 1, *TNFSF* TNF super family, *TRACP* tartrate-resistant acid phosphatase, *VCAM* vascular cell adhesion protein, *VEGFA* vascular endothelial growth factor A

chemokine ligand (CCL)3, CCL2, CRP, soluble intercellular adhesion molecule 1 (sICAM-1), TNF, YKL-40, TNF receptor 1 (TNFR1), and interleukin 17A (IL-17A). Increased patient-reported pain was positively associated with sICAM-1, IL-17A, TNFR1, LEPTIN, serum amyloid-related protein A (SAA), and CRP. A significant positive association with fatigue (higher FACIT-F measurements represent less fatigue) was identified for TNFR1, CRP, LEPTIN, SAA, and IL-18, and inverse relationships were identified between fatigue and bone alkaline phosphatase (BALP) and immunoglobulin G (IGG). The strongest positive correlation between disease activity measure and baseline biomarkers was between DAS28(CRP) and CRP, with rho = 0.52 (Fig. 2).

A number of inflammatory and matrix remodeling biomarkers were also associated with modified total Sharp score (mTSS) at baseline, including the pleiotropic inflammatory cytokine IL-6, yet association of mTSS with CRP was notably absent. The bone and matrix remodeling markers cross-linked C-telopeptide (CTX), cross-linked N-telopeptide of type 1 collagen (NTX), BALP, and tartrate-resistant acid phosphatase were associated almost exclusively with mTSS and other joint scores at baseline but not with general clinical RA scores. CXC motif chemokine ligand 13 (CXCL13) and CXCL10 were associated with baseline mTSS, perhaps reflecting the stage of damage within tissues and the potential utility of cytokine-targeted treatments.

## Filgotinib Effects on Disease Activity-Associated Biomarkers

IL-6, CXCL13, and TNFα are involved in inflammation and cell migration [14]. Filgotinib was associated with significant dose-dependent reductions in these cytokines at weeks 4 and 12. Patients who received adalimumab also had significant decreases in IL-6, CXCL13, and TNFα at weeks 4 and 12 compared with baseline. IL-6, CXCL13, and TNFα were similar at weeks 4 and 12 compared with baseline in patients receiving methotrexate monotherapy (Fig. 3). Interestingly, the biomarkers related to bone and cartilage turnover NTX and CTX-1 showed a marked reduction after 12 weeks of filgotinib treatment, whereas the effect of adalimumab treatment on these markers was minimal.

## Patterns of Biomarker Response

Statistically significant reductions in multiple biomarkers that were associated with baseline disease activity were observed in patients who received filgotinib at weeks 4 and 12. Figure 4 illustrates the changes in biomarkers from baseline within each treatment arm, and Table 1 lists the significant biomarker changes by study drug treatment compared to the

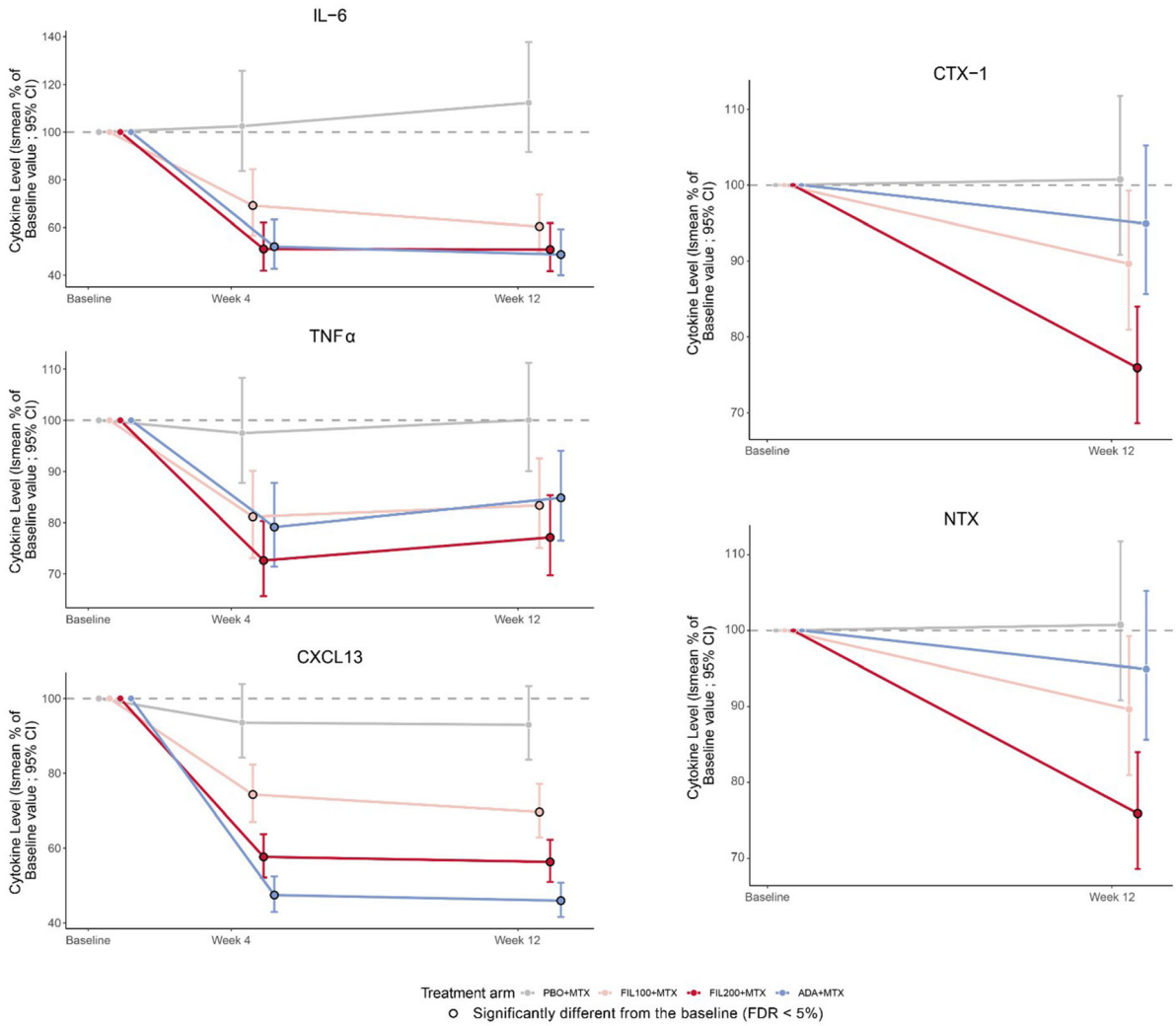



**Fig. 3** Time- and dose-dependent changes in IL-6, TNFα, CXCL13, CTX-1, and NTX. Outlined circles represent statistically significant changes from baseline compared with placebo based on linear mixed-effects models adjusting for age, sex, race, and baseline biomarker level. *ADA* adalimumab, *CI* confidence interval, *CTX-1* collagen cross-linked C-telopeptide 1, *CXCL* CXC motif chemokine ligand, *FDR* false discovery rate, *FIL* filgotinib, *IL* interleukin, *LS* least squares, *MTX* methotrexate, *NTX* N-telopeptide of type 1 collagen, *PBO* placebo, *TNFα* tumor necrosis factor α

placebo group at week 12. Overall, filgotinib treatment showed a broad reduction in systemic inflammatory cytokines over 12 weeks, with marked reductions comparable to adalimumab in IL-6 and SAA. At weeks 4 and 12, filgotinib 200 mg induced a significantly greater reduction in lymphocyte migration-related biomarkers, such as TNFR1, TNF superfamily 13B, IL-18, and YKL-40, when compared with adalimumab. Additional biomarkers exhibiting a stronger reduction with filgotinib 200 mg when compared with adalimumab included IL-23A; RESISTIN (immune cell inflammatory signaling); the synovial inflammation-related markers vascular cell adhesion molecule-1, vascular endothelial growth factor A (VEGFA), and matrix metalloproteinases 1 (MMP-1); and the collagen biomarkers CTX-1 and NTX. No significant changes in these biomarkers were

observed at weeks 4 or 12 in patients who received methotrexate monotherapy.

### No Baseline Predictors of Therapeutic Response Could be Identified

Potential relationships between clinical response to filgotinib and baseline biomarkers were investigated using linear regression modeling. No significant association of individual biomarkers or the four identified biomarker clusters was observed after correction for multiple testing.

## DISCUSSION

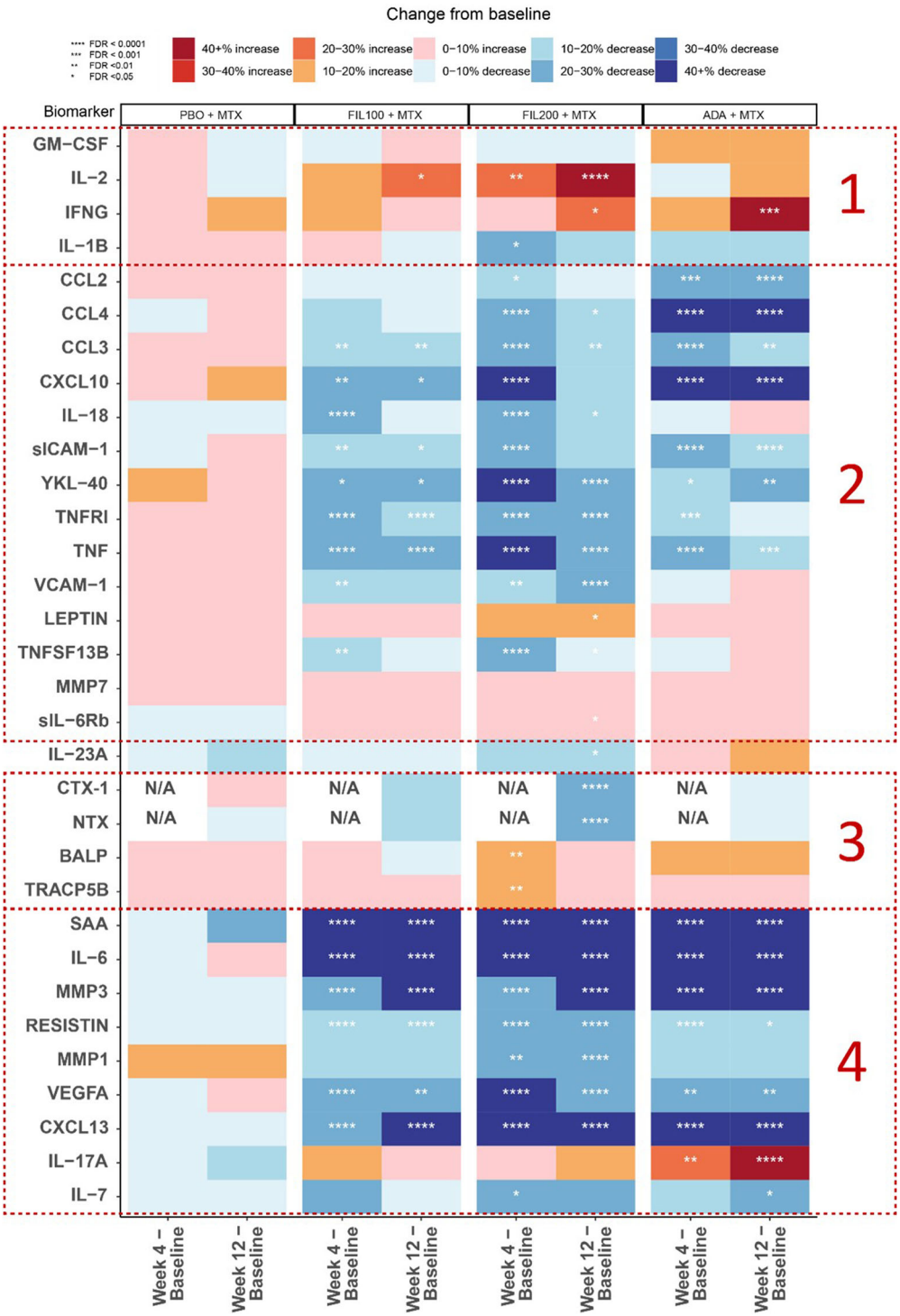
For the last several decades, methotrexate has been the standard-of-care treatment for RA, with approximately 30–40% of patients achieving low disease activity with methotrexate monotherapy [15–17]. In this hypothesis-generating study of patients with active RA who had inadequate response to methotrexate, plasma and serum samples were used to assess the effects of RA treatment (filgotinib or adalimumab with methotrexate) on several protein biomarkers related to disease activity and signaling. The study also assessed the relationship between these biomarkers at baseline and radiographic and disease activity measures associated with RA severity.

Four biomarker clusters were identified at baseline: lymphocyte differentiation, lymphocyte migration, bone erosion, and cartilage damage and synovial proliferation. No association was identified between any of the baseline biomarker clusters and subsequent therapeutic response, suggesting that filgotinib was comparably efficacious across the molecular subtypes characterized in this study.

Consistent with the observed therapeutic efficacy of filgotinib, significant dose-dependent reductions were observed in biomarkers associated with RA disease activity compared with methotrexate alone. Changes in biomarkers associated with JAK/STAT signaling, inflammatory signaling, immune cell migration, and bone resorption were observed as early as week 4, consistent with the emergence of clinical efficacy in the FINCH 1 study [18–20]. Changes for biomarkers contained within the cluster annotated for bone erosion were largest for patients treated with filgotinib 200 mg plus methotrexate compared with those treated with adalimumab plus methotrexate. Of particular note, the bone-related biomarkers NTX and CTX-1 showed a marked reduction after 12 weeks of filgotinib treatment, whereas the effect of adalimumab treatment on these markers was minimal. Both targeted treatment modalities demonstrated significant mean structural damage inhibition in comparison with methotrexate in the FINCH 1 study [11], but these findings may indicate a greater potential for filgotinib over adalimumab for disease modification in rapidly progressing patients [21]. Additionally, the observed improvements in disease activity-associated biomarkers may provide additional insight into the rapid and profound pain relief associated with JAK inhibitor therapy, as JAK inhibition interrupts signaling of multiple cytokines implicated in pain transduction pathways [22].

The changes in peripheral biomarkers associated with filgotinib treatment in methotrexate-experienced patients are consistent with changes observed in both methotrexate-naïve [23] and bDMARD-experienced [24] RA populations. The reductions in IL-6, CXCL13, and TNFα observed in filgotinib-treated patients in this study were also observed in the DARWIN 1 (NCT01888874) and DARWIN 2 (NCT01894516) Phase 2 studies in which patients received filgotinib or placebo as an add-on to methotrexate or as monotherapy [25]. In addition, whole blood samples from patients with RA with prior inadequate response to methotrexate showed that filgotinib mediated disease activity-associated gene expression related to JAK/STAT signaling and pathways associated with T-cell receptor signaling, immune cell cytotoxicity, and migration [26].

The biomarker analysis in this study was only conducted up to the primary endpoint, week 12, precluding conclusions about relations to disease activity or progression that may have manifested only at later time points. Several stratified analyses by poor prognostic factors were also performed as part of this study,


Change from baseline
**** FDR < 0.0001
*** FDR < 0.001
** FDR <0.01
* FDR <0.05
40+% increase
30–40% increase
20–30% increase
10–20% increase
0–10% increase
0–10% decrease
10–20% decrease
20–30% decrease
30–40% decrease
40+% decrease
Biomarker
PBO + MTX
FIL100 + MTX
FIL200 + MTX
ADA + MTX
GM–CSF
IL–2
IFNG
IL–1B
CCL2
CCL4
CCL3
CXCL10
IL–18
sICAM–1
YKL–40
TNFRI
TNF
VCAM–1
LEPTIN
TNFSF13B
MMP7
sIL–6Rb
IL–23A
CTX-1
NTX
BALP
TRACP5B
SAA
IL–6
MMP3
RESISTIN
MMP1
VEGFA
CXCL13
IL–17A
IL–7
N/A
1
2
3
4
Week 4 – Baseline
Week 12 – Baseline

◀**Fig. 4** Pattern of dose- and time-dependent changes in disease activity-associated biomarkers within treatment arms. Heatmap showing the percent change of biomarker values at week 4 and week 12 from baseline by treatment group. *Red* and *blue tiles* indicate an increase or decrease in biomarker values from baseline, respectively. Biomarkers are in *rows*, and treatment groups are in *columns*. Biomarker order is consistent with Fig. 1, and the 4 baseline biomarker clusters are identified using dotted red boxes. N/A represents biomarkers for which no measurements were available. *ADA* adalimumab, *BALP* bone alkaline phosphatase, *CCL* chemokine ligand, *CTX* collagen cross-linked C-telopeptide, *CXCL* CXC motif chemokine ligand, *FDR* false discovery rate, *FIL* filgotinib, *GM-CSF* granulocyte–macrophage colony-stimulating factor, *IFNG* interferon gamma, *IL* interleukin, *MMP* matrix metalloproteinase, *MTX* methotrexate, *N/A* not applicable, *NTX* N-telopeptide of type 1 collagen, *PBO* placebo, *SAA* serum amyloid A, *sICAM* soluble intercellular adhesion molecule, *TNF* tumor necrosis factor, *TNFR1* TNF receptor 1, *TNFSF* TNF super family, *TRACP* tartrate-resistant acid phosphatase, *VCAM* vascular cell adhesion protein, *VEGFA* vascular endothelial growth factor A

including rheumatoid factor (RF) positivity (serum RF > 15 IU/ml) vs. RF negativity (serum RF < 15 IU/ml). Results from these stratified analyses were similar to those of the overall analyses but showed fewer significant associations due to smaller sample sizes (data not shown). This study was also limited by the use of plasma and serum samples as measurements for disease-associated processes related to RA, with the profiling of peripheral samples an imperfect surrogate for inflammatory processes in distal joints and bones. Furthermore, this study did not include patients who experienced prior bDMARD failure or patients who were not previously exposed to methotrexate, limiting the extension of these findings to those populations. Finally, a high proportion of placebo-treated patients (about 50%) achieved low-level efficacy-related endpoints in the clinical study, which has further complicated predictive biomarker analysis.

**Table 1** Significant biomarker changes by study drug treatment compared with placebo at week 12 (FDR <0.01)

| Cluster | Direction | FIL100 | FIL200 | ADA |
|---|---|---|---|---|
| Lymphocyte migration-related | ↓ | **CCL3, CXCL10, TNF, TNFR1** | **CCL3,** CCL4, **YKL-40, TNF, TNFR1,** TNFSF13B, **VCAM-1** | **CCL2, CCL3,** CCL4, **CXCL10, sICAM-1, TNF** |
| Bone erosion | ↓ | | CTX-1, NTX | |
| Cartilage damage and synovial proliferation | ↓ | **CXCL13, IL-6, MMP3, RESISTIN, SAA** | **CXCL13, IL-6,** MMP1, **MMP3, RESISTIN, SAA, VEGFA** | **CXCL13, IL-6,** MMP1, **MMP3, SAA** |
| Lymphocyte differentiation | ↑ | | IL-2 | |
| Cartilage damage and synovial proliferation | ↑ | | | **IL-17A** |
| | ↑ | | | IL-23A |

Biomarkers in bold were associated with DAS28(CRP) at baseline
*ADA* adalimumab, *CCL* chemokine ligand, *CRP* C-reactive protein, *CXCL* CXC motif chemokine ligand, *DAS28(CRP)* Disease Activity Score in 28 Joints with CRP, *FIL* filgotinib, *IL* interleukin, *MMP* matrix metalloproteinase, *NTX* cross-linked N-telopeptide of type 1 collagen, *SAA* serum amyloid-related protein A, *sICAM* soluble intercellular adhesion molecule, *TNF* tumor necrosis factor, *TNFR* TNF receptor, *TNFSF* TNF super family, *VCAM* vascular cell adhesion protein, *VEGFA* vascular endothelial growth factor A, *YKL* tyrosine lysine leucine

## CONCLUSIONS

These data demonstrate dose-dependent effects of preferential JAK1 inhibition by filgotinib on peripheral blood protein biomarkers implicated in the pathobiology of patients with RA who had prior inadequate response to methotrexate. Many of the observed reductions in disease-associated biomarkers over time are consistent with the clinical efficacy demonstrated for both adalimumab and filgotinib treatment. In contrast, the bone-related biomarkers NTX and CTX-1 showed a marked reduction after 12 weeks of filgotinib treatment but minimal change with adalimumab treatment. No baseline biomarkers were significantly associated with therapeutic response to filgotinib.

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***Data Availability.*** Gilead Sciences shares anonymized individual patient data upon request or as required by law or regulation with qualified external researchers based on submitted curriculum vitae and reflecting non conflict of interest. The request proposal must also include a statistician. Approval of such requests is at Gilead Sciences' discretion and is dependent on the nature of the request, the merit of the research proposed, the availability of the data, and the intended use of the data. Data requests should be sent to datarequest@gilead.com.



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