original research

Pharmacodynamic effects of filgotinib treatment driving clinical improvement in patients with active psoriatic arthritis enrolled in the EQUATOR trial

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ABSTRACT

Objectives The goal of this study was to identify protein and transcriptional biomarkers and pathways associated with baseline disease state, the effect of filgotinib (FIL) treatment on these biomarkers, and to investigate the mechanism of action of FIL on clinical improvement in patients with active psoriatic arthritis (PsA).

Methods The phase II EQUATOR (NCT03101670) trial evaluated the efficacy of FIL, a Janus kinase 1-preferential inhibitor, in patients with PsA. Peripheral protein and gene expression levels in association with clinical state at baseline and post-treatment were assessed in 121 patients using linear mixed effects models for repeated measures analyses. Mediation analysis and structural equation modelling (SEM) were performed to investigate the mechanism of action of FIL at week 4 on downstream clinical improvement at week 16.

Results Baseline analyses showed that markers of inflammation were significantly associated with multiple PsA clinical metrics, except for Psoriasis Area and Severity Index (PASI), which corresponded to Th17 markers. FIL treatment resulted in sustained transcriptional inhibition of immune genes and pathways, a sustained increase in B-cell fraction and mature B-cells in circulation, and a transient effect on other cell fractions. Mediation analysis revealed that changes in B cells, systemic inflammatory cytokines and neutrophils at week 4 were associated with changes in clinical metrics at week 16. SEM suggested that FIL improved PASI through reduction of IL-23 p19 and IL-12 p40 proteins.

Conclusions Our results revealed that FIL treatment rapidly downregulates inflammatory and immune pathways associated with PsA disease activity corresponding to clinical improvement in PsA.

Trial registration number NCT03101670.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Filgotinib (FIL), a Janus kinase (JAK1)-preferential inhibitor, simultaneously blocks multiple inflammatory pathways and is efficacious in treating psoriatic arthritis (PsA).

⇒ The molecular mechanism by which FIL improves signs and symptoms of patients with PsA has not been well studied.

WHAT THIS STUDY ADDS

⇒ This large study with 121 patients uses extensive biomarker profiling to demonstrate the mechanism of action of FIL in patients with PsA.

⇒ Treatment with FIL downregulated immune-related pathways, such as IL6-JAK-STAT3, inflammatory response, complement, coagulation and interferon α/γ response pathways.

⇒ FIL treatment-induced changes to inflammatory cytokines, B cells, cholesterol and neutrophil-associated biomarkers at week 4 were associated with changes in several PsA clinical metrics (except Psoriasis Area and Severity Index (PASI)) at week 16.

⇒ FIL improves PASI through reduction of IL-23 p19 and IL-12 p40.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The study provides insights into the mechanisms of action of FIL and JAK1 inhibition in PsA and identifies potential biomarkers of arthritis and skin response to treatment with FIL in PsA.

INTRODUCTION

Psoriatic arthritis (PsA) is a chronic, immune-mediated inflammatory musculoskeletal (MSK) disease associated with cutaneous psoriasis. It affects men and women similarly with a peak age at onset between 40 and 50 years. PsA is a heterogeneous disease affecting multiple organs, including peripheral and axial joints, entheses, skin and nails. PsA is associated with other inflammatory diseases such as uveitis and inflammatory bowel disease and with comorbidities such as metabolic syndrome, diabetes, cardiovascular disease and depression.1 Patients with active PsA are typically treated with conventional
and newer targeted immunomodulatory therapies with the aim of reaching a state of low disease activity or remission.2

Many cytokines contribute to the inflammation of the skin and joints in patients with PsA. The Janus kinase (JAK) family of tyrosine kinases (JAK1, JAK2, JAK3 and TYK2 in humans) are key signalling proteins that are critical for cytokine-mediated intracellular signal transduction.3 Inhibition of JAK proteins has the potential to simultaneously block multiple inflammatory pathways and alleviate disease pathology, with varying efficacy and safety attributed to different JAK family members.4,5

The phase II EQUATOR trial (NCT03101670) was designed to evaluate the efficacy of filgotinib (FIL), a JAK1-preferential inhibitor, in patients with PsA. FIL showed significant improvements in clinical signs and symptoms of PsA versus placebo (PBO) over 16 weeks and was reported to improve quality of life.6,7 While previous studies have shown FIL’s mechanism of action in other diseases such as rheumatoid arthritis, such studies have not yet been conducted in PsA.8 Using peripheral blood samples collected from this study, we aimed (1) to identify proteins, transcriptional biomarkers and pathways associated with baseline disease state and the impact of FIL treatment on these biomarkers in PsA and (2) to investigate the mechanism of action of FIL on clinical improvement.

METHODS

Study population and sample collection

EQUATOR was a 16-week multicentre, phase II, double-blind, PBO-controlled study in subjects with moderate-to-severe active PsA (defined as at least five swollen joints and at least five tender joints) satisfying CLASSification criteria for Psoriatic Arthritis who previously had an inadequate response or were intolerant to conventional disease-modifying therapy.6 Patients were randomised to FIL 200 mg daily or matched PBO at a 1:1 ratio. Blood samples for biomarker analysis were obtained from patients who consented to sample collection. All patients provided informed consent for publication of research results.

Patient and public involvement

Patients and the public were not involved in the design, conduct, reporting or dissemination of this research.

Clinical assessments and outcome metrics

Clinical assessments were performed across all time points (baseline, weeks 1, 4 and 16). Assessments included swollen and tender joint counts (SJC66 and TJC68), Leeds Enthesitis Index, Psoriasis Area and Severity Index (PASI) in patients with at least 3% body surface area affected by psoriasis,6 patient-reported pain, Physician’s Global Assessment of Disease (PGA), the 36-item Short Form Health Survey Physical Component Summary score (SF-36) and Health Assessment Questionnaire-Disability Index. The Psoriatic Arthritis Disease Activity Score (PASDAS) was used to measure overall PsA disease activity and the Disease Activity Index for Psoriatic Arthritis (DAPSA) was used to measure peripheral arthritis.

Whole blood transcriptome sample collection and processing

Whole blood samples from patients were collected using PaxGene tubes across all time points. Illumina TrueSeq Stranded mRNA (2 x 100 bp pair-end) was generated for 452 samples from 121 patients and the Illumina HiSeq V1.5 (BCX2) platform was used for sequencing. Gene-level quantification of RNA-seq counts and Transcripts Per Million (TPM) were conducted using Salmon (v.0.8.2) based on version 25 of the transcriptome from GENCODE. Information on quality control of transcriptomic samples is detailed in online supplemental figures 1 and 2.

Pathway analysis was performed using single sample gene set enrichment analysis (ssGSEA),9 based on Hallmark 50 pathways from the Molecular Signature Database (https://www.gsea-msigdb.org/gsea/index.jsp). Differential expression analyses were conducted using the limma R package; voom-limma at the gene level and limma at the pathway level.10

Peripheral blood protein biomarker collection and processing

Serum samples were collected from patients across all time points and 136 serum biomarkers from MSD, Nordic, Millipore and PacBio were analysed from 121 patients. Of these, 108 biomarkers were within their manufacturer-specified dynamic range and were included for analysis. A full list of peripheral biomarkers, respective platforms and quality control information are detailed in online supplemental tables 1 and 2.

Complete blood count

Complete blood count samples were measured across all time points. In addition, counts of CD19+B cells, CD3+T cells, CD4+T cells, CD8+T cells and CD16+CD56+ NK cells were evaluated using standard flow cytometry at week 4 and week 16.

Baseline analyses

Baseline correlations between clinical metrics and three separate datasets (genes, pathways and peripheral blood biomarkers) were calculated using Spearman rank correlation. False discovery rate (FDR) was controlled using the Benjamini-Hochberg method.

Post hoc analysis of treatment effects

Treatment effects on differential expression on genes, pathways and peripheral blood biomarkers were evaluated, comparing weeks 1, 4 and 16 with baseline, using mixed-effect repeated measures analyses as follows:

\[
\log_2(\text{fold change}) \sim \text{Treatment} + \text{Visit} + \text{age} + \text{sex} + \\
+ \log_2(\text{baseline biomarker value}) + (1 | \text{subject})
\]

The treatment effect of FIL was then calculated using the following contrast:
Using 10 variables, using the SEM function from the lavaan package to perform mediation analyses with more than one mediator and to draw statistical inference of causal association.

Exploratory factor analysis (EFA) was performed to examine common underlying factors among the union of significant gene signatures across all time points compared with baseline. Four lines of evidence were employed to biologically annotate significant factors. First, factor loadings were applied to our proprietary peripheral blood mononuclear cell (PBMC) atlas. Second, the cell specificity for the top five genes within the highest loadings were evaluated. Third, annotations were made using correlations between factors and all possible ssGSEA scores and correlations with blood cell fractions. Lastly, the biological function of the top loading genes in each factor was used to annotate the factors. These derived RNA-seq factors were subsequently treated as additional biomarker variables (see the Causal associations section).

Causal associations of derived factors, peripheral biomarkers and clinical labs to clinical disease scores

Mediation analysis was performed using the mediation package in R, with a nonparametric bootstrap with 5000 Monte Carlo draws. The causal model evaluated whether the clinical improvement by treatment at week 16 could be driven through an indirect path via the biomarker state at week 4. We postulated that each clinical improvement made by JAKI inhibition is driven by changes in the corresponding causal molecular pathway in affected tissues, which although not directly observable, may drive changes in the blood. Thus, biomarkers values at week 4, including all significant derived gene expression markers, pathways, factors, peripheral biomarkers and clinical labs, were grouped together using EFA, and then used to examine downstream associations with clinical changes at week 16. The average causal mediation effect (ACME) was defined as the expected difference in the potential outcome when the mediator took the value that would realise under the treatment condition as opposed to the control condition, while the treatment status itself was held constant. The p value for ACME was used to draw statistical inference of causal association.

Structural equation modelling (SEM) was used to perform mediation analyses with more than one mediator variable, using the SEM function from the lavaan package using 10000 bootstrap samples. PASI was subjected to arcsinh transformation to handle zero values.

RESULTS

Of 130 patients included in the EQUATOR study, blood samples from 121 patients were available for biomarker analyses (FIL n=60; PBO: n=61). Baseline demographics and disease characteristics were similar between the two groups (table 1).

Baseline associations

mRNA expression of 41 genes at baseline were significantly associated with at least one clinical metric (online supplemental figure 3). Most genes were correlated with C reactive protein (CRP) at baseline, including FAM20A, a known CRP-associated gene (online supplemental figure 3). Seven genes were positively associated with either TJC68 or SJC66, while 11 were negatively correlated with PASI and CRP. Several immune-related Hallmark 50 pathways (ie, IL6-JAK-STAT3-signalling, inflammatory response, coagulation and complement) were significantly correlated with CRP, PASDAS, DAPSA and patient-reported pain (online supplemental figure 4). As CRP is a component of both the PASDAS and DAPSA scores, these findings were not unexpected, but reinforces the clinical utility of CRP as a circulating biomarker of inflammation. Among circulating protein biomarkers, we found that systemic inflammatory markers, such as CRP, IL-6 and SAA, correlated with DAPSA (online supplemental figure 5), whereas markers from the Th17 pathway, such as IL-17A and IL-22, correlated with PASI, forming two discrete clusters of biomarkers.

FIL treatment effects

Gene expression changes with FIL treatment

Gene level

Overall, 945 genes were differentially expressed between FIL and PBO at week 1 compared with baseline; 886 at week 4 compared with baseline, and 373 at week 16 compared with baseline with FDR<0.05 (figure 1). Across all time points, genes associated with CRP, including FAM20A, MS4A4A, NRG1, ASGR2 and the SOCS family genes, CISH and SOCS2, were among the most strongly downregulated genes by FIL treatment, indicating that FIL treatment reduces inflammatory signalling. B-cell marker genes, including BANK1, CD79B, FCRLA, CD19, BLK and BLNK, were the most strongly upregulated genes (figure 1).

Pathway level

Hallmark pathways that were significantly affected by FIL treatment showed a similar pattern as the differentially expressed genes: FIL had a rapid (from week 1) and most prominent effect in significantly downregulating immune-related pathways, such as IL6-JAK-STAT3; inflammatory response, complement, coagulation, interferon α/γ response pathways (figure 2; for all 50 hallmark pathways, see online supplemental figure 6). The only two pathways negatively correlated with CRP at baseline, WNTβ-catenin-signalling and TGFβ-signalling (online supplemental figure 4), were upregulated by FIL treatment at week 4 (p<0.05; figure 2). Consistent with the observed changes in gene expression, we found that after an initial change at week 1 and/or at week 4, the effect was seemingly reduced in nearly all affected hallmark pathways by week 16 (figure 2).
Blood cell dynamics following FIL treatment

The reduction in the number of differentially expressed genes over time with FIL treatment was somewhat unexpected, as FIL previously displayed sustained clinical response over 16 weeks of treatment. However, gene expression in blood may be severely affected by changing leukocyte cell fractions.

Cell count data showed FIL treatment caused an increase in the absolute number of B-cells, a decrease in total white blood cell count and absolute neutrophil counts up to week 4, as well as a transient decrease in absolute monocyte count (figure 3A). Similar changes were seen in lymphocytes, monocytes and neutrophils when normalised to total leukocytes (figure 3B). Thus, the transient effect of FIL on lymphocyte cell fraction, monocyte fraction and neutrophil fraction can be expected to contribute towards the observed decline in the number of differentially expressed genes over time and particularly at week 16 as RNAseq signal is normalised to the total amount of mRNA. To investigate, we further analysed the effect of FIL on blood cell dynamics and successfully integrated information from both gene expression and cell count data (see the Treatment effects of FIL on gene expression factors section).

Treatment effects of FIL on gene expression factors

To reduce the very large number of genes and to extract more interpretable latent variables on clinical changes, EFA was performed on 305 significant differentially expressed genes across all time points (online supplemental figure 7). Factor analysis on these genes resulted in 12 latent factors, of which 8 were significantly affected by FIL treatment with FDR<0.05 (figure 4; online supplemental figure 8). Four factors were driven by the effect of FIL on blood cell fractions: sustained increase in % B cells, sustained increase in % effector B cells, transient increase in % T cells, and transient decrease in % monocytes (figure 4). The other four factors displayed mostly transcriptional impacts of FIL: transient decrease of complement and type I interferon, sustained decrease in the factor representing inflammation driven by CRP-associated genes (FAM20A, ASGR2, MS4A4A, NRG1) and the feedback of inflammation, driven by CISH and SOC2 genes.

Circulating protein biomarkers changed with FIL treatment

Peripheral protein biomarker analysis revealed clusters of biomarkers reduced with FIL treatment. Rapid and substantial decreases in systemic inflammatory proteins,
including SAA, CRP and IL-6, were seen as early as 1 week following treatment and indicate a fast onset of action of FIL and rapid mitigation of inflammation at the molecular level (figure 5). Other early response cytokines with modestly decreased fold-changes include several cytokines involved in Th1 development and IFNγ response, and cellular adhesion. Decreases in TNFR and markers of cell differentiation showed a delayed response at week 4, which was sustained through week 16. It is likely these week 4 decreases are not directly downstream of JAK1 signalling but a result of a deferred cascade of inflammatory inhibition initiated by FIL treatment.

Causal associations of derived factors, peripheral biomarkers and clinical labs with clinical disease scores
To investigate the mechanism of action of FIL on PsA clinical improvement, mediation analysis was performed to derive potential causal associations. Mediation analyses showed that six main groups of biomarkers grouped by EFA of FIL treatment-induced changes at week 4 were associated with changes in several clinical metrics at week 16 (figure 6; see online supplemental table 3). In general, the state of inflammatory cytokines, B cells, cholesterol and neutrophil-associated biomarkers at week 4 were associated with changes in a wide range of clinical scores. A group consisting of neutrophils and neutrophil-related factors (IL-16, calprotectin) was the largest contributor to multiple composite scores such as PASDAS and DAPSA. The systemic inflammation markers CRP, IL-6 and SAA associated with changes in SJC66 and TJC68, DAPSA and PGA. Interestingly, systemic inflammation was not significantly associated with changes in PASDAS. In contrast, PASDAS was highly associated with neutrophils, cholesterol and B cell percentages in peripheral blood. The JAK/STAT pathway feedback components CISH and SOCS2 were also associated with change in PASDAS. Improvement in PASI was not associated with any biomarkers at week 4.

As no biomarkers at week 4 were associated with clinical improvement of PASI, we explored additional possible mediation by other biomarkers at week 16 to explain the mechanism of PASI improvement. As we observed a strong indirect effect of FIL at week 16 on both subunits of IL-23, IL-12 p40 and IL-23 p19, we tested for a possible mediation effect of FIL on these proteins. SEM was employed to incorporate two mediators. This model suggested that PASI improvement was mediated by inhibition of both subunits of IL-23.

DISCUSSION
The mechanisms by which conventional and targeted disease-modifying antirheumatic drugs improve signs and symptoms of PsA has not been well studied, partly due to the difficulty of accessing target tissues within joints. We addressed this gap using an unbiased approach to explore changes in gene expression and protein biomarkers to understand the association between these biomarkers at baseline and the changes induced by treatment with FIL.
Notably, from our extensive biomarker study on 121 patients with PsA, we demonstrate that at baseline, disease pathways including inflammatory response, IL6-JAK-STAT3 signalling, coagulation, cholesterol homeostasis and epithelial-mesenchymal transition (EMT) are associated with PASDAS, DAPSA and pain scores. Compared with PBO, FIL treatment for 16 weeks led to significantly decreased proinflammatory cytokines, adhesion molecules and markers of matrix remodelling. We also suggest that changes in both peripheral cell composition and inflammation-related pathways drive FIL effects on gene expression in whole blood. Mediation analysis revealed broad associations between B cells, systemic inflammatory cytokines and neutrophils with a wide range of clinical metrics that improved with FIL treatment.

At baseline, we noted distinct differences in the association between the biomarkers and MSK-related outcomes and skin responses (online supplemental figures 3 and 5). Systemic inflammation markers were associated with MSK measures, whereas Th17-related markers were associated with skin measures. Patient-reported outcomes including pain and SF-36 PCS were also associated with...
similar markers. Given these correlations, as expected, there were significant associations between the markers and composite indices such as the DAPSA and PASDAS that include the above measures.

Few studies have evaluated peripheral blood gene expression in PsA on this scale. In a recently published observational study including 30 PsA patients, similar pathways including inflammatory response, complement, IFNα and IFNγ response, oxidative phosphorylation, adipogenesis, fatty acid metabolism, EMT, angiogenesis and signalling cascades related to WNTβ catenin and TGFβ were identified. Another observational study of 60 patients also identified inflammation, immune response, apoptosis, cell cycle regulation and proliferation, cell migration and invasion, extracellular matrix remodelling, bone remodelling, angiogenesis, signal transduction as important pathways reflected in the peripheral blood cells of PsA patients. Thus, our results provide insights to biomarkers and pathways driving disease activity in PsA and highlights the importance of IL6-JAK-STAT3 signalling.

Our primary purpose was to identify biomarkers and pathways associated with FIL treatment effects in PsA. As previous studies on FIL in PsA and other indications demonstrated a sustained FIL clinical effect, we expected to also see sustained changes in gene expression. However, we found a reduction in the number of differentially expressed genes from week 1 to week 16. Further analyses indicated that changes in cell fractions including a sustained increase in B cell counts, a transient increase of lymphocyte count, and a transient decrease of neutrophil and monocyte counts may explain the observed decrease in total gene expression at week 16 and the increase in B cell-related genes. Similar increases in the number of circulating CD19+B cells have been previously observed on treatment with the JAK inhibitors in stable kidney allograft recipients and in RA patients. It is speculated that increased B cell population are driven by increased regulatory B cells.

Along with B, T, macrophage and dendritic cells, neutrophilic infiltration is prominent in psoriatic synovium. Neutrophils play a well documented but underappreciated role in the pathogenesis of PsA. Neutrophil-related factors (IL-16, calprotectin) are markers of activated neutrophils. Our analyses indicate that activated neutrophils may play an important role in PsA disease activity. Reduction in neutrophil activity may lead to a reduction in clinical PsA disease activity. As expected, genes related...
to CRP (markers of inflammation) as well as SOCS family genes (cytokine-inducible negative regulators of cytokine receptor signalling via the JAK/STAT pathway) were strongly downregulated by FIL.20 Pathway-level analyses based on hallmark pathways and EFA also showed similar patterns.

Evaluating circulating protein markers, the most dramatic decreases following FIL treatment were observed in systemic inflammatory proteins, including SAA, CRP and IL-6 as well as the collagen fragments C1M and C3M. These changes in peripheral biomarker levels coupled with shifting cellular fractions reflect FIL’s proposed mechanism of action. Our results are consistent with changes observed with other JAK inhibitors in rheumatoid arthritis.21 22

While the findings described above resulted from association analyses, we also conducted mediation analyses to investigate potential causal relationships between molecular pathways and clinical endpoints.23 Multiple PsA treatment response measures make interpreting the results challenging. Although most of the grouped biomarkers influenced multiple arthritis-related outcomes, some were unique. Overall, neutrophils and neutrophil-related factors (IL-16, calprotectin) and the systemic inflammation markers CRP, IL-6 and SAA seemed to be most impactful. In this analysis, no significant association with PASI was found. However, SEM with PASI as the outcome, demonstrated that FIL potentially improves PASI through reduction of IL-23 p19 and its IL-12 p40 subunit. While these results need to be verified in independent studies, these findings are consistent with the proven efficacy of guselkumab, risankizumab, tildrakizumab and ustekinumab in PsA.

Limitations of our study include the fact that biopsies were not performed in the trial; therefore, molecular effects of FIL on the pathobiology of PsA were inferred from indirect effects observed from circulating protein biomarkers rather than directly from disease tissue. Moreover, as analyses performed here were post hoc, findings on the mechanistic effect of FIL will need to be tested and validated as prespecified hypotheses in subsequent clinical studies.

Our study also has several strengths. This biomarker study is to our knowledge, the largest and most extensive biomarker study to-date in patients with PsA, and uncovers the mechanism of FIL in PsA, which has not been previously shown. Additionally, the analyses were performed in the context of a randomised phase II trial, thus minimising confounders related to allotment of treatment. The biomarkers included transcriptomic

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**Figure 4** Filgotinib treatment effects on significant gene expression factors. The union of gene signatures (FDR<2e-3) at week 1, week 4 and week 16, across all available samples after the QC adjustment, were used as the input for the EFA, from which twelve latent variables were identified. Subsequent analyses revealed that 8 out of 12 gene expression factors were significantly affected by the filgotinib treatment with FDR<0.05 at a minimum of at least one time point. The top row shows factors mostly driven by cell fractions; while the bottom row reflects transcriptional regulation. EFA, exploratory factor analysis; FDR, false discovery rate. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001; ns: not significant.
Figure 5 Filgotinib treatment effects on circulating biomarkers. Log fold change of filgotinib treatment effects on circulating protein biomarkers between each week (weeks 1, 4 and 16) and baseline, compared with placebo. Hierarchical clustering was performed using correlation distance between all circulating biomarkers across all time points to capture proteins with similar action. Grey tiles indicate missing values as these proteins were added in after week 1. Only proteins with significant changes from baseline shown; see online supplemental material for the full list of analysed proteins.
Causal modelling between early biomarkers changes by FIL treatment, at week 4, and subsequent improvement of clinical endpoints at week 16. (A) We postulated that each clinical improvement by JAK1 inhibition by FIL is mediated by the corresponding causal molecular pathway in disease tissues, which may not be observable in circulating biomarkers in blood. However, some circulating biomarkers in blood may mirror the processes in the disease tissue and display correlated behaviour to these processes. Consequently, these biomarkers were expected to demonstrate statistical significance in the mediation model and indicate a significant 'indirect' treatment effect. Using the changes in biomarkers from baseline to week 4, the model suggests an association with changes in clinical characteristics at week 16, thereby inferring an indirect effect on clinical measurements. (B) Biomarkers at week 4 associated with clinical improvement were grouped together using exploratory factor analysis. The width of lines depicts percent of indirect effect of total drug effect (see online supplemental material for full table). Only statistically significant associations (FDR<0.1) are shown. CRP, C reactive protein; DAPSA, Disease Activity Index for Psoriatic Arthritis; FDR, false discovery rate; FIL, filgotinib; PASDAS, Psoriatic Arthritis Disease Activity Score; PGA, Physician’s Global Assessment; SJC, swollen joint count; TJC, tender joint count.

**Figure 6** Causal modelling between early biomarkers changes by FIL treatment, at week 4, and subsequent improvement of clinical endpoints at week 16. (A) We postulated that each clinical improvement by JAK1 inhibition by FIL is mediated by the corresponding causal molecular pathway in disease tissues, which may not be observable in circulating biomarkers in blood. However, some circulating biomarkers in blood may mirror the processes in the disease tissue and display correlated behaviour to these processes. Consequently, these biomarkers were expected to demonstrate statistical significance in the mediation model and indicate a significant 'indirect' treatment effect. Using the changes in biomarkers from baseline to week 4, the model suggests an association with changes in clinical characteristics at week 16, thereby inferring an indirect effect on clinical measurements. (B) Biomarkers at week 4 associated with clinical improvement were grouped together using exploratory factor analysis. The width of lines depicts percent of indirect effect of total drug effect (see online supplemental material for full table). Only statistically significant associations (FDR<0.1) are shown. CRP, C reactive protein; DAPSA, Disease Activity Index for Psoriatic Arthritis; FDR, false discovery rate; FIL, filgotinib; PASDAS, Psoriatic Arthritis Disease Activity Score; PGA, Physician’s Global Assessment; SJC, swollen joint count; TJC, tender joint count.
markers assayed in an untargeted manner using RNA-seq and many relevant circulating protein markers. Analyses at both individual and pathway levels built on prior knowledge and provided insights into the effect of JAK1 inhibition by FIL in humans with PsA. In addition, mediation analyses allowed us to attempt causal inference and derive a few interesting findings.

In conclusion, results from biomarker analyses on subjects participating in the phase II EQUATOR trial evaluating the efficacy of FIL in moderate-to-severe active PsA demonstrate that treatment with FIL rapidly down-regulates inflammatory and immune pathways associated with PsA disease activity as a result of changes in inflammatory gene expression and alterations in circulating cellular composition.

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Portions of this work were previously presented: (1) Gladman et al. Long-term efficacy of the oral selective Janus kinase 1 inhibitor filgotinib in psoriatic arthritis: Week 52 response patterns in individual patients from an open-label extension (OLE) study (EQUATOR2). Abstract at the European League Against Rheumatism, EULAR, European E-Congress of Rheumatology 2020. (2) Gladman et al. Filgotinib treatment leads to rapid and sustained reductions in inflammatory biomarkers in patients with moderate to severe psoriatic arthritis. Abstract at the European League Against Rheumatism, EULAR, European E-Congress of Rheumatology 2020. (3) Gladman et al. Whole blood transcriptional changes following treatment with Filgotinib in patients with Psoriatic Arthritis. Abstract presented at the American College of Rheumatology Convergence Conference 2021.

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**Contributors**

Conception and design: MT, AH, VC and DG; methodology: AH and VAM; data analyses: VAM, KLI, YL, LV and OKY; interpretation of analyses: all authors: writing, reviewing and editing: KLI, VC, DG, VAM, AH and JL; Guarantor: VAM

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**Competing interests**

VAM, KLI, YL, LV, OKY, JL, MT and AH are/were employees of Gilead Sciences and owners of company stock, MT and LV were affiliated with Gilead Sciences at the time of the trial. MT is currently affiliated with Amgen, and LV is currently affiliated with Novo Nordisk. The trial was designed and conducted by the sponsor (Gilead Sciences and Galapagos) in collaboration with the principal investigators and in accordance with the protocol and amendments. The sponsor collected data, monitored trial conduct and performed the statistical analyses. VC has received research grants from AbbVie, Amgen and Eli Lilly and has received honoraria for advisory board member roles from AbbVie, Amgen, BMS, Eli Lilly, Janssen, Novartis, Pfizer, and UCB. His spouse is an employee of AstraZeneca. He is supported by a Pfizer Chair in Rheumatology salary award. DG received grants and/or consulting fees from Abbvie, Amgen, BMS, Gilead, Galapagos, Eli Lilly, Janssen, Novartis, Pfizer and UCB.

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**Ethics approval** This study involves human participants and the study protocol was reviewed and approved by the central or individual independent ethics committee in each participating country. Please contact the corresponding author for the full list of ethics committees in all participating countries. Participants gave informed consent to participate in the study before taking part.

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**Data availability statement** Data are available on reasonable request. Any requests for use of data in this study must be sent to the corresponding author. The request will be examined with respect to the informed consent form relevant for this clinical trial.

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