Brief Report

DOI: 10.5582/ddt.2026.01000

Effects of Janus kinase inhibition and interleukin 6 inhibition on serum cytokine/chemokine in idiopathic multicentric Castleman disease

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SUMMARY: Idiopathic multicentric Castleman disease (iMCD) is a rare lymphoproliferative disease characterized by systemic inflammation. Although IL-6 receptor blockade with tocilizumab is an established treatment, Janus kinase (JAK) inhibition may offer broader immunomodulation by targeting multiple cytokine signaling pathways. This comparative longitudinal study evaluated 41 serum cytokines/chemokines in 10 patients with plasma-cell-type iMCD (five treated with filgotinib (JAK1 preferential inhibitor) for 52 weeks and five treated with tocilizumab for a median of 28 months) to compare the cytokine/chemokine suppression profiles between these two mechanistically distinct therapies. Patient values were normalized to those of healthy controls (n = 101) using Z-scores. At baseline, both groups exhibited marked elevations in cytokines, including IL-12p70, IL-22, IFN-γ, and IL-6. Both treatments resulted in significant changes in multiple cytokines, with 12 cytokines showing significant changes in each group. Between-group comparison revealed only three cytokines with differential responses: IL-6 (receptor blockade artifact), IL-15 (greater suppression with filgotinib), and PDGF-AA (greater suppression with tocilizumab). Overall, cytokine suppression was equivalent between the treatments (median ΔZ-score: −0.32 (filgotinib) *vs.* −0.27 (tocilizumab), p = 0.74). Principal component analysis demonstrated parallel treatment trajectories toward normalization. These exploratory findings from this small-sample study suggest that JAK inhibition and IL-6 receptor blockade may achieve comparable broad-spectrum cytokine/chemokine suppression in iMCD, despite the different mechanisms of action. However, the discordance between cytokine suppression and clinical improvement with filgotinib suggests that complete IL-6 pathway blockade remains critical for the clinical response in iMCD, highlighting the need to identify additional pathogenic drivers beyond the measured cytokine network.

Keywords: multicentric Castleman disease, cytokine profile, filgotinib, tocilizumab, JAK inhibitor, biomarker

1. Introduction

Idiopathic multicentric Castleman disease (iMCD) is a rare lymphoproliferative disease characterized by polyclonal lymph node hyperplasia, distinctive histopathological features, and systemic inflammatory symptoms (*1,2*). iMCD is a rare and refractory disease that annually affects ~900-4,200 people in the US (*3*) and ~1,500 in Japan (*4*). Patients typically present with generalized lymphadenopathy accompanied by constitutional symptoms, including fever, fatigue, weight loss, and night sweats. Laboratory abnormalities are hallmarks of the disease and include elevated C-reactive protein (CRP) levels, anemia of chronic inflammation, hypoalbuminemia, and elevated erythrocyte sedimentation rate. The constellation of clinical and laboratory findings reflects the systemic inflammatory state characteristic of iMCD. Without effective treatment, iMCD can progress to life-threatening complications, such as multiorgan damage and secondary amyloidosis.

The pathogenesis of iMCD remains incompletely understood; however, interleukin-6 (IL-6) has been identified as a central driver of disease manifestations through extensive research. Elevated serum IL-6 levels have been reported in patients with Castleman disease (*5*). In addition, single-cell RNA sequencing and spatial enhanced resolution omics sequencing demonstrated that IL-6 pathway signals were dominant in nodal fibroblastic

reticular cells and endothelial cells in the lymph nodes of iMCD (*6*). Anti-IL-6 therapies, including the IL-6 receptor antibody tocilizumab (approved in Japan) (*7*) and the IL-6-neutralizing antibody siltuximab (approved in the United States, Europe, and other regions) (*8*), have transformed the patient outcomes. However, a significant proportion of patients do not benefit from anti–IL-6 treatment, and additional therapeutic options are needed for non-responders, especially severely afflicted patients (*9*).

The inflammatory milieu of iMCD extends beyond IL-6, encompassing a complex network of a pleomorphic cytokine profile, and the disease is not driven by IL-6. Serum proteomic analyses have revealed a "chemokine storm" in iMCD, with the simultaneous elevation of numerous inflammatory mediators that may perpetuate disease activity (*10*). This observation has prompted interest in therapeutic strategies that target broader inflammatory networks rather than individual cytokines.

To regulate broader inflammatory networks, Janus kinases (JAKs), which are downstream of cytokine receptors, including IL-6 (*11*), appear to be promising treatment targets for iMCD. JAKs contribute to the signal transducer and activator of transcription (STAT) 3 activation in iMCD pathogenesis (*12*), suggesting that JAK-STAT signaling inhibition is a potential treatment approach. This rationale led to a phase Ib investigator-led clinical trial of filgotinib, a JAK1 preferential inhibitor, for iMCD, which was conducted across Japan in 2024 (*13*).

Filgotinib preferentially inhibits JAK1-dependent cytokine signaling *in vitro* (*14*) and has been approved for use in rheumatoid arthritis (RA) (*15*) and ulcerative colitis (*16*). JAK1 inhibition theoretically offers broader immunomodulation by simultaneously suppressing the signaling of multiple cytokine receptors that utilize JAK1 for signal transduction. This mechanistic difference raises the fundamental question of whether JAK1 inhibition might achieve more comprehensive cytokine suppression than selective IL-6 receptor blockade, potentially offering advantages for patients with complex cytokine dysregulation.

In an evaluation at eight weeks, treatment with filgotinib did not show apparent efficacy on the CHAP score and its components (CRP, hemoglobin, and albumin) (*13*). Evaluation of multiple cytokines and chemokines demonstrated differences in the serum levels of FGF-2, IL-4, IL-6, TNF-β, and VEGF-A at eight weeks between filgotinib- and tocilizumab-treated patients (*17*). However, the evaluation at eight weeks seemed too early to draw a definitive conclusion.

This comparative longitudinal study aimed to comprehensively characterize the cytokine and chemokine profiles of both treatments, with normalization to healthy controls. Our approach enables a direct comparison of different cytokines and chemokines on a common scale using Z-score normalization, facilitating the interpretation of the relative magnitude of abnormalities and treatment effects. Specifically, we sought to (1) compare the breadth and magnitude of cytokine/chemokine suppression between filgotinib and tocilizumab across the entire inflammatory network; (2) identify cytokine/chemokine biomarkers associated with clinical parameters and treatment response; (3) determine whether JAK1 inhibition achieves broader immunomodulation than IL-6 receptor blockade; and (4) characterize the specific cytokines that show differential responses to these two mechanistically distinct therapies. Understanding these cytokine/chemokine dynamics has important implications for future therapeutic strategies for this challenging rare disease.

2. Materials and Methods

This comparative longitudinal study evaluated serum cytokine and chemokine dynamics in two cohorts of patients with iMCD diagnosed according to the Japanese Ministry of Health, Labour and Welfare criteria (designated intractable diseases notice no. 331, effective April 1, 2018). The filgotinib cohort comprised 5 patients enrolled in a prospective, single-arm, open-label Phase Ib clinical trial (registered in the Japan Registry of Clinical Trials (https://jrct.niph.go.jp/) as jRCT2071230108 approved by the Nagasaki University Hospital Institutional Review Board, approval No. 123-002 and jRCTs071230120 approved by the Nagasaki University Hospital Institutional Review Board, approval No. CRB23-008). Patients with a total score on the CHAP (C-reactive protein (CRP), Hemoglobin, Albumin, + Performance Status (PS, Eastern Cooperative Oncology Group [ECOG])) (*4*) that was ≥ 2 points in total with hemoglobin or albumin ≥ 1 point and CRP ≥ 1 point at baseline were included. Patients with iMCD-TAFRO (thrombocytopenia, anasarca, fever, reticulin fibrosis, renal insufficiency, and organomegaly clinical subtype), as defined by the validated international definition (*18*), were excluded. These patients received filgotinib (Gilead Sciences, Inc., Foster City, CA, USA) 200 mg orally once daily for 52 weeks, and serum samples were collected. The tocilizumab cohort comprised five patients receiving standard tocilizumab (Chugai Pharmaceutical Co., Ltd., Tokyo, Japan) therapy according to the approved dosing regimen, with serum samples collected at baseline and post-treatment status (median 28 months [minimum 11 - maximum 73 months]). All patients had plasma-cell-type histological features on lymph node biopsy and were human herpesvirus 8 (HHV-8) negative. The baseline characteristics are summarized in Table 1.

This study complied with the Declaration of Helsinki and was approved by the Nagasaki University Hospital Institutional Review Board (approval No. 25111306). An opt-out approach was used to obtain patient consent for the study.

The sera of the patients were analyzed using the

Table 1. Characteristics of patients with idiopathic multicentric Castleman disease treated with either tocilizumab or with filgotinib at baseline and after treatment

Characteristics	Tocilizumab (*n* = 5)	Filgotinib (*n* = 5)
At baseline		
Age, years, median (min−max)	62 (43−69)	60 (37−61)
Sex, female, *n* (%)	2 (40)	2 (40)
Height, cm, median (min−max)	160 (152−172)	170 (152−176)
Body weight, kg, median (min−max)	55.7 (46.1−72.5)	73.7 (50.9−75.0)
Treatment-naïve, *n* (%)	5 (100)	3 (60)
Previous immunosuppressant, *n* (%)		
Prednisolone	0 (0)	2 (40)
Others	0 (0)	0 (0)
Concomitant immunosuppressant, *n* (%)		
Prednisolone 10 mg/day	0 (0)	1 (20)
Others	0 (0)	0 (0)
Histology, *n* (%)		
Hyaline vascular type	0 (0)	0 (0)
Plasma cell type	5 (100)	5 (100)
Mixed type	0 (0)	0 (0)
CRP, mg/dL, median (min−max)	4.90 (3.70−7.22)	6.73 (2.47−7.45)
Hemoglobin, g/dL, median (min−max)	9.3 (8.5−11.6)	10.1 (8.1−11.8)
Albumin, g/dL, median (min−max)	2.4 (2.4−2.7)	2.9 (2.5−3.3)
ECOG-PS, *n* (%)		
0	4 (80)	2 (40)
1	1 (20)	3 (60)
CHAP score, median (min−max)	5 (3−7)	5 (3−6)
Platelet count, $\times 10^3/\mu L$, median (min−max)	338 (262−456)	339 (226−556)
Immunoglobulin G, mg/dL, median (min−max)	4,436 (3,733−4,996)	5,357 (4,446−5,968)
At 52 weeks* or later**		
CRP, mg/dL, median (min−max)	0.01 (0.01−0.10)	3.30 (1.92−6.31)
Hemoglobin, g/dL, median (min−max)	14.1 (12.6−14.3)	11.1 (9.4−12.3)
Albumin, g/dL, median (min−max)	4.2 (4.0−4.3)	3.3 (2.8−3.6)
ECOG-PS, *n* (%)		
0	5 (100)	4 (80)
1	0 (0)	1 (20)
CHAP score, median (min−max)	0 (0−0)	3 (1−4)

Abbreviation: CHAP: CRP, hemoglobin, albumin, + PS (ECOG), CRP: C-reactive protein, ECOG-PS: Eastern Cooperative Oncology Group-Performance Status. * for the filgotinib group, ** for the tocilizumab group.

Milliplex® MAP Human Cytokine/Chemokine Magnetic Bead Panel-Premixed 41 Plex panel (Merck Millipore, Billerica, MA, USA) and MAGPIX® with xPONENT® software (Luminex Corp., Austin, TX, USA). The levels of 41 cytokines/chemokines were measured as follows: epidermal growth factor (EGF), CCL11/eotaxin, basic fibroblast growth factor (FGF-2/bFGF), FMS-like tyrosine kinase 3 ligand (FLT-3 L), fractalkine, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), chemokine (C-X-C motif) ligand 1 (CXCL1/GRO-α), interferon (IFN)-α2, IFN-γ, interleukin (IL)-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17A, IL-17F, IL-18, IL-1 receptor antagonist (IL-1RA), IL-1α, IL-1β, IL-2, IL-22, IL-27, IL-4, IL-5, IL-6, IL-7, IL-8, C-X-C motif chemokine ligand 10 (CXCL10/IP-10), monocyte chemoattractant protein-1 (MCP-1/CCL2), MCP-3, macrophage-derived chemokine (MDC), CCL3/macrophage inflammatory protein (MIP)-1α, CCL4/MIP-1β, platelet-derived growth factor (PDGF)-AA, transforming growth factor (TGF)-α, tumor necrosis factor (TNF)-α, TNF-β, vascular endothelial growth factor (VEGF)-A, and soluble CD40 ligand (sCD40L).

Residents of the town of Saza in Nagasaki prefecture who underwent specific health checkups in 2016 were used for the 41 Plex panel as controls to calculate the normal limits with a 95% confidence interval (approved by the Ethics Committee of the Nagasaki University Graduate School of Biomedical Sciences, project registration No.: 14051404). All healthy donors had no past or present medical history of inflammatory disease (*n* = 101 (59 women), mean age 58 (standard deviation: 10) years).

For each cytokine/chemokine, patient values were normalized to healthy controls using Z-scores calculated as follows: Z = (patient value − healthy mean) / healthy SD. This normalization allowed for the direct comparison of different cytokines on a common scale, with a Z-score of $|Z| \leq 2$ defined as within the normal range (corresponding to approximately 95% of healthy individuals).

Changes in cytokine/chemokine Z-scores from baseline to week 52 (ΔZ-scores) within each treatment group were assessed using paired *t*-tests. Between-group comparisons of ΔZ-scores were performed using Welch's *t*-test, which does not assume equal variances. Pearson's

correlation coefficients were used to evaluate inter-cytokine/chemokine relationships and the relationships between cytokines/chemokines and clinical parameters at baseline across all patients. Given the exploratory nature of the study, statistical significance was set at $p < 0.05$ without multiple testing corrections. All analyses were performed using R (version 4.5.0; R Foundation for Statistical Computing, Vienna, Austria).

3. Results and Discussion

The baseline characteristics of both treatment groups are summarized in Table 1. The filgotinib cohort included five patients (median age 60 years, range 37-61; three males) with plasma-cell-type iMCD. The tocilizumab cohort also included five patients (median age 62 years, range 43-69; three males). While all patients receiving tocilizumab were treatment-naïve, two patients receiving filgotinib had prior prednisolone exposure, and one was receiving concomitant prednisolone (10 mg/day). Both groups had similar baseline disease activity, as reflected by the median CHAP score of 5 in both groups. In the filgotinib group, CRP decreased from 6.73 to 3.30 mg/dL and albumin increased from 2.9 to 3.3 g/dL, while CRP decreased from 4.90 to 0.01 mg/dL, hemoglobin increased from 9.3 to 14.1 g/dL, albumin increased from 2.4 to 4.2 g/dL, and CHAP score improved from 5 to 0 in the tocilizumab group.

Figure 1 shows the time series of 41 cytokines and chemokines and clinical parameters (CRP, hemoglobin, albumin, and CHAP score). At baseline, both treatment groups showed marked elevation of multiple cytokines compared to healthy controls (Figure 1, green shaded area: mean ± 2 SD of healthy control). The most prominently elevated cytokines included IL-12p70 (Z = 31.7), IL-22 (Z = 10.0), IFN-γ (Z = 9.5), IL-6 (Z = 9.1), and TNF-β (Z = 7.3) (Figure 2A). Additional notably elevated cytokines and chemokines included GM-CSF (Z = 4.4), TNF-α (Z = 3.7), MCP-3 (Z = 3.1), Fractalkine (Z = 3.0), FGF-2 (Z = 2.7), PDGF-AA (Z = 2.5), and IL-12p40 (Z = 2.1).

3.1. Cytokine/chemokine correlations at baseline

We analyzed the correlations among 41 serum cytokines at baseline in 10 patients. Pearson's correlation analysis revealed several statistically significant cytokine clusters (Figure 2B). IL-6 showed significant positive correlations with TNF-α and TNF-β levels. IL-13 and IL-22 also had significant positive correlations with IL-6, TNF-α, and TNF-β (lower right corner of Figure 2B). Meanwhile, IL-10, TGF-α, IL-15, and IL-1α had significant positive correlations with each other (upper left corner of Figure 2B). These separate correlation clusters suggest the presence of multiple inflammatory axes within the iMCD cytokine/chemokine network that may respond differently to targeted therapies.

3.2. Cytokine/chemokine correlations with clinical parameters at baseline

We analyzed the correlations between cytokine/chemokine and clinical parameters (Figure 2C). CRP levels at baseline were significantly negatively correlated with G-CSF levels. Meanwhile, baseline hemoglobin and albumin levels were significantly positively correlated with MCP-1 and IL-4 levels, respectively. No cytokines or chemokines were significantly correlated with the CHAP score at baseline.

3.3. Cytokine/chemokine changes in two groups

In the filgotinib group, paired *t*-tests identified 12 cytokines with statistically significant changes from baseline to week 52 ($p < 0.05$, Table 2, Figure 2D). The most significant decreases were observed for IL-6 (change of Z score from baseline to 52 weeks (ΔZ)= − 2.24, $p = 0.001$), IL-1RA (ΔZ = − 0.44, $p = 0.003$), and IFN-γ (ΔZ = − 5.12, $p = 0.005$). Additional cytokines that showed significant decreases included IL-2, FGF-2, MCP-3, IL-15, VEGF-A, EGF, IL-17A, and IL-12p40. MCP-1 showed a significant increase (ΔZ = + 0.45, $p = 0.013$). In the tocilizumab group, paired *t*-tests identified 12 cytokines with statistically significant changes ($p < 0.05$, Table 2, Figure 2D). PDGF-AA showed the most significant decrease (ΔZ = − 1.89, $p = 0.011$), followed by MCP-3 (ΔZ = − 3.50, $p = 0.013$), VEGF-A (ΔZ = − 2.11, $p = 0.023$). Additional cytokines that showed significant decreases included IL-4, IL-17F, TGF-α, IL-22, TNF-β, FLT-3L, and FGF-2. As expected with IL-6 receptor blockade, serum IL-6 levels showed a significant increase (ΔZ = + 126.77, $p = 0.047$) due to impaired receptor-mediated clearance. MCP-1 also increased significantly (ΔZ = +1.00, $p = 0.029$).

3.4. Between-group comparison of cytokine/chemokine changes

A direct comparison of ΔZ-scores between treatment groups using Welch's *t*-test identified only three of 41 cytokines with statistically significant differences ($p < 0.05$, Figure 2E). IL-6 showed the largest difference (ΔZ: − 2.24 in filgotinib *vs.* + 126.8 in tocilizumab, $p = 0.045$), reflecting the pharmacological artifact of IL-6 accumulation following receptor blockade. PDGF-AA showed greater suppression with tocilizumab (ΔZ: − 0.50 *vs.* −1.89, $p = 0.028$), whereas IL-15 showed greater suppression with filgotinib (ΔZ: − 0.94 *vs.* −0.12, $p = 0.040$).

3.5. Overall cytokine/chemokine change comparison

When comparing the overall cytokine/chemokine changes between the treatments, both achieved equivalent effects on cytokine/chemokine changes (Figure 2F).

Figure 1. Time-course changes in serum cytokines/chemokines and clinical parameters in patients with iMCD treated with filgotinib or tocilizumab. Serum levels of 41 cytokines/chemokines are shown for patients treated with filgotinib (blue line) and tocilizumab (red dashed line) during the treatment period. The green shaded area indicates the normal range (mean (dashed green lines) ± 2 SD of healthy controls, n = 101). The lower panels show the corresponding clinical parameters: C-reactive protein (CRP), hemoglobin (Hb), albumin (Alb), and CHAP score. For the filgotinib cohort, measurements were obtained at baseline and at weeks 4, 8, 18, 26, 34, and 52. For the tocilizumab cohort, measurements were obtained at baseline and post-treatment (Post-Tx; median, 28 months; range, 11-73 months).

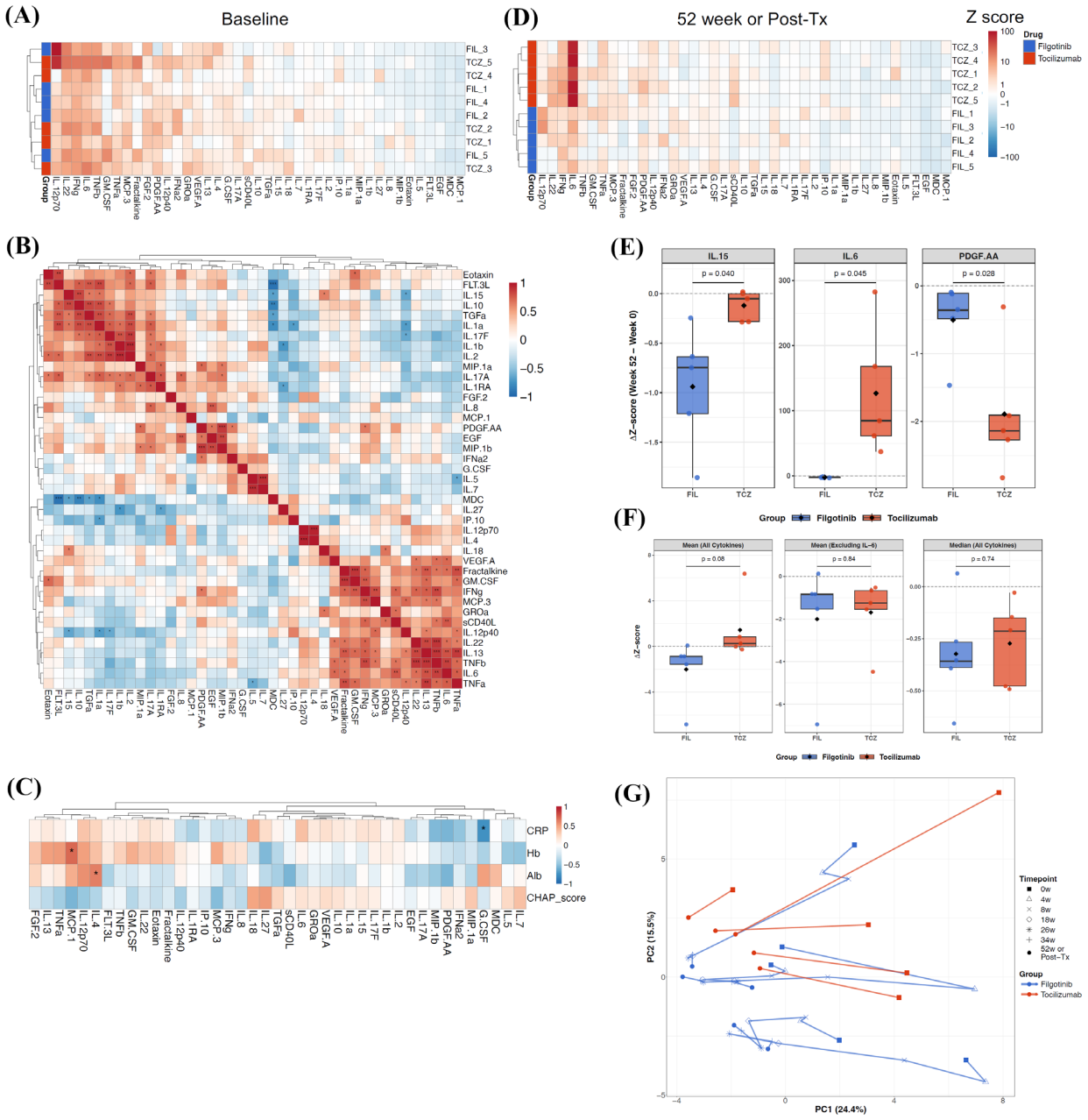

Figure 2. Comprehensive cytokine/chemokine analysis comparing filgotinib and tocilizumab treatment in iMCD. (**A**) Z-score-normalized baseline cytokine/chemokine profiles of all 10 patients. Blue: filgotinib, red: tocilizumab. (**B**) Correlation matrix of the 41 cytokines/chemokines at baseline across all patients. Colors indicate Pearson's correlation coefficients (red: positive correlation; blue: negative correlation). Asterisks denote statistical significance (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). (**C**) Correlation between baseline cytokine/chemokine levels and clinical parameters (CRP, hemoglobin, albumin, and CHAP scores). Significant correlations are indicated by asterisks (*$p < 0.05$). (**D**) Z-score-normalized cytokine/chemokine profiles of all 10 patients at 52 weeks. Blue: filgotinib, red: tocilizumab. (**E**) Between-group comparison of ΔZ-scores for all 41 cytokines/chemokines. IL-6, IL-15, and PDGF-AA levels were significantly different between the treatments (Welch's *t*-test, $p < 0.05$). (**F**) Overall comparison of cytokine/chemokine changes between the treatment groups. Box plots show the distribution of ΔZ-scores across all cytokines for each treatment, with and without IL-6. (**G**) Principal component analysis (PCA) trajectory plot of the 41 cytokines/chemokines. Arrows indicate the treatment trajectories from baseline to the endpoint for each patient. Filgotinib-treated patients are shown with solid blue arrows (7 timepoints); tocilizumab-treated patients are shown with dashed red arrows (2 timepoints).

Using all 41 cytokines/chemokines with mean ΔZ-scores, filgotinib showed − 2.01 ± 2.76 versus tocilizumab + 1.44 ± 2.79 ($p = 0.085$), but this difference was driven entirely by the IL-6 artifact in the tocilizumab group. When IL-6 was excluded from the analysis, the mean ΔZ-scores were also equivalent: − 2.01 ± 2.83 versus − 1.70 ± 1.61 ($p = 0.84$). Using median ΔZ-scores, which are robust to outliers, both groups showed comparative cytokine/chemokine changes(− 0.32 ± 0.26 (filgotinib) *vs.* −0.27 ± 0.21 (tocilizumab), $p = 0.74$). These findings suggest that JAK1 inhibition and IL-6 receptor blockade may achieve comparable broad-spectrum cytokine suppression in iMCD, despite differences in their molecular mechanisms of action.

Table 2. Statistically significant within-group cytokine changes (paired *t*-test, $p < 0.05$)

Filgotinib					Tocilizumab				
Cytokine/chemokine	Baseline	At 52 weeks	ΔZ	*p* value	Cytokine/chemokine	Baseline	Post-treatment	ΔZ	*p* value
IL-6	6.64	4.40	-2.24	0.001	PDGF-AA	3.88	1.99	-1.89	0.011
IL-1RA	0.28	-0.16	-0.44	0.003	MCP-3	4.37	0.87	-3.50	0.013
IFN-γ	7.49	2.38	-5.12	0.005	VEGF-A	1.60	-0.51	-2.11	0.023
MCP-1	-1.32	-0.86	0.45	0.013	IL-4	0.68	0.07	-0.61	0.025
IL-2	0.29	-0.16	-0.45	0.021	MCP-1	-1.43	-0.43	1.00	0.029
FGF-2	3.25	0.01	-3.24	0.026	IL-17F	0.04	-0.10	-0.14	0.030
MCP-3	1.82	0.20	-1.62	0.027	TGF-α	0.34	-0.05	-0.39	0.033
IL-15	0.69	-0.25	-0.94	0.027	IL-22	11.30	3.52	-7.78	0.037
VEGF-A	1.32	0.65	-0.67	0.030	TNF-β	9.79	1.26	-8.54	0.041
EGF	-1.40	-1.50	-0.10	0.032	FLT-3L	-0.67	-0.76	-0.09	0.043
IL-17A	0.65	-0.02	-0.67	0.038	FGF-2	2.13	0.52	-1.62	0.046
IL-12p40	1.35	0.16	-1.19	0.043	IL-6	11.49	138.27	126.77	0.047

ΔZ = Week 52 (filgotinib) or Post-treatment (tocilizumab) Z-score − Baseline Z-score.

3.6. Sensitivity analysis

To address the potential confounding effect of concomitant prednisolone use in one filgotinib-treated patient, we performed a sensitivity analysis excluding this patient (filgotinib $n = 4$ *vs*. tocilizumab $n = 5$). Between-group comparison identified three cytokines with statistically significant differences: FLT-3L (ΔZ: + 0.11 in filgotinib *vs.* − 0.09 in tocilizumab, $p = 0.002$), PDGF-AA (ΔZ: − 0.54 *vs.* − 1.89, $p = 0.039$), and IL-6 (ΔZ: − 2.08 *vs.* + 126.8, $p = 0.045$). IL-15 (ΔZ: − 0.71 *vs.* − 0.12, p = 0.053) became borderline. The overall cytokine suppression equivalence was maintained: mean ΔZ-score − 2.12 ± 3.18 (filgotinib) *vs.* + 1.44 ± 2.79 (tocilizumab) ($p = 0.13$); mean ΔZ-score excluding IL-6: − 2.12 ± 3.25 *vs.* − 1.70 ± 1.61 ($p = 0.82$); median ΔZ-score − 0.31 ± 0.30 *vs.* − 0.27 ± 0.21 ($p = 0.82$). Within-group significant cytokines in the filgotinib group decreased from 12 to 8, consistent with reduced statistical power. The detailed within-group comparison is presented in Supplementary Table S1 (*https://www.ddtjournal.com/action/getSupplementalData.php?ID=293*).

3.7. Principal component analysis with trajectories

Principal component analysis (PCA) of the 41 cytokines and chemokines revealed treatment trajectories in the multidimensional cytokine space (Figure 2G). PC1 and PC2 accounted for 24.4% and 15.5% of the total variance, respectively. At baseline, patients in both treatment groups clustered in the upper-right quadrant, reflecting an elevated inflammatory cytokine state. Over the course of treatment, both filgotinib-treated (seven time points, solid blue arrows) and tocilizumab-treated patients (two time points, dashed red arrows) demonstrated directional movement toward the center-left region of the plot, representing the normalization of cytokine profiles toward healthy control values. The treatment trajectories were largely parallel between the two groups, indicating that JAK1 inhibition and IL-6 receptor blockade induce similar patterns of cytokine modulation, despite their distinct molecular mechanisms of action. PCA trajectory analysis provides compelling visual evidence that filgotinib and tocilizumab achieve comparable immunomodulatory effects in iMCD from the viewpoint of serum cytokines and chemokines.

A notable and clinically important finding was the differential clinical response between the two treatment groups, despite equivalent cytokine/chemokine suppression. This disparity likely reflects the distinct mechanisms of action of these therapies and the specific role of IL-6 in the regulation of acute-phase proteins.

IL-6 is the primary driver of hepatic acute-phase protein synthesis, including CRP, serum amyloid A, and fibrinogen (*19*). IL-6 induces the production of positive acute-phase proteins while simultaneously inhibiting the synthesis of negative acute-phase proteins, such as albumin and transferrin (*20*). IL-6 also suppresses erythropoiesis by promoting hepcidin synthesis, resulting in hypoferremia and anemia (*21*). Tocilizumab achieves complete IL-6 receptor blockade by competitively inhibiting IL-6 binding to both membrane-bound and soluble IL-6 receptors (*22*), thereby comprehensively suppressing the acute phase response. In contrast, filgotinib inhibits JAK1-mediated signaling downstream of the IL-6 receptor but does not completely block the pathway, which may lead to residual IL-6 signaling sufficient to maintain elevated CRP levels, suppress albumin synthesis, and cause anemia. Additionally, the shorter duration of follow-up in the filgotinib cohort (52 weeks) compared with that in the tocilizumab cohort (median 28 months) may have contributed to the observed differences.

A direct comparison of ΔZ-scores between treatment groups using Welch's *t*-test identified only three of the 41 cytokines, IL-6, PDGF-AA, and IL-15, with statistically significant differences (Figure 2E). Because the elevation of IL-6 by tocilizumab is a well-known artifact, we concentrated on IL-15 and PDGF-AA.

The preferential suppression of IL-15 by filgotinib

is consistent with its mechanism of action as a JAK1 inhibitor and provides important insights into the broader immunomodulatory effects of JAK1 inhibition. Upon IL-15 binding, JAK1 and JAK3 are recruited, leading to the phosphorylation of STAT3 *via* JAK1 and STAT5 *via* JAK3 (*23*). In addition, the correlation between IL-15 and other pro-inflammatory cytokines (IL-10, TGF-α, IL-1α) observed in our baseline analysis suggests that IL-15 may participate in a distinct inflammatory axis within the iMCD cytokine network that operates independently of the IL-6 pathway. However, the clinical significance of IL-15 suppression by filgotinib remains to be elucidated. Despite achieving substantial IL-15 reduction, patients treated with filgotinib showed only modest improvements in CRP, hemoglobin, and albumin levels compared to the apparent normalization observed with tocilizumab. This dissociation between IL-15 suppression and modest clinical response suggests that IL-15 may not be a critical driver of core pathophysiology in iMCD with plasma-cell-type histology.

Conversely, the greater suppression of PDGF-AA by tocilizumab warrants careful consideration in the context of iMCD pathophysiology. PDGF-AA transduces potent mitogenic signals and induces actin reorganization (*24*). In iMCD, PDGF-AA may contribute to the characteristic lymph node histopathology and vascular proliferation observed in the affected tissues. However, because serum PDGF-AA levels in iMCD were comparable to those in healthy controls in a previous study (*25*), further research on the role of PDGF-AA in the pathogenesis of iMCD is warranted.

This study has several important limitations that must be considered when interpreting the results. First, the small sample size (n = 5 per group) severely limits statistical power and the generalizability of the findings. Second, the non-randomized design—combining a prospective single-arm trial (filgotinib) with a retrospective cohort (tocilizumab)—introduces potential selection bias. Third, the mismatched follow-up durations between the two cohorts (52 weeks *vs.* a median of 28 months) may have contributed to the more pronounced clinical improvement observed in the tocilizumab group as longer treatment exposure could allow for greater therapeutic benefit. Fourth, concomitant prednisolone use in one filgotinib-treated patient (10 mg/day) represents a confounding factor, as prednisolone possesses broad anti-inflammatory and cytokine-modulating properties that could obscure the true treatment effect of filgotinib. Although sensitivity analyses excluding this patient confirmed the robustness of the primary findings, the contribution of concomitant prednisolone use cannot be entirely excluded in the primary analysis. Given these constraints, the present findings should be considered exploratory and hypothesis-generating. Large-scale, prospective, randomized controlled trials are warranted to validate these observations.

In conclusion, this exploratory analysis from a small-sample study suggests that filgotinib may achieve broad-spectrum cytokine and chemokine suppression comparable to that of tocilizumab in patients with iMCD. The discordance between cytokine/chemokine improvement and clinical parameter improvement suggests the need to identify the important drivers of iMCD pathogenesis in addition to IL-6.

Acknowledgements

The authors thank the patients who participated in this study and their families for their cooperation.

Funding: This study was sponsored by Gilead Sciences, Inc. (CO-JP-986-7009), which provided funding and filgotinib. The funding source had no role in the study design, data collection, analysis, interpretation, manuscript writing, or decision to submit for publication.

Conflict of Interest: The authors have no conflicts of interest to disclose.

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Received January 3, 2026; Revised March 23, 2026; Accepted March 29, 2026.

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Released online in J-STAGE as advance publication March 31, 2026.