



ORIGINAL RESEARCH

Filgotinib, a JAK1 Inhibitor, Modulates Disease-Related Biomarkers in Rheumatoid Arthritis: Results from Two Randomized, Controlled Phase 2b Trials

Jacqueline M. Tarrant · René Galien · Wanying Li · Lovely Goyal ·
Yang Pan · Rachael Hawtin · Wangshu Zhang · Annegret Van der Aa ·
Peter C. Taylor

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ABSTRACT

Introduction: The Janus kinase (JAK) inhibitor therapeutic class has shown significant clinical benefit in the treatment of rheumatoid arthritis (RA). We sought to gain insight into the mode of action and immunological effects of filgotinib, a JAK1 selective inhibitor, in active RA by analyzing secreted and cell-based biomarkers

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J. M. Tarrant · W. Li · L. Goyal · Y. Pan · R. Hawtin
· W. Zhang
Gilead Sciences, Inc., Foster City, CA, USA

R. Galien
Galapagos SASU, Romainville, France

A. Van der Aa
OCTIMET Oncology, Beerse, Belgium

P. C. Taylor (✉)
Botnar Research Centre, Nuffield Department of
Orthopaedics, Rheumatology and Musculoskeletal
Sciences, University of Oxford, Oxford, UK
e-mail: peter.taylor@kennedy.ox.ac.uk

Present Address:
W. Li
MyoKardia, South San Francisco, CA, USA

key to RA pathophysiology in two phase 2b trials of filgotinib in active RA.

Methods: Immune cell subsets and 34 serum biomarkers were analyzed longitudinally over 12 weeks using blood samples collected from patients with active RA receiving filgotinib (100 or 200 mg once daily) or placebo (PBO) in the two phase 2b trials (DARWIN 1, on a background of methotrexate, and DARWIN 2, as monotherapy).

Results: Consistently across both studies, filgotinib treatment decreased multiple immune response biomarkers that have key roles in RA for immune response, and decreased markers that promote matrix degradation, angiogenesis, leukocyte adhesion, and recruitment. Filgotinib did not significantly modulate T and natural killer (NK) lymphoid subsets, but slightly increased B cell numbers after 12 weeks. Multiple correlations were observed for changes in biomarkers with disease activity score 28-CRP. MIP1 β showed modest predictivity at baseline for ACR50 response at 12 weeks in the 100 mg filgotinib dose across both studies (AUROC, 0.65 and 0.67, $p < 0.05$).

Conclusions: Filgotinib regulates biomarkers from multiple pathways, indicative of direct and indirect network effects on the immune system and the stromal response. These effects were not associated with reductions of major circulating lymphoid populations.

Trial Registration: ClinicalTrials.gov, NCT01888874, NCT01894516.

Keywords: Biomarkers; Cytokines; DARWIN1; DARWIN2; Filgotinib; Rheumatoid arthritis

Key Summary Points

Why carry out this study?

Multiple pathophysiological pathways of inflammation, immunity, tissue extracellular matrix turnover, and joint remodeling are involved in active rheumatoid arthritis.

Evaluation of a broad panel of circulating biomarkers representing these pathways would aid in generating a hypothesis for the mechanism of action of JAK1 inhibition by filgotinib.

What was learned from the study?

Filgotinib regulates biomarkers from multiple pathways, indicative of direct and indirect network effects on the immune system and the stromal response. These effects were not associated with reductions of major circulating lymphoid populations.

from membrane receptors to the nucleus via activation of the signal-transducer and activator of transcription (STAT) factors. JAK enzymes dimerize as homo- or heterodimers that interact with transmembrane receptors of the type I/II cytokine family to regulate cell expansion, hematopoiesis, and immune responses [2]. JAK1 modulates a subset of proinflammatory cytokines within the JAK-STAT pathway including interleukin-6 (IL-6). JAK1 inhibition may be primarily responsible for the therapeutic efficacy of pan-JAK inhibitors in immune-mediated diseases, supporting the use of selective inhibition of JAK1 as a therapeutic strategy [3].

Filgotinib is a once-daily, orally administered, highly selective JAK1 inhibitor small molecule in development for the treatment of inflammatory disorders, including RA, Crohn's disease, and ulcerative colitis [4–7]. The efficacy and safety of filgotinib as both an add-on to methotrexate (MTX) and a monotherapy have been demonstrated in two 24-week phase 2b studies in adult patients with moderately-to-severely active RA and an inadequate response to MTX (MTX-IR) [5, 6]. These results were confirmed and extended in three phase 3 randomized clinical trials in MTX-IR, MTX-naïve, and biologic disease-modifying anti-rheumatic drug-IR moderately-to-severely active RA, which met 12- or 24-week primary endpoints for efficacy [8–10].

Our aim with this study was to generate a hypothesis for the mechanism of action of JAK1 inhibition in active RA by measuring a broad panel of circulating biomarkers chosen to represent multiple pathophysiological pathways of inflammation, immunity, tissue extracellular matrix turnover, and joint remodeling, in addition to circulating immune cell subsets. The selection of these biomarkers was based on several criteria. Pharmacodynamic activity of filgotinib was characterized by measuring JAK1 cytokine ligands and downstream biomarkers. Immune effects of filgotinib were also captured with key T-cell, B-cell, and innate cell cytokines and their recruiting chemokines, together with a cell-based biomarker readout of major immune cell subsets in the blood by flow cytometry immunophenotyping. Pathogenic effectors of tissue matrix damage and cross-talk

INTRODUCTION

Rheumatoid arthritis (RA) is a debilitating, heterogeneous, autoimmune disease characterized by chronic, systemic inflammation as well as synovitis and progressive joint destruction. Despite recent advances in the treatment of RA, a considerable number of patients experience persistent disease activity manifesting as chronic synovitis, progressive destruction of articular cartilage and bone, and functional impairment and disability [1].

Both innate and adaptive immune cellular responses mediated by cytokine activity contribute to inflammation and joint injury that characterize RA pathology. Janus kinases (JAKs) are a family of four intracellular cytoplasmic tyrosine kinases that mediate cytokine signaling

between synovial fibroblasts, endothelial cells, cartilage, and bone were founded on a clinically validated panel of soluble biomarkers that associate with RA disease activity [1, 11]. Finally, several biomarkers that have been implicated in predicting clinical therapeutic response were also included [11, 12]. By using highly sensitive multiplex immunoassays that minimized sample blood volume burden to patients, we could profile serial time points for an extensive characterization of a new molecular entity in the next generation of targeted RA therapeutics.

METHODS

Study Design

DARWIN1 and DARWIN2 (clinicaltrials.gov identifiers: NCT01888874 and NCT01894516) are previously reported 24-week, multicenter, randomized, double-blind, phase 2b, dose-finding studies of orally administered filgotinib or placebo (PBO) as an add-on to MTX (DARWIN1) or as monotherapy (DARWIN2) [5, 6]. Institutional review boards (IRBs)/ethics committees (ECs) from each country reviewed and approved the protocol (see Supplementary Material for a list of the IRBs or ECs). The study was performed in compliance with the ethical principles of good clinical practice and according to the ICH Harmonised Tripartite Guideline. Patients provided written informed consent to participate in the study and had the right to withdraw at any time.

Biomarker Analysis

Biomarkers were measured in available serum samples collected at baseline (BL), and at weeks 4 and 12 in the 100 mg, 200 mg, or PBO treatment groups and stored at -80°C until analysis. The biomarker analyses and patient characteristic summaries were performed using the biomarker analysis set, comprising randomized subjects who received ≥ 1 dose of study treatment and have ≥ 1 serum biomarker with evaluable measurement available at BL. Samples

were analyzed for 34 biomarkers by validated, commercially available, single- or multiplex immunoassays (Crescendo Bioscience Vectra[®] DA, South San Francisco, CA, USA; Merck-Millipore Milliplex high-sensitive T-cell panel immunoassay, Burlington, MA, USA; Meso Scale Discovery, Rockville, MD, USA; and R&D Systems, Minneapolis, MN, USA). The biomarkers analyzed were IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12-p70, IL-13, IL-17A, IL-21, IL-23, B-cell activating factor (BAFF), interferon- γ (IFN γ), epidermal growth factor (EGF), granulocyte-macrophage colony-stimulating factor (GM-CSF), vascular endothelial growth factor (VEGF), tumor necrosis factor alpha (TNF α), soluble TNF receptor type I (TNF-RI), chemokine C-X-C motif ligands (CXCL10 and CXCL13), intercellular adhesion molecule-1 (ICAM1), leptin, macrophage inflammatory proteins (MIP-1 α and MIP-1 β), matrix metalloproteinases (MMP1 and MMP3), monocyte chemoattractant protein-1 (MCP1), resistin, serum amyloid A (SAA), C-reactive protein (CRP), soluble glycoprotein 130 (SGP130), vascular cell adhesion molecule-1 (VCAM1), and human cartilage glycoprotein (YKL40, also known as Chitinase-3 L1). For biomarkers below the limit of quantification of the Milliplex multiplex assay (i.e., concentrations that cannot be back-calculated from standard curves), those missing values were imputed (with concentration level of the first standard/2). Values below the lower limit of quantification (LLOQ) for other immunoassays were imputed with LLOQ-1 significant digit. Biomarker data from the IL-4 assay were excluded from further analysis as less than 50% of values at BL were measurable by the Milliplex assay.

Flow Cytometry Analysis

Immunophenotyping analysis was conducted on data from the intent-to-treat population. Whole blood was collected into BD TruCount tubes (BD Biosciences, San Jose, CA), and all testing was conducted within a centralized laboratory network with harmonized protocols. Lymphocyte subpopulations (CD3⁺ T cells, CD3⁺CD4⁺ T-helper cells, CD3⁺CD8⁺ cytotoxic

T cells, CD19⁺ B cells, and CD16⁺CD56⁺ natural-killer [NK] cells) were quantified at BL, weeks 1, 2, 4, 8, and 12 by flow cytometry using the BD MultiTEST 6 Colour TBNK reagents on a BD FACSCanto. The time points for flow cytometry were conducted as part of the hematology and clinical chemistry laboratory assessments and were therefore more frequent than the biomarker collections.

Statistics

For each study, analysis of covariance model (ANCOVA) was fitted for weeks 4 and 12, respectively, to estimate the log ratio of each biomarker from BL by treatment group, after adjusting for prior biologic use and region (stratification factors), and the log-transformed BL biomarker value.

The model estimated percent change from PBO in post-BL ratio of each biomarker in filgotinib treatment arms, and the associated *P* values were adjusted for multiple doses per biomarker time point using Hommel's method. Correlations between percent change in biomarkers and changes in the Disease Activity Score in 28 joints (ADAS28)-CRP were evaluated by Spearman correlation. The area under the receiver operating characteristics curve (AUROC) was used to assess the predictive value of individual serum biomarkers at BL for treatment response (American College of Rheumatology [ACR] 20, 50, 70) at week 12. Combinations of biomarkers were also explored for identifying a predictive signature of the filgotinib treatment response by using hybrid stepwise model selection method over logistic/linear models. Wilcoxon rank-sum tests were conducted to compare the percent change of immune cells in the filgotinib-treated arm with PBO at both week 2 and week 12. All *P* values were reported from two-sided tests and < 0.05 were considered significant; adjustments of *P* values for multiplicity were not implemented, except for the ANCOVA models used to assess the treatment effect on biomarkers at multiple doses as specified above.

RESULTS

Demographics, Baseline Disease Characteristics, and Biomarker Levels

Key demographics and BL disease characteristics were balanced across treatment groups within each study for the biomarker analysis set (411 patients) (Supplementary Table 1). Notably, BL biomarker levels were generally balanced across treatment groups within each study (Supplementary Table 2). Median biomarker levels at BL were similar in DARWIN1 and DARWIN2 (pooling all treatment groups), with the exception of IL-1 β , IL-10, IL-21, GM-CSF, and TNF α , which were > 2-fold higher in DARWIN 2 (Fig. 1). The elevated cytokine levels at BL in the DARWIN2 trial were likely attributable to the absence of the immunosuppressive agent MTX.

Changes from Baseline in Cytokines, Chemokines, and Markers of Tissue Degradation

Following treatment with filgotinib, there were significant reductions in cytokines important for the expansion and activity of T-cell subsets: T_H1 (IFN γ , IL-2, IL-12, and TNF α), T_H2 (IL-5 [DARWIN2 only] and IL-13), T_H17 (IL-1 β , IL-6, IL-21, IL-23, and IL-17A [DARWIN 2 only]), B cells (CXCL13, IL-7, and IL-21), B_{reg}/T_{reg} (IL-10), and myeloid cells (GM-CSF) when compared with PBO (Fig. 2, Supplementary Table 3, and biomarker change from BL by treatment group in online Supplementary Figure A). The largest reductions (\geq 49% median reduction from BL in 200 mg filgotinib groups) were observed for the proinflammatory markers IL-6 and SAA. Other biomarkers with a large effect size (\geq 25% median reduction from BL in 200 mg filgotinib groups) were related to immune cell recruitment (CXCL10 and CXCL13), tissue matrix degradation (MMP1, MMP3, and YKL40), and angiogenesis (VEGF). These effects were mostly dose related, apparent at week 4, and sustained or further suppressed at week 12. Between studies, the magnitude of the percent reduction from BL to week 12 in the broad panel of cytokines was remarkably similar for filgotinib

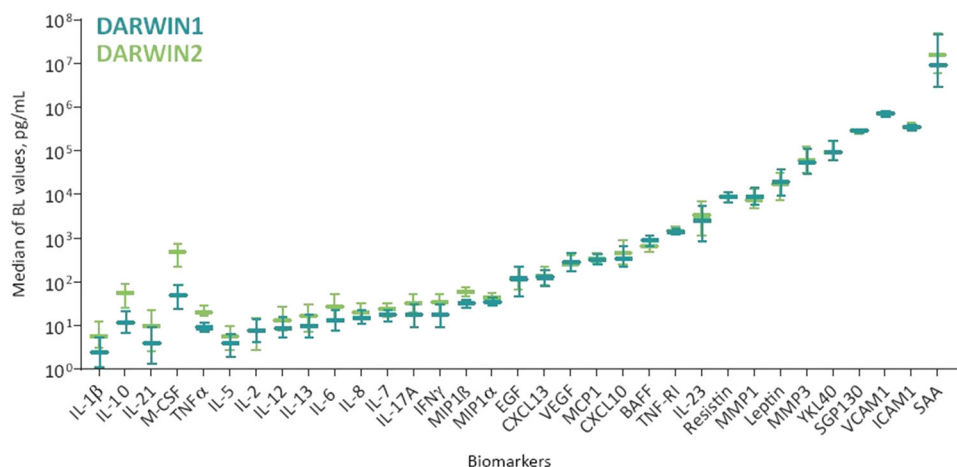


Fig. 1 Baseline levels (median, interquartile range) of biomarkers in DARWIN 1 (filgotinib on MTX background therapy) and DARWIN 2 (filgotinib monotherapy)

in combination with MTX or as monotherapy at both 100 mg and 200 mg once-daily doses (Fig. 3, Supplementary Table 3).

Correlation Between Composite Disease Activity Measures and Individual Biomarkers

Correlations between percent change from BL in biomarker levels and Δ DAS28-CRP at week 4 or week 12 were inconsistent across the two studies, doses, and time points (Table 1). Only BAFF and ICAM1 were significant at week 12 in both studies (albeit at different doses of filgotinib). YKL40 was correlated with Δ DAS28-CRP at weeks 4 and 12 with filgotinib monotherapy. In contrast, more biomarkers were correlated with Δ DAS28-CRP in the MTX PBO group of DARWIN1. This has been previously noted, and several of these biomarkers are used in a clinically validated panel for disease activity measurement in RA [13].

Predictivity of BL Biomarker for Filgotinib Therapeutic Response

AUROC analysis demonstrated weak and inconsistent predictive values of individual serum biomarkers measured at BL for ACR20, 50, and 70 responses at week 12 (Table 2). Based on the ACR50 disease improvement measure,

IL-6 (AUROC 0.65 95% CI [0.52, 0.78]) was modestly predictive for filgotinib 200 mg monotherapy and MIP1 β (AUROC 0.65 95% CI [0.49, 0.8]) for 100 mg filgotinib monotherapy. IL-13 (AUROC 0.67, 95% CI [0.53, 0.8]) and MIP1 β (AUROC 0.67, 95% CI [0.53, 0.8]) were significant predictors for 200 mg or 100 mg filgotinib in combination with MTX, respectively. No combination of these tested biomarkers better predicted treatment response as evaluated with any ACR responses or Δ DAS28-CRP (data not shown).

Immune Cell Phenotyping

Absolute lymphocyte counts at BL were comparable across treatment arms in both studies. There were transient, dose-related increases of all lymphocyte subsets in filgotinib-treated groups in the first 2 weeks of dosing that recovered to BL levels by week 12, with the exception of a sustained increase in B-cell numbers in DARWIN1 (Fig. 4). Median and interquartile ranges for all major immune cell subsets measured (B, total T, helper T, cytotoxic T, and NK cells) remained within the reference interval throughout the study period (Supplementary Figure B).

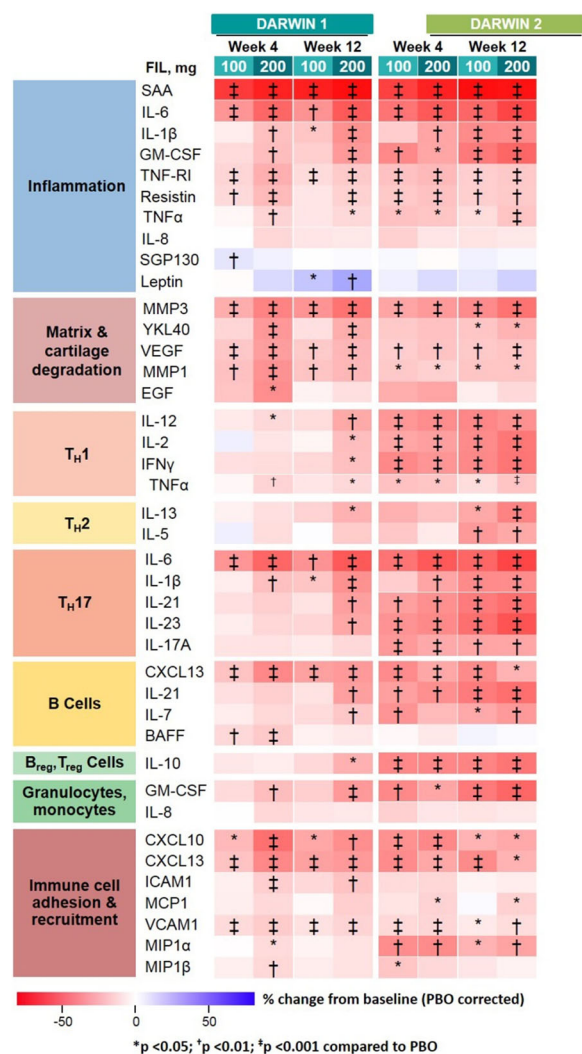


Fig. 2 Early and sustained biomarker changes with filgotinib in DARWIN 1 and DARWIN 2 based on a model of estimated percent change from placebo in post-BL ratio of each biomarker

DISCUSSION

In this study, we assessed the effects of a selective JAK1 inhibitor on an array of circulating immune and tissue biomarkers in active RA. Treatment of RA patients with filgotinib resulted in significant reductions in the levels of a broad range of biomarkers involved in multiple pathophysiological pathways (Fig. 5). Despite important differences in the study populations with regard to the presence (DARWIN1) or

absence (DARWIN2) of MTX as background therapy, we demonstrated remarkably similar biomarker modulations over 12 weeks of filgotinib treatment, comparable to the efficacious clinical responses (Fig. 3). A greater relative reduction of some T_H1- (IL-2, IFNγ, and IL-12) and T_H2-related (IL-5 and IL-13) cytokines by filgotinib monotherapy in DARWIN2 (Fig. 2) may reflect the lack of background MTX in this study compared to DARWIN1 (Supplementary Table 3). It is interesting to note that although

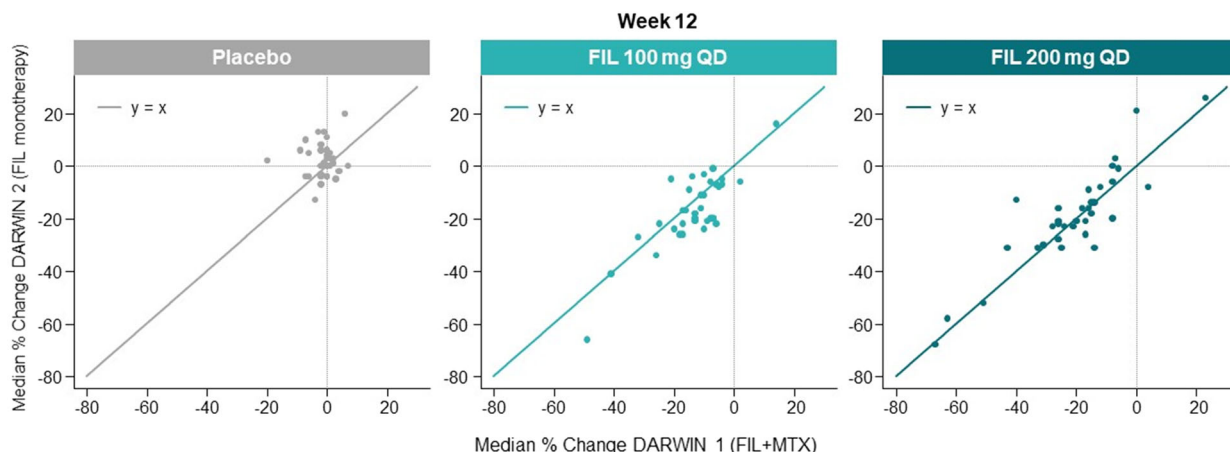


Fig. 3 Percent change of biomarkers from baseline in filgotinib-treated arms were comparable in DARWIN 1 (filgotinib [FIL] + methotrexate [MTX]) and DARWIN 2 (FIL monotherapy)

MTX reduces IL-6 in MTX-naïve patients [14], in the setting of MTX-IR, the JAK inhibitor (JAKinib) class retains the ability to markedly lower systemic levels of this key pathogenic mediator in RA [15, 16].

Filgotinib treatment induced a dose-dependent and significant decrease in a variety of biomarkers implicated in RA pathogenesis. The reduction in cytokines was significant by 4 weeks of treatment, consistent with the early onset of clinical efficacy in these phase 2b studies [5, 6]. The biomarkers that include SAA, IL-6, MMP3, CXCL10, and CXCL13 were biomarkers that showed a median reduction from BL levels $\geq 20\%$ at both dose levels in both studies. The diversity of affected biomarkers establishes that filgotinib moderates cell activation proximally through JAK1 signaling (e.g., IL-6) and distally by reducing downstream biomarkers subsequently produced by impacted cells that go on to signal via non-JAK pathways (e.g., MMP3, CXCL10, TNF α , IL-17, and IL-1 β). This process has been referred to as a network effect on immune responses [17].

The highest magnitude reductions were seen with IL-6 and its downstream targets, SAA, and probably also CXCL10, MMP1, and MMP3 [18], indicative of inhibition of JAK1 signaling and cellular biomarker output. IL-6, a key pathogenic mediator in RA, is produced by immune cells (T and B lymphocytes, macrophages), with a substantial contribution by stromal fibroblast-

like synoviocytes (FLS) that involves a newly described leukemia inhibitory factor-JAK1-TYK2 autocrine amplification of IL-6 [19–21]. IL-6 was predictive of 200 mg filgotinib monotherapy ACR50 clinical response; however, this was not the case for filgotinib in combination with MTX, indicating that the serum concentration of IL-6 (either BL or post-treatment reduction) was not the sole determinant of filgotinib efficacy.

We noted significant reductions in markers that have important functional roles for activated B cells including the B-cell chemoattractant CXCL13, survival and activation marker BAFF, and biomarkers that direct germinal center and plasma-cell differentiation (IL-2, IL-5, IL-7, and IL-21). These observations are despite an increase in circulating B-cell number following filgotinib treatment. Down-modulation of BAFF may also impact the activation, proliferation, and cytokine production of other pathogenic cells in the joint, including macrophages, dendritic cells, CD4⁺ T cells, and FLS [22].

We observed decreases in several immune cell recruitment biomarkers (CXCL10, CXCL13, VCAM, and ICAM1). Reduced serum CXCL10 [23, 24] and CXCL13 [24, 25] levels were reported following treatment with other RA therapeutics, and CXCL10 was also reduced locally in the synovium of RA patients in response to the pan-JAK inhibitor, tofacitinib [23].

Table 1 Spearman correlations between percent change from baseline at weeks 4 and 12 of selected biomarkers (those with $p \leq 0.05$) and Δ DAS28-CRP scores in the DARWIN 1 and DARWIN 2 studies

Study	DARWIN 1		DARWIN 2	
	Week 4 Biomarker: rho (95% CI)	Week 12 Biomarker: rho (95% CI)	Week 4 Biomarker: rho (95% CI)	Week 12 Biomarker: rho (95% CI)
Placebo	CXCL13: 0.35 (0.13, 0.53) [†] IL-1 β : 0.32 (0.11, 0.51) [†] IL-2: 0.24 (0.02, 0.44)* IL-6: 0.34 (0.12, 0.53) [†] IL-21: 0.28 (0.06, 0.48)* SAA: 0.32 (0.08, 0.53)*	CXCL13: 0.32 (0.11, 0.51) [†] ICAM1: 0.27 (0.05, 0.47)* IFN γ : -0.23 (-0.43, -0.01)* IL-6: 0.30 (0.08, 0.49) [†] IL-17A: -0.24 (-0.44, -0.02)* MMP1: 0.43 (0.21, 0.61) [‡] VEGF: 0.27 (0.02, 0.48)*	No biomarkers meeting criteria	MMP1: 0.32 (0.04, 0.56)* SAA: 0.45 (0.18, 0.65) [†] SGP130: -0.31 (-0.53, -0.07)*
FIL 100 mg	Resistin: 0.31 (0.07, 0.52)* TNF-RI: 0.30 (0.05, 0.51)*	IL-13: 0.33 (0.1, 0.53) [†] MMP1: 0.27 (0.02, 0.49)* SAA: 0.27 (0.02, 0.49)* TNF-RI: 0.31 (0.06, 0.52)*	IL-17A: -0.27 (-0.49, -0.03)* YKL40: 0.32 (0.04, 0.55)*	BAFF: 0.30 (0.02, 0.53)* CXCL10: 0.36 (0.11, 0.56) [†] ICAM1: 0.30 (0.05, 0.52)* MIP1 α : -0.28 (-0.5, -0.04)*
FIL 200 mg	BAFF: 0.33 (0.08, 0.55)* CXCL13: 0.31 (0.08, 0.5) [†] ICAM1: 0.27 (0.05, 0.47)*	BAFF: 0.43 (0.19, 0.62) [‡] CXCL13: 0.24 (0.01, 0.45)* ICAM1: 0.33 (0.11, 0.52) [†] MMP1: 0.28 (0.04, 0.48)* MMP3: 0.30 (0.06, 0.5)*	No biomarkers meeting criteria	YKL40: 0.31 (0.05, 0.53)*

Biomarkers without a statistically significant correlation are not shown

* $p \leq 0.05$, [†] $p \leq 0.01$, [‡] $p \leq 0.001$

Reductions in biomarkers previously associated with the stromal response (ICAM1, VCAM, MMP1, and MMP3) have been demonstrated in the synovium after treatment with other effective RA therapies [23, 26]. Together with the significant decrease in VEGF by filgotinib,

these effects could act to decrease infiltration of leukocytes at sites of synovial inflammation. Collectively, our data show that filgotinib displays effects on immune factors that are common to other treatments with demonstrated efficacy in RA. Furthermore, these findings also

Table 2 Predictive and prognostic performance of BL serum concentrations of selected biomarkers in DARWIN1 (FIL + MTX) and DARWIN2 (FIL monotherapy) studies for clinical outcomes at week 12

Biomarker	Efficacy measure	Placebo		FIL 100 mg	
		Biomarker level, median (Q1, Q3)		Biomarker level, median (Q1, Q3)	
		Responder	Non-responder	Responder	Non-responder
DARWIN1					
MIP1β (pg/ml)	ACR20	31.3 (25.1, 39.1)	31.3 (27.2, 41.3)	33.4 (27.4, 43.9)	26.3 (20.8, 31.6)
	ACR50	33.8 (20.5, 41.5)	31.3 (26.9, 39.1)	35.2 (28.2, 44.7)	31.0 (23, 35.5)
	ACR70	39.1 (34.3, 47.7)	30.3 (26.1, 39.1)	41.9 (30.1, 44.8)	31.2 (24.4, 37.0)
IL-6 (pg/ml)	ACR20	15.1 (9.7, 20.6)	13.6 (8.3, 23.0)	13.9 (8.2, 24.4)	14.1 (8.2, 32.2)
	ACR50	12.1 (9.7, 20.6)	14.7 (9.1, 22.7)	15.0 (6.5, 30.8)	13.4 (10.2, 19.0)
	ACR70	15.1 (10.1, 21.2)	14.2 (8.7, 21.7)	12.8 (5.9, 42.1)	14.1 (9.5, 21.1)
IL-13 (pg/ml)	ACR20	10.9 (6.6, 20.1)	8.5 (4.3, 13.6)	11.6 (6.1, 23.3)	10.5 (7.6, 14.8)
	ACR50	10.6 (6.0, 13.9)	8.8 (5.0, 16.8)	10.2 (6.1, 21.4)	10.9 (6.5, 22.1)
	ACR70	11.4 (7.1, 17.4)	8.8 (5.0, 16.6)	10.2 (6.2, 22.4)	10.7 (5.7, 22.0)
ICAM1 (ng/ml)	ACR20	325 (292, 394)	328 (284, 384)	357 (293, 406)	290 (269, 370)
	ACR50	379 (302, 395)	324 (284, 384)	372 (300, 406)	322 (272, 388)
	ACR70	373 (253, 387)	326 (284, 387)	388 (349, 436)	323 (273, 390)
CXCL13 (pg/ml)	ACR20	130 (79, 182)	117 (87, 143)	127 (68, 182)	103 (80, 121)
	ACR50	130 (71, 182)	119 (86, 160)	116 (68, 190)	107 (73, 142)
	ACR70	182 (107, 243)	119 (79, 163)	116 (82, 186)	107 (69, 152)
DARWIN2					
MIP1β (pg/ml)	ACR20	57.4 (44.2, 63.4)	64.7 (49.1, 85.3)	57.5 (46.5, 74.9)	65.4 (55.5, 76.8)
	ACR50	55.2 (46.1, 79.5)	62.6 (48.6, 78.3)	54.0 (43.7, 70.1)	63.2 (54.4, 76.4)
	ACR70	61.5 (44.6, 78.5)	62.0 (49.1, 78.0)	54.1 (44.3, 70.2)	61.4 (52.6, 76.2)

Table 2 continued

Biomarker	Efficacy measure	Placebo		FIL 100 mg			
		Biomarker level, median (Q1, Q3)				Biomarker level, median (Q1, Q3)	
		Responder	Non-responder	AUROC (95% CI)	P value	Responder	Non-responder
IL-6 (pg/ml)	ACR20	28.8 (16.5, 36.0)	29.7 (14.4, 57.0)	0.54 (0.39, 0.68)	0.624	25.2 (10.0, 52.7)	22.0 (11.5, 44.1)
	ACR50	22.8 (11.7, 30.5)	31.1 (15.3, 57.5)	0.66 (0.46, 0.84)	0.175	25.2 (9.0, 53.1)	22.0 (10.1, 46.8)
	ACR70	16.8 (9.7, 23.9)	29.8 (15.4, 55.6)	0.73 (0.39, 1)	0.283	25.2 (7.4, 63.1)	23.6 (10.1, 50.0)
IL-13 (pg/ml)	ACR20	16.6 (8.5, 28.0)	17.6 (4.8, 24.2)	0.42 (0.28, 0.58)	0.355	23.4 (10.1, 39.7)	15.2 (8.4, 30.0)
	ACR50	7.6 (7.3, 37.8)	17.4 (6.0, 25.8)	0.45 (0.2, 0.69)	0.692	23.9 (11.5, 39.7)	18.2 (7.1, 34.6)
	ACR70	29.3 (18.4, 40.2)	17.2 (6.1, 25.7)	0.64 (0.25, 0.98)	0.53	20.3 (10.9, 39.4)	23.6 (8.5, 39.2)
ICAM1 (ng/ml)	ACR20	408 (345, 543)	375 (311, 454)	0.56 (0.4, 0.72)	0.466	355 (304, 438)	390 (317, 423)
	ACR50	574 (357, 636)	378 (313, 449)	0.67 (0.39, 0.91)	0.151	399 (315, 438)	361 (304, 423)
	ACR70	507 (431, 584)	382 (313, 454)	0.67 (0.31, 0.98)	0.43	409 (332, 446)	359 (304, 425)
CXCL13 (pg/ml)	ACR20	143 (76, 226)	138 (90, 226)	0.49 (0.34, 0.66)	0.924	114 (76, 188)	183 (97, 261)
	ACR50	102 (64, 179)	141 (91, 234)	0.61 (0.38, 0.84)	0.359	96 (79, 200)	157 (81, 201)
	ACR70	126 (92, 161)	138 (90, 233)	0.6 (0.22, 0.97)	0.641	84 (79, 188)	154 (82, 203)
Biomarker	Efficacy measure	FIL 100 mg		FIL 200 mg			
		Biomarker level, median (Q1, Q3)				Biomarker level, median (Q1, Q3)	
		AUROC (95% CI)	P value	Responder	Non-responder	AUROC (95% CI)	P value
DARWINI MIP1β (pg/ml)	ACR20	0.68 (0.52, 0.82)	0.037	32.9 (25.6, 38.0)	24.5 (20.7, 34.4)	0.65 (0.5, 0.79)	0.054
	ACR50	0.67 (0.53, 0.8)	0.021	33.1 (26.3, 38.5)	27.9 (21.7, 34.5)	0.62 (0.48, 0.74)	0.089
	ACR70	0.67 (0.49, 0.84)	0.057	32.2 (26.0, 33.7)	30.7 (23.1, 39.0)	0.52 (0.37, 0.66)	0.8
IL-6 (pg/ml)	ACR20	0.5 (0.33, 0.67)	0.96	12.8 (6.1, 20.5)	12.9 (9.1, 31.7)	0.55 (0.4, 0.71)	0.507
	ACR50	0.54 (0.38, 0.69)	0.62	12.7 (6.3, 19.9)	12.9 (6.3, 25.0)	0.52 (0.39, 0.65)	0.761
	ACR70	0.47 (0.27, 0.66)	0.733	12.6 (8.2, 17.7)	13.0 (6.1, 25.2)	0.55 (0.42, 0.69)	0.504

Table 2 continued

Biomarker	Efficacy measure	FIL 100 mg		FIL 200 mg			
		Biomarker level, median (Q1, Q3)		Biomarker level, median (Q1, Q3)			
		AUROC (95% CI)	P value	Responder	Non-responder	AUROC (95% CI)	P value
IL-13 (pg/ml)	ACR20	0.54 (0.39, 0.69)	0.64	12.2 (4.6, 20.1)	8.0 (4.1, 11.2)	0.65 (0.52, 0.76)	0.057
	ACR50	0.54 (0.4, 0.69)	0.604	16.6 (5.7, 22.3)	7.5 (4.0, 12.6)	0.67 (0.53, 0.8)	0.012
	ACR70	0.52 (0.34, 0.7)	0.798	16.1 (5.0, 19.2)	8.8 (4.2, 15.7)	0.6 (0.45, 0.74)	0.185
	ACR20	0.78 (0.6, 0.93)	0.01	324 (274, 430)	322 (304, 420)	0.61 (0.43, 0.78)	0.212
	ACR50	0.58 (0.39, 0.76)	0.394	339 (291, 444)	315 (268, 378)	0.49 (0.33, 0.66)	0.912
	ACR70	0.64 (0.44, 0.81)	0.188	313 (288, 420)	325 (278, 427)	0.59 (0.41, 0.74)	0.371
	ACR20	0.76 (0.61, 0.9)	0.02	135 (88, 200)	113 (66, 194)	0.51 (0.31, 0.69)	0.958
CXCL13 (pg/ml)	ACR50	0.67 (0.48, 0.83)	0.076	153 (99, 200)	109 (66, 189)	0.57 (0.41, 0.73)	0.431
	ACR70	0.55 (0.35, 0.74)	0.626	175 (112, 251)	116 (67, 189)	0.58 (0.4, 0.75)	0.388
DARWIN2							
MIP1β (pg/ml)	ACR20	0.59 (0.45, 0.73)	0.275	51.35 (42.5, 65.8)	53.8 (48.2, 67.4)	0.59 (0.44, 0.74)	0.29
	ACR50	0.65 (0.49, 0.8)	0.039	51.5 (44.4, 66.8)	51.2 (44.4, 61.6)	0.5 (0.36, 0.64)	0.989
	ACR70	0.63 (0.44, 0.82)	0.142	52.8 (46.7, 69.7)	51.5 (44.4, 61.6)	0.54 (0.34, 0.74)	0.712
IL-6 (pg/ml)	ACR20	0.52 (0.37, 0.67)	0.811	22.4 (9.9, 35.2)	29.5 (19.6, 58.4)	0.65 (0.49, 0.78)	0.081
	ACR50	0.53 (0.38, 0.67)	0.722	18.4 (7.5, 32.8)	25.9 (16.0, 51.6)	0.65 (0.52, 0.78)	0.041
	ACR70	0.52 (0.34, 0.71)	0.809	25.5 (10.6, 33.4)	23.4 (12.8, 41.0)	0.53 (0.25, 0.7)	0.795
IL-13 (pg/ml)	ACR20	0.56 (0.4, 0.71)	0.469	13.0 (4.8, 22.4)	19.6 (8.7, 32.4)	0.61 (0.46, 0.77)	0.183
	ACR50	0.55 (0.41, 0.7)	0.481	11.4 (4.8, 17.2)	17.8 (7.0, 30.4)	0.63 (0.49, 0.75)	0.083
	ACR70	0.53 (0.36, 0.7)	0.751	10.9 (7.6, 15.8)	15.1 (6.1, 28.8)	0.56 (0.38, 0.74)	0.583
ICAM1 (ng/ml)	ACR20	0.56 (0.4, 0.72)	0.466	352 (289, 421)	368 (329, 379)	0.51 (0.35, 0.65)	0.946
	ACR50	0.55 (0.4, 0.69)	0.51	325 (285, 393)	360 (308, 398)	0.56 (0.42, 0.71)	0.435
	ACR70	0.58 (0.41, 0.74)	0.391	368 (317, 424)	352 (288, 390)	0.58 (0.36, 0.8)	0.478

Table 2 continued

Biomarker	Efficacy measure	FIL 100 mg		FIL 200 mg		P value	
		Biomarker level, median (Q1, Q3)		Biomarker level, median (Q1, Q3)			
		AUROC (95% CI)	P value	Responder	Non-responder		
		AUROC (95% CI)	AUROC (95% CI)				
CXCL13 (pg/ml)	ACR20	0.66 (0.51, 0.81)	0.05	113 (72, 204)	153 (97, 260)	0.59 (0.43, 0.75)	0.266
	ACR50	0.56 (0.41, 0.7)	0.444	95 (68, 235)	135 (91, 188)	0.55 (0.4, 0.7)	0.483
	ACR70	0.59 (0.41, 0.76)	0.346	78 (58, 201)	129 (78, 229)	0.64 (0.39, 0.86)	0.205

Biomarkers not shown were not predictive at any time point in either study

support a network effect of JAK1 inhibition that contributes to filgotinib mechanism of action.

Individual biomarkers in this study were modestly associated with disease activity or clinical response when using the standard composite scores, Δ DAS28-CRP and ACR response (Tables 1 and 2). The predictive ability of BL levels of IL-6 or MIP1 β for filgotinib monotherapy and IL-13 or MIP-1 for filgotinib with MTX will need to be reproduced in follow-up studies for these therapeutic regimens. Multivariate analysis of biomarker combinations did not reveal predictors of clinical response for 12 weeks of filgotinib treatment. These data are consistent with other investigations seeking predictive biomarkers for RA, and, to date, there are no clinically valid predictors of response to any therapeutic. Recently, the levels of serum ICAM1 and CXCL13 have been shown as potential BL predictors of TNF α inhibitor, tocilizumab (anti-IL-6), or therapeutic response, respectively [12, 27]. We explored the impact of filgotinib on these biomarkers using a similar approach, but failed to demonstrate any predictive value for response in our MTX-IR population. Conspicuously, neither serum IL-6 nor TNF α are predictive for treatment response to targeted therapies that specifically block these cytokines [28]. We conclude that the heterogeneous and complex pathophysiology of RA, in addition to the composite clinical scores used as the outcome measure, continue to challenge the search for a predictive biomarker panel.

Mild, transient increases in total lymphocyte count, T cells (CD3, CD4, CD8), and NK cells were largely confined to the first 2 weeks of treatment with filgotinib. Significant elevation of B-cell numbers was sustained through to 12 weeks of treatment in the setting of MTX co-therapy, and a trend was evident also with filgotinib monotherapy. In the absence of major numerical changes in the examined lymphoid subsets, the observed cytokine changes could reflect a down-modulation of the pathogenic activity of these cell subsets. Furthermore, peripheral blood cytokine levels may be influenced by inflammatory activity localized to affected joints. Conversely, arthritic disease expression may be dominated by paracrine

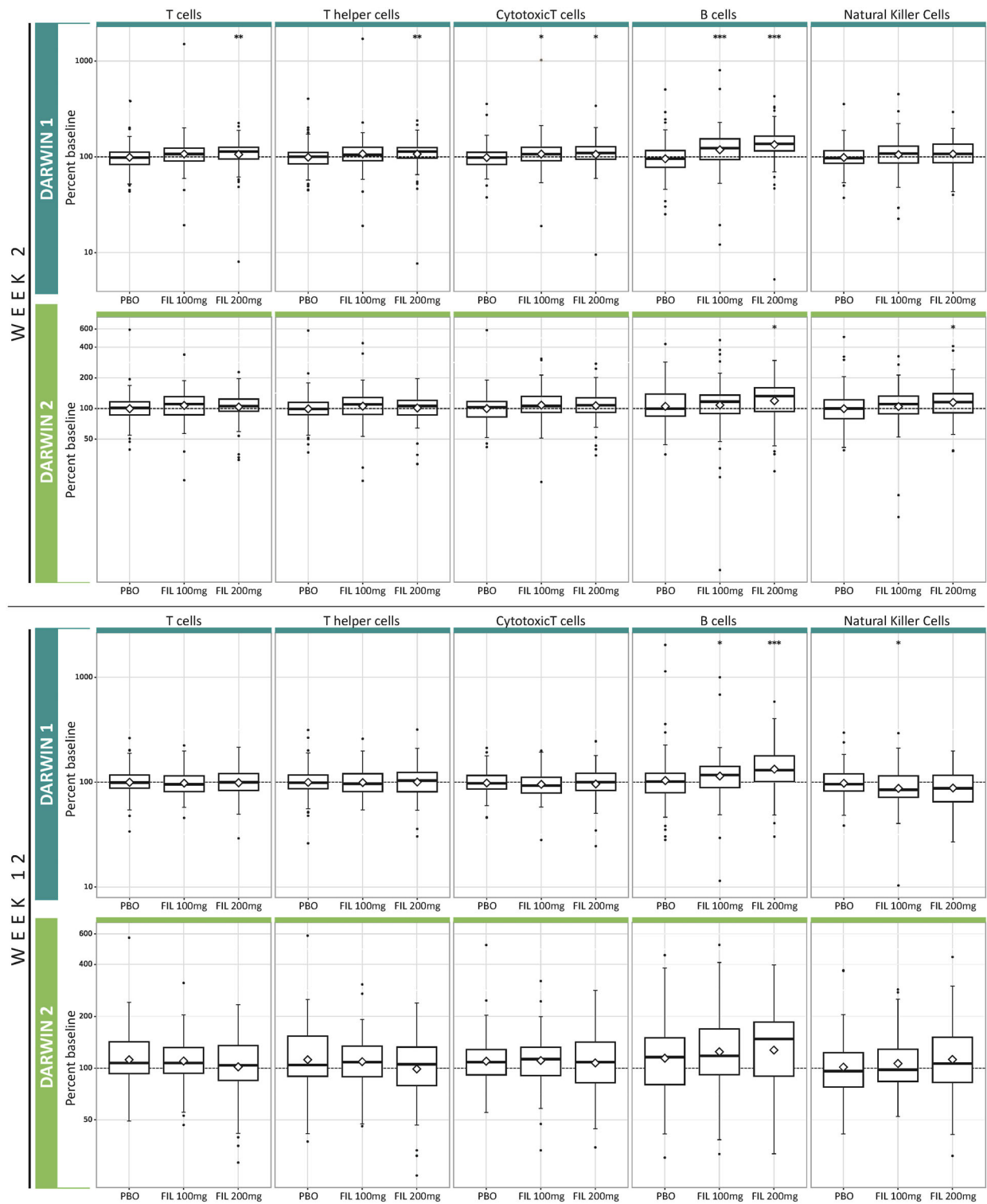
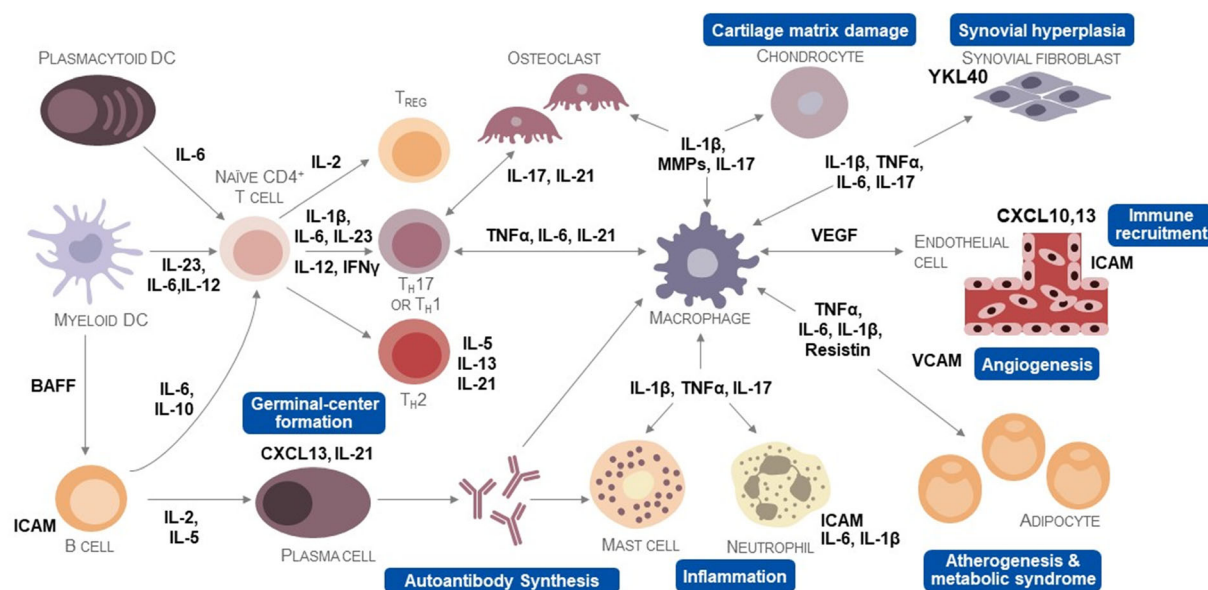


Fig. 4 Box plots of percentage changes from baseline in absolute lymphocyte subpopulation counts at weeks 2 and 12 in the DARWIN 1 and DARWIN 2 studies



*Immune cell and joint cell interactions in RA mediated by soluble factors; only cytokines that were impacted by FIL monotherapy in RA patients are depicted. Other biomarkers assessed, but not shown are CRP, EGF, GM-CSF, IL-7, IL-8, IL-13, leptin, MIP-1α and MIP-1β, MCP1, SAA, SGP130 and TNF-RI. DC, dendritic cell.

Fig. 5 Overview of the cytokine-mediated regulation of synovial interactions. Adapted from Ref. [37]

cytokine activity, which may not be fully apparent in peripheral blood [29].

Immunophenotyping of the major immune cell populations also allows indirect comparison with the reported effects of JAK inhibitors of different selectivities. Total lymphocyte count is also transiently increased by other JAK inhibitors [14, 30–33]. Sustained B-cell increases of similar magnitude to week 24 have been noted with baricitinib (JAK1/2 inhibitor) [33], while tofacitinib (JAK1/2/3 inhibitor) increased B cells in healthy subjects, and subjects with RA or psoriasis after 12 weeks of therapy, but this effect was not evident at week 24 in longer duration studies in RA [34–36]. The numerical increase in B-cell numbers is as of yet unexplained, and our data indicate that B-cell increases are not due to cytokine-driven enhanced survival or expansion, as key factors in these processes were down-regulated (IL-7 and BAFF) with treatment. Transient increases in T cells that return to pre-dose levels occur with filgotinib and tofacitinib, in contrast to the progressive reduction of total T-cell count to week 24 with baricitinib [33, 35, 36]. The differential effects on NK-cell numbers provide the

most striking differences amongst JAK inhibitors. Other JAK inhibitors have decreased NK-cell numbers by week 12 of treatment; in contrast, filgotinib relatively spared NK-cell count [14, 31, 35, 36]. The decrease in NK-cell numbers with tofacitinib therapy was also associated with attenuation of functional activity, which was not investigated in our studies [36]. It is not clear why NK-cell counts were not significantly altered by filgotinib, but the counts likely reflect a balance of JAK selectivity, potency, and duration of inhibition that differs between JAK inhibitors.

There were several limitations in this study, including the approach of measuring systemic cytokine levels as a surrogate for local processes in the arthritic joint and immunophenotyping that was confined to major immune cell populations (with no further B-cell or T-cell subset analysis or cell function studies). Additionally, earlier time points may help identify key drivers of the observed network effects. Despite these limitations, blood-based biomarker panels, including the markers we analyzed in this report, have been correlated with contemporaneous clinical disease activity measures in RA

(with some RA therapies), supporting mechanistic understanding of disease from selective profiling of the systemic compartment [13]. Further, as a systemic disease, with multiple organ involvement in subgroups of patients, profiling the immune status in the periphery remains a relevant analysis and may serve to better characterize the disease.

The strength of this work is the reproducibility in findings over two large, independent, placebo-controlled, randomized clinical studies. The robustness of these findings has in part been achieved by minimizing analytical variability by conducting biomarker testing at the same central laboratories using identical assays and platforms within a short time period. This is the first time that such a wide range of disease-relevant biomarkers have been profiled for a targeted synthetic disease-modifying anti-rheumatic drug and could set the standard for mechanistic profiling for new medications in active RA.

CONCLUSIONS

Our data demonstrate the ability of selective JAK1 inhibition to down-modulate several key inflammatory mediators of signaling pathways in RA, independent of MTX background therapy. This confirms the strong network effects (on JAK1 and downstream non-JAK pathways) of the JAK1 node in autoimmunity, matrix and cartilage degradation, angiogenesis, and leukocyte adhesion and recruitment (Fig. 5). Such broad profiling of cytokines may provide insight into the optimal JAK inhibition profile for effective treatment of RA and other autoimmune diseases.

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- Genovese M, Li W, Goyal L, et al. Effects of the JAK1-selective inhibitor filgotinib on multibiomarker disease activity scores in patients with active rheumatoid arthritis and an inadequate response to methotrexate. *Annals of the Rheumatic Diseases*. 2017;76:281.
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- Genovese MC, Galien R, Pan Y, et al. Correlation of multi-biomarker disease activity score with clinical disease activity measures for the JAK1-selective inhibitor filgotinib as monotherapy and in combination with methotrexate in rheumatoid arthritis patients [abstract]. *Arthritis Rheumatol*. 2017;69(suppl 10). <https://acrabstracts.org/abstract/correlation-of-multi-biomarker-disease-activity-score-with-clinical-disease-activity-measures-for-the-jak1-selective-inhibitor-filgotinib-as-monotherapy-and-in-combination-with-methotrexate-in-rheumat/>.
- Taylor PC, Galien R, Van der Aa A, et al. Monotherapy with filgotinib, a JAK1-selective inhibitor, reduces disease-related biomarkers in rheumatoid arthritis patients [abstract]. *Arthritis Rheumatol*. 2017;69(suppl 10). <https://acrabstracts.org/abstract/monotherapy-with-filgotinib-a-jak1-selective-inhibitor-reduces-disease-related-biomarkers-in-rheumatoid-arthritis-patients/>.

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provided written informed consent to participate in the study and had the right to withdraw at any time.

Data Availability. The data supporting the authors' conclusions are included in the article and additional file.

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