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RESEARCH

Clinical and Immunological Impact of JAK Inhibition in Concurrent Down Syndrome and STAT1 Gain of Function

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Abstract

Purpose Down syndrome (DS) and STAT1 gain-of-function (GOF) share clinical and molecular features, including persistent inflammation. We aimed to investigate whether the coexistence of DS and a STAT1 GOF mutation in a patient synergistically enhances interferon (IFN) signaling and exacerbates inflammatory responses, posing additional management challenges. Two patients (P1 and P2) were studied: P1, with DS and a heterozygous p.P326S STAT1 variant, and P2, with the STAT1 p.P326S variant only. Individuals with isolated DS or STAT1 GOF served as controls. IFN receptor subunits (IFNγR1/R2 and IFNαR1/R2) and responses to IFNα/γ stimulation were analyzed using flow cytometry and RT-PCR. Whole blood type I IFN signature and serum cytokines were evaluated using NanoString and Luminex assays. P1 experienced recurrent infections, chronic mucocutaneous candidiasis, interstitial pneumonitis, and pulmonary hypertension. P2 presented with esophageal candidiasis, dysphagia, and stenosis. The p.P326S variant led to increased STAT1/pSTAT1 levels in response to IFNα/γ. Both patients showed significant clinical improvement with the Janus kinase (JAK) inhibitor ruxolitinib. However, P1's key biomarkers (STAT1 levels, IFN signature, TNFα, IL-6) remained altered, indicating persistent inflammation despite clinical improvement. This first report of a STAT1 GOF variant in DS provides a unique "experiment of nature", offering insights into the interplay between trisomy 21 and STAT1-mediated immune dysregulation. Although ruxolitinib demonstrated clinical benefits, the persistent inflammation observed in P1 highlights the need for further strategies to achieve complete immune resolution. These findings emphasize the importance of comprehensive genetic and immunological assessments in individuals with DS, particularly when immune dysfunction is suspected.

Keywords Down syndrome · Inborn errors of immunity · STAT1 transcription factor · STAT1 gain-of-function · Interferon · JAK inhibitors · Ruxolitinib

Introduction

Down syndrome (DS) is a prevalent genetic disorder that arises from an additional copy of chromosome 21, either partial or complete [1]. This condition has been linked to neurodevelopmental delay, hypotonia, congenital cardiac and gastrointestinal abnormalities, and a higher risk of leukemia and Alzheimer-like disease [2]. Individuals with DS have an increased predisposition for the development of autoimmune and autoinflammatory diseases, including type 1 diabetes, hypothyroidism, arthritis, and skin conditions [1, 3]. While their susceptibility to viral infections is not inherently high, the incidence of morbidity and mortality associated with such infections is disproportionately elevated compared to the general population [4, 5]. DS has recently been recognized as an interferonopathy characterized by persistent interferon (IFN) hyperactivity contributing to chronic sterile inflammation [6, 7]. This phenomenon is partly attributed to the gene dosage effect, as four out of six IFN receptor subunits are encoded on chromosome 21 resulting in an enhanced Janus kinase/signal

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transducer and activator of transcription (JAK/STAT) signaling [8–13] (Figure S1). Furthermore, increased levels of total and phosphorylated STAT1 (pSTAT1) have been observed in peripheral blood mononuclear cells (PBMCs) compared with the control groups [6, 8, 14]. A recent multi-omic analysis revealed that individuals with DS who exhibited stronger IFN signaling pathway activity were more prone to encounter major comorbidities such as congenital heart disease and hypothyroidism [15]. Recently, encouraging outcomes with JAK inhibitors (JAKi) have been reported in DS-associated autoimmune conditions, including alopecia areata and psoriatic arthritis [15–20]. Moreover, ongoing clinical trials (NCT04246372, NCT05662228) aim to assess the therapeutic potential of modulating IFN responses with JAK1/JAK3 inhibitor tofacitinib in DS.

A clinical overlap between certain DS individuals and those patients harboring gain-of-function (GOF) variants in *STAT1* has been described as both groups share features such as chronic mucocutaneous candidiasis (CMC), poor infection outcomes and autoimmunity [21]. Furthermore, non-immunological complications, including cerebral aneurysms, neurological features, and cognitive disability, have been observed in patients with STAT1 GOF variants at slightly higher rates [21]. The molecular hallmark of STAT1 GOF is characterized by increased total STAT1 and pSTAT1 in response to stimulation with type I/II IFNs [22–24] (Figure S1). The use of JAKi has yielded favorable outcomes in both adult and pediatric patients with *STAT1* GOF variants [25–28], although prospective studies are needed to evaluate long-term treatment outcomes and to identify effective biomarkers to monitor therapeutic efficacy.

We describe here for the first time, the case of a child with DS who exhibited severe clinical manifestations resulting in a substantial impact on her quality-of-life (QoL). Notably, she was found to carry a previously unreported STAT1 GOF variant (p.P326S). Treatment with ruxolitinib led to sustained clinical improvement, although key inflammatory markers remained elevated, indicating persistent subclinical inflammation.

Methods

Study Participants

The study was approved by the ethics committees of Hospital Universitario Virgen Macarena and Hospital Universitario Virgen del Rocío (PI19/01471, 0641-N-20) and conducted in accordance with the ethical principles set forth in the Declaration of Helsinki.

Genetic Analysis

Genes associated with mendelian susceptibility to mycobacterial disease (*IFNGR1*, *IFNGR2*, *IL12RB1*, *IL12B*, *IRF8*, *ISG15*, *STAT1*, *RORC* and *TYK2*) were analyzed using a next-generation sequencing (NGS) approach. This analysis was performed using an AmpliSeq strategy for the library preparation, and sequencing was conducted on the Ion Torrent PGM platform. The identification of a variant in the *STAT1* gene was validated through Sanger sequencing.

Lung Section Staining

A lung fragment measuring 1 cm was formalin-fixed (10%), paraffin-embedded, sectioned (3–8 μm) and stained with hematoxylin-eosin, Grocott, PAS-Diastase, and Masson's trichrome. Immunohistochemistry was performed using the following antibodies (Roche Diagnostics): CD3 (2GV6), CD4 (SP35), CD8 (SP57), CD20 (L26), CD68 (KP-1), cytomegalovirus (8B1.2, 1G5.2, 2D4.2), and Epstein-Barr virus (CS1-4).

Intracellular Staining for STAT1 and pSTAT1

Quantification was performed as previously described [29]. Briefly, whole blood was stimulated (15 min, 37 °C) with IFNγ (400 UI/mL) or IFNα (100 ng/mL) with or without ruxolitinib (100, 500 or 1000 nM). Following the lysis of erythrocytes and ice-cold methanol permeabilization, the cells were stained with fluorochrome-conjugated human monoclonal antibodies against CD14 (FITC; clone M5E2, Becton Dickinson, BD), CD3 (APC-H7; clone SK7, BD), CD4 (BV711; clone SK3, BioLegend), CD8 (PE-Cy7; clone SK1, BioLegend), STAT1 (Alexa Fluor 647; clone 1/STAT1, BD) and pSTAT1 (pTyr701-PerCP-Cy5.5; clone 4 A, BD). Data were collected using the BD LSR Fortessa™ and analyzed with the FlowJo software package (Becton Dickinson).

Dephosphorylation Assay

Whole blood samples were incubated with anti-human CD14-FITC monoclonal antibody (FITC; clone M5E2, Becton Dickinson, BD) and subsequently stimulated with IFNγ for 15 min at 37 °C. Ruxolitinib (1000 nM) was added and the samples were incubated for additional 15, 30, 60, 90–120 min before fixation and staining as previously described [24].

Gene Expression Analysis

The transcription levels of STAT1-dependent genes were evaluated as previously described [29]. Briefly, peripheral blood mononuclear cells (PBMCs) from the STAT1 GOF patient (P2) and a healthy donor were stimulated with IFNγ (400 UI/mL) or IFNα (100ng/ml) ± ruxolitinib (100, 500 or 1000 nM) for 4 h at 37 °C. An unstimulated sample was included as a baseline reference. *STAT1* mRNA levels were evaluated after normalization to beta actin as an internal control.

Western Blot

STAT1 and pSTAT1 protein levels were quantified as described [29]. PBMCs from P2 and a healthy donor were stimulated with IFNα (100 ng/mL) ± ruxolitinib (1 µM; 30 min, 37 °C). Proteins were subjected to sodium dodecyl sulfate-10% polyacrylamide gel electrophoresis, transferred to PVDF membranes and incubated overnight (4 °C) with STAT1 (Cell Signaling 9172), pSTAT1 (pY701; Cell Signaling 9167) or β-actin (Cell Signaling 4967) antibodies. Detection was performed using horseradish peroxidase-conjugated secondary antibody (1 h, room temperature) and chemiluminescence detection.

Type I Interferon Signature

The evaluation of the type I IFN signature was conducted using whole blood specimens collected into Blood RNA Tempus™ (Thermofisher) tubes in accordance with the manufacturer's instructions. Quantification of a subset of IFN-response genes was performed using the nCounter Elements Technology System (NanoString), including 28 genes representative of the type I IFN response (28-IRG) [30] and 2 additional genes (CXCL9 and STAT1) relevant to the IFN response although not exclusively to type I response. Z-score calculation for each gene was conducted: Z-score = (counts of gene A - average count of gene A in healthy controls)/standard deviation of gene A in healthy controls. A Z-score exceeding 1.73 for the 28-IRG set and 1.96 for the 6-IRG (*IFI 27*, *IFI44L*, *IFIT1*, *ISG15*, *RSAD2*, *SIGLEC1*) was defined as indicative of an upregulation of type I IFN-response genes.

Cytokines Analysis

Serum cytokines (IFNα, IFNγ, IL-1Ra, IL-6, IL-8, IL-7, IL-9, IL-10, IL-12p70, IL-17 A, IL-18, IL-23, IP-10, TNFα and CXCL5) were quantified using Procarta-Pex™ panels, (Invitrogen) and Bio-Plex 200 System (Bio-Rad).

Clinical Assessment Before and During JAKi Therapy Using the Immune Deficiency and Dysregulation Activity (IDDA) Score

This score is a physician-reported outcome measure that evaluates the severity of involvement in 12 organ systems affected by immune dysregulation, together with two additional features: failure to thrive and severe infections. It also accounts for the proportion of hospitalization days and the need for supportive therapies and care. The score was further expanded by including "any other organ or immune dysfunction" and "any relevant chronic or recurrent infestation or infection," as previously described [31].

Data Analysis

Flow cytometry data were analyzed in raw values of the geometric mean of fluorescence intensity (gMFI). Graphs and statistics (Shapiro-Wilk, Wilcoxon, Kruskal-Wallis tests; significance $p < 0.05$) were performed using the Prism software (version 8, GraphPad Software) and IMB SPSS Statistics 26.

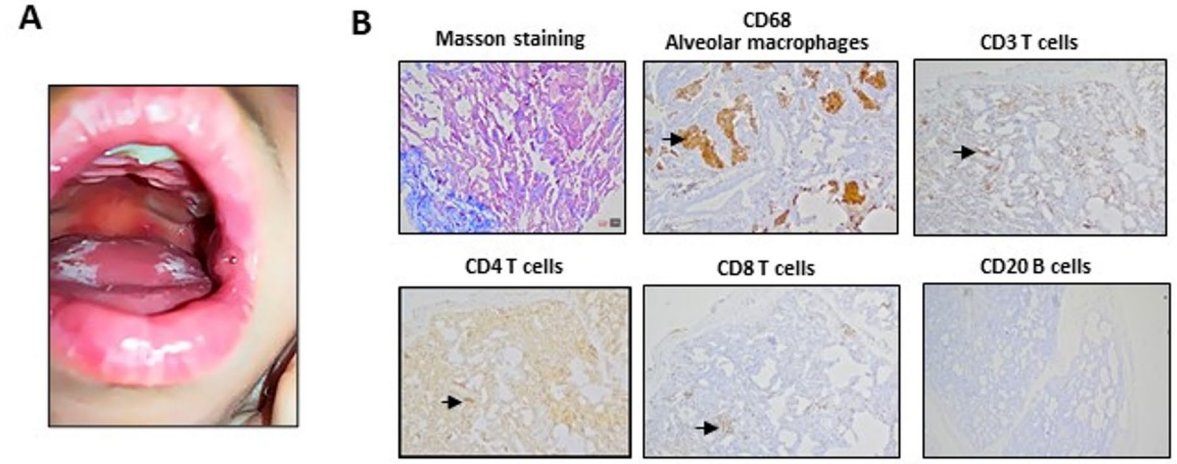
Results

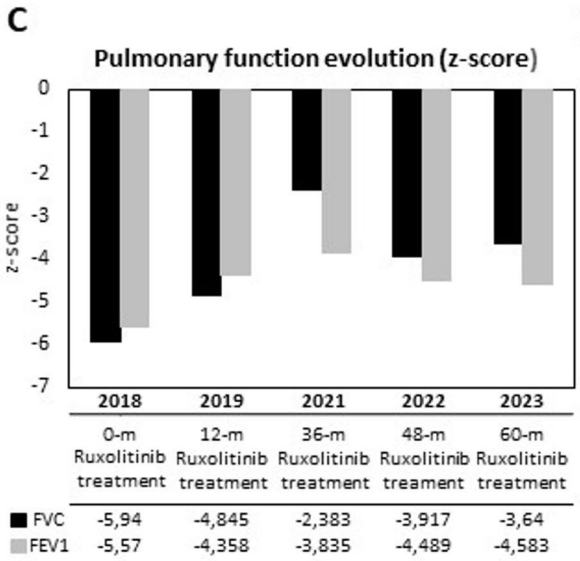
Clinical Evaluation, Genetic Analysis and Diagnosis

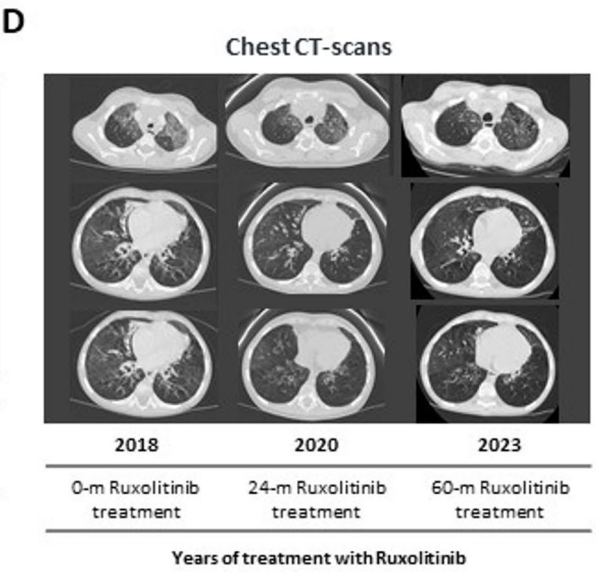
A 5-year-old girl (P1) with DS presented with a 2-year history of recurrent bacterial infections in the upper respiratory tract, oral (Fig. 1A) and vaginal *Candida albicans* infections, lymphadenopathies, progressive interstitial pneumonitis with bronchiectasis, and secondary pulmonary hypertension. She required antibiotic and antifungal prophylaxis (including sulfamethoxazole and trimethoprim (SMX-TMP), and fluconazole), intermittent nebulized and systemic steroid therapy, 24-h oxygen supplementation, and pulmonary antihypertensive therapy with, sildenafil and iloprost (Table 1).

A comprehensive immunological workup revealed reduced memory B cells whereas immunoglobulin levels were within the normal range for age (Table S1). Histopathological analysis showed bronchiolar submucosal fibrosis and alveolar septal widening, as well as increased alveolar macrophages (CD68+) and T cells (CD3+; balanced CD4+/CD8 + ratio) but scarce B cells (CD20+). No signs of vasculitis or thrombosis were observed, and screening for fungal, CMV and other microorganisms was negative (Fig. 1B).

NGS analysis identified a heterozygous c.976 C >T variant in the *STAT1* gene that leads to an amino acid exchange p.P326S in the DNA-binding domain of the protein (Fig. 2A) [26, 28]. Intrafamilial segregation analysis revealed







E

6 Minute Walk Test

	2019	2020	2022	2023
	12-m Ruxolitinib treatment	24-m Ruxolitinib treatment	48-m Ruxolitinib treatment	60-m Ruxolitinib treatment
Age (years)	8	9	11	12
Distance (m)	404,8	410,8	455,4	506
z-score (age)	-3,21	-3,11	-3,24	-2,66
z-score (height)	-0,8	-0,85	-1,07	-0,7

F

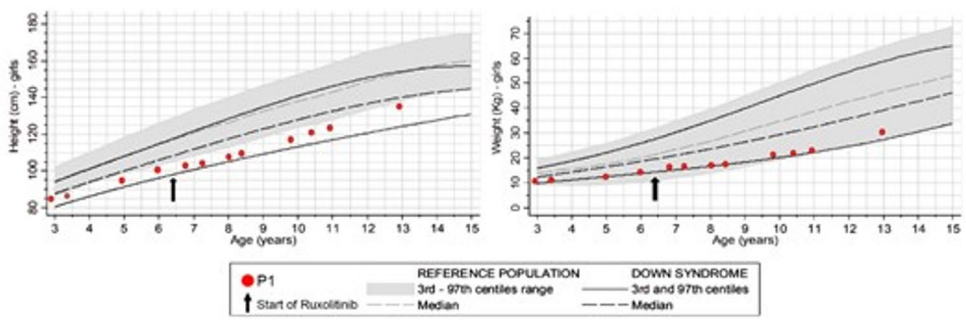


Fig. 1 Clinical manifestations of P1. (**A**) Oral candidiasis at the age of 5 years old (2016). (**B**) Immunohistological staining of lung tissue. Masson staining was performed for collagen fibers detection and differentiation between connective and lung tissue samples. Immune cell infiltration in lung tissue was assessed by immunochemistry targeting CD68+ alveolar macrophages, CD3+ cells, CD4+ T cells, CD8+ T cells and CD20+ B cells. (**C**) Pulmonary function over time. P1's pulmonary function was measured through spirometry for 60 months after the start of treatment with ruxolitinib. Results are shown as z-score based on forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV1). (**D**) Chest CT-scans. P1 presented with lung involvement including bronchiectasis and ground-glass opacification due to continued inflammation and infection. P1's lung structure was analyzed by chest CT-scans over time since the start of treatment with ruxolitinib. (**E**) 6-minute walk test. Physical condition was measured through 6-minute walk tests for 60 months after the start of treatment with ruxolitinib. (**F**) P1's weight and height using 3rd-97th centile ranges using Growth Charts for Children with Down syndrome [58]

that this variant was inherited from the patient's mother (P2). At that time, the mother was 42 year-old and referred for childhood onset CMC including the esophagus which resulted in esophageal stenosis requiring endoscopic dilatation. In silico tools classified this variant as deleterious (including Sift: 0.05; Polyphen 0.991) (Table S2). In addition, functional assays using peripheral blood from P2 showed increased STAT1 levels in monocytes and increased pSTAT1 upon IFNγ stimulation, with normal dephosphorylation kinetics (Fig. 2B-C) [32]. These findings confirmed P326S as a pathogenic STAT1 GOF variant.

Ex Vivo Effects of Ruxolitinib and Long-term Clinical Benefits in STAT1 GOF Syndrome

We evaluated the ex vivo effect of ruxolitinib on IFNγ-induced pSTAT1 levels in P2's monocytes. At concentrations of 0.1–0.5 µM, ruxolitinib normalized pSTAT1 levels (Fig. 2D) as well as the relative STAT1 expression (Fig. 2E) to those observed in the control group (Fig. 2E). Similarly, PBMCs from P2 stimulated with IFNα displayed increased pSTAT1 levels, which were inhibited by 1 µM ruxolitinib (Fig. 2F). Based on these findings, and prior positive clinical reports [25], ruxolitinib was initiated in P1 (age 6.5 years) at 3 mg every 12 h (0.4 mg/kg/day; March 2018). Given the good tolerance of the drug, the dose was subsequently increased to 5 mg every 12 h (0.66 mg/kg/day; May 2018) and later adjusted to her current regimen of 5 mg every 8 h (0.66 mg/kg/day; May 2024). P2 (age 43 years), who presented with milder symptoms, started ruxolitinib at 5 mg every 12 h (February 2021), which was later increased to 7.5 mg every 12 h to achieve complete symptom control—a regimen she continues to date.

P1´s clinical response to ruxolitinib was remarkable and has been sustained for 7 years. CMC resolved within 4–6 weeks, allowing the withdrawal of the antifungal prophylaxis. Steroids and oxygen supplementation were successfully weaned within 6 and 12 months, respectively. Pulmonary hypertension improved, enabling a 66% reduction of phosphodiesterase 5 (PDE5) inhibitors within 36 months (Table 1). Overall, the pulmonary function was stabilized and partially improved (Fig. 1C-D). In contrast to the 30 non-elective hospitalizations recorded between the diagnosis of STAT1 GOF in October 2016 and the initiation of JAKi therapy in March 2018, she did not require any hospital admissions since starting ruxolitinib therapy. Her exercise tolerance assessed by the 6-minute walk test, also significantly improved (Fig. 1E) [33].

The IDDA score decreased from 40 before ruxolitinib treatment to 8.1 after 40 months [31]. Nutritional support was discontinued after 20 months, and she continued to thrive along the 3rd percentile (Fig. 1F).

Of note, despite the observed clinical benefits, progressive lymphopenia affecting T, B, and NK cells has been noted (Table S1).

Ruxolitinib Modulates PSTAT1 Activation in P1 Cells But Fails to Normalize IFNAR and Total STAT1 Levels

As a unique and challenging case combining two complex diseases associated with IFN signaling dysregulation (DS and STAT1 GOF), and since individuals with DS exhibit elevated IFN receptor levels compared with healthy controls, we hypothesized a synergistic IFN hyperactivity in P1 exceeding that seen in isolated DS or STAT1 GOF cases. Baseline experimental tests could not be performed in P1 before ruxolitinib initiation. However, analysis of the IFN receptor subunit was performed 60 months after treatment. Despite clinical improvement (Table 1; Fig. 1), P1 exhibited persistently elevated IFNAR2 levels comparable to those of individuals with DS ($n = 8$, DS1–DS6 and DS8–DS9; clinical characteristics in Table S3), whereas the remaining subunits were similar to those of healthy controls and P2, who had received ruxolitinib for 30 months (Figure S2).

Due to limited sample availability, we analyzed P1's IFN response at 18, 24-, 36-, 60-, and 70-months post-treatment, comparing STAT1 and pSTAT1 levels to healthy controls, DS individuals, naïve STAT1 GOF patients (GOF1-4, P2 pre-treatment), and STAT1 GOF patients on ruxolitinib (P2 at 7 months, GOF5 at 60 months) (Fig. 3, Tables S3-S4).

Although ruxolitinib reduced STAT1 levels in P1 compared to *naïve* STAT1 GOF patients, they remained elevated relative to healthy controls (Fig. 3A, Figure S3A). However, pSTAT1 levels post-IFN stimulation were notably reduced in P1 (Fig. 3B, Figure S4). A similar

Table 1 Clinical course and treatment response

	Oct. 2016 5 y-old	Mar 2018 6,5 years old	Mar 2020 8,5 years old	Mar 2021 9,5 years old	Mar 2022 10,5 years old	Mar 2023 11,5 years old
	STAT1 GOF diagnosis	Start on ruxolitinib	24-m ruxolitinib treatment	36-m ruxolitinib treatment	48-m ruxolitinib treatment	60-m ruxolitinib treatment
SABA	On demand	On demand	On demand	On demand	On demand	On demand
LABA/IC (mcg/day)*	100/500	100/500				
LTRA	4 mg	4 mg				
Short Acting anti-cholinergic	On demand	On demand	On demand	On demand	On demand	On demand
Long Acting Anti-cholinergic			5mcg/24 hours	5mcg/24 hours	5mcg/24 hours	5mcg/24 hours
Antibiotic (prophylaxis)	SMX/TMP 100/20mg/day	SMX/TMP 100/20mg/day	SMX/TMP 200/40mg/day	SMX/TMP 200/40mg/day	SMX/TMP 200/40mg/day	SMX/TMP 200/40mg/day
Immunoglobulin	7,5gr/4-weeks i.v.$	7,5gr/4-weeks i.v.$	5gr/3-weeks s.c."	5gr/3-weeks s.c."	5gr/3-weeks s.c. "	5gr/3-weeks s.c."
Anti-fungal (prophylaxis)	Fluconazole 6mg/kg/day	Fluconazole 6mg/kg/day	Topical Nystatin	Topical Nystatin	Topical Nystatin	Topical Nystatin
Ruxolitinib		3mg/12 hours	5 mg/12 hours	5 mg/12 hours	5 mg/12 hours	5 mg/12 hours
Oxygen (hours/per day)	24	24	0	0	0	0
Oxygen L/per min	0,75-2	1-3	0	0	0	0
Gastro-esophagic reflex	PBI+	PBI+	Surgical treatment			
PDE5 inhibitors[a]	1mg/kg/8hours&	1mg/kg/8hours&	1mg/kg/8hours&	1mg/kg/day&	1mg/kg/day%	1mg/kg/day%
Iloprost	2,5ng (x5 /day)	2,5ng (x5 /day)				

SABA Short-Acting Beta Agonist, *LABA* Long-Acting Beta-Agonist, *IC* Inhaled Corticosteroid, *LTRA* Leukotriene Receptor Antagonist, *SMX/TMP* Sulfamethoxazole and Trimethoprim

*salmeterol/fluticasone;+Proton Bomb Inhibitors; "Subcutaneous immunoglobulin; $Intravenous immunoglobulins; PDE5 inhibitors [a]aphosphodiesterase type 5 inhibitor, %Tadalafil; &Sildenafil

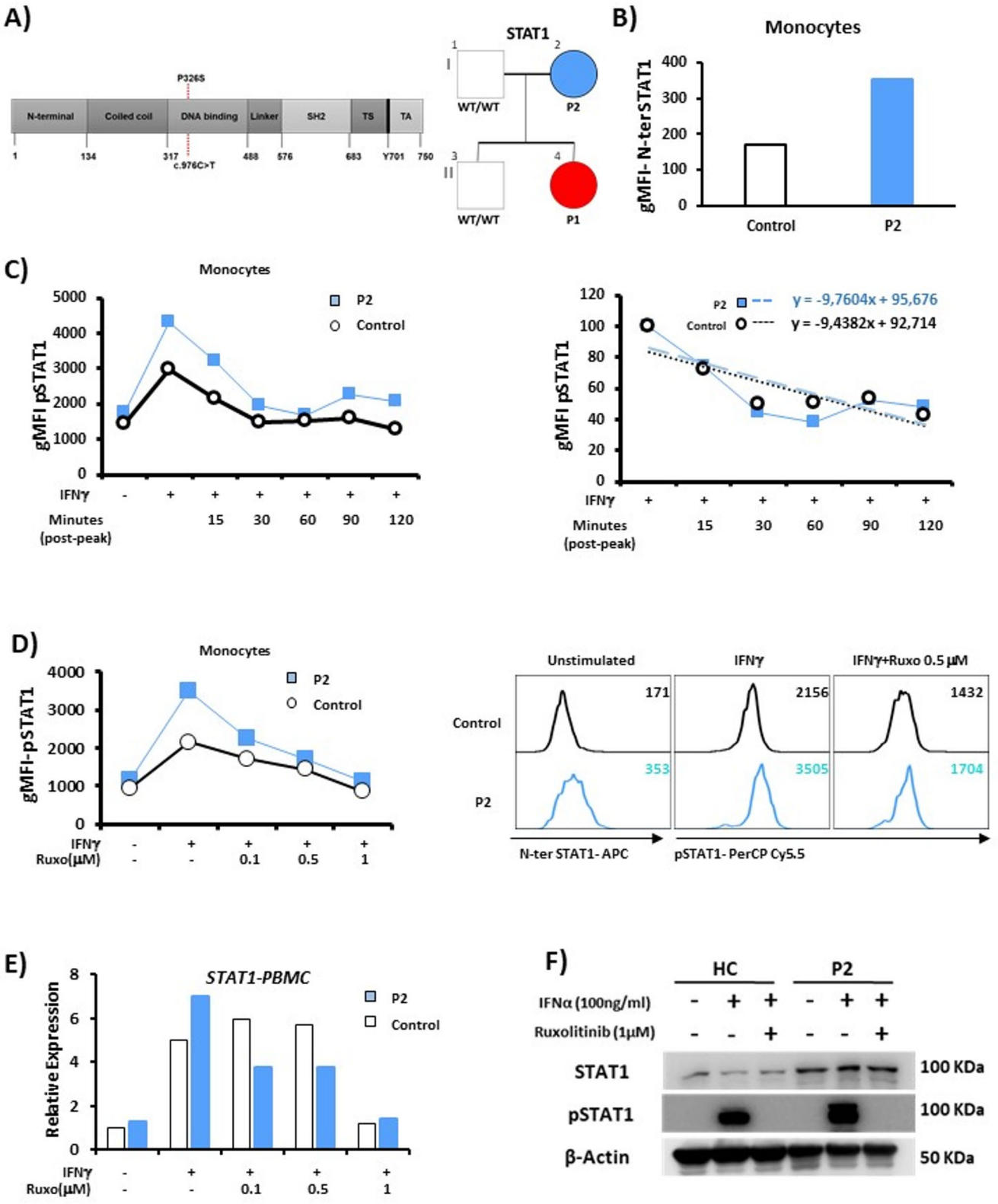
pattern was observed in P2 and GOF5 during ruxolitinib treatment: total STAT1 remained high, but pSTAT1 normalized upon IFNα and IFNγ stimulations (Fig. 3, Figure S3D, 5).

Altogether, these findings suggest that, although ruxolitinib effectively modulates pSTAT1 activation in an individual with concurrent DS and STAT1 GOF, it does not fully normalize IFNAR or total STAT1 levels. The persistent STAT1 elevation in P1, P2 and GOF5 despite ruxolitinib, may suggest that JAKi have a limited effect on restoring the STAT1 homeostasis.

Limited Efficacy of Ruxolitinib in Normalizing the Atypical IFN Signature in P1

We next assessed the IFN signature in P1's peripheral blood, comparing it with that of naïve individuals (DS, P2 before treatment) and STAT1 GOF patients receiving ruxolitinib (P2, GOF5; Table 2). At 36 months post-treatment, P1 continued to display a persistently positive IFN signature, comparable to naïve P2 and DS-9. Elevated expression of specific STAT1 target genes, including CXCL9 and CXCL10, was detected in naïve P2, in 50% of DS individuals, and in both P1 and GOF5. Whereas ruxolitinib normalized the expression of most genes in P2 and GOF5, P1 showed increased expression in 22 of the 32 analyzed genes, notably those encoding CXCL9, CXCL10, ISG15, and USP18.

Fig. 2 P326S STAT1 variant identification and validation and the ex vivo effect of ruxolitinib. **A**) Schematic representation of the STAT1 protein domains including the mutation. **B**) Geometric mean fluorescence intensity (gMFI) of STAT1 in resting $CD3^+$, $CD4^+$ and $CD8^+$ T cells and $CD14^+$ monocytes of healthy controls (white bars) and P2 (blue bars). **C**) Levels of pSTAT1 after IFNγ stimulation for 15 min and 15–120 min after reaching peak levels in the presence of ruxolitinib (1 μM) using monocytes of P2 (blue) and healthy controls (white). **D**) Dose-related effect of ruxolitinib (0.1, 0.5 and 1 μM) on pSTAT1 levels of monocytes from P2 (blue) and healthy controls (white) after stimulation with IFNγ. Representative histograms of the gMFI obtained for STAT1 (APC) and pSTAT1 (PercP Cy5.5) are shown at right. **E**) Relative expression of *STAT1* after 4 h-stimulation of PBMCs with IFNα in the presence of different concentrations of ruxolitinib (0.1 μM, 0.5 μM or 1 μM). Relative expression was calculated after normalization to unstimulated d sample of healthy control using the comparative $2^{-\Delta\Delta ct}$ method. **F**) Immunoblot analysis of lysates of PBMCs from P2 and the healthy control. Cells were left unstimulated or stimulated with IFNα with or without the presence of 1 μM ruxolitinib


A)
STAT1
P326S
N-terminal
Coiled coil
DNA binding
Linker
SH2
TS
TA
1
134
317
c.976C>T
488
576
683
Y701
750
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4
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D)
Monocytes
gMFI-pSTAT1
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P2
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IFNγ - + + + +
Ruxo(μM) - - 0.1 0.5 1
Unstimulated
IFNγ
IFNγ+Ruxo 0.5 μM
Control
P2
171
2156
1432
353
3505
1704
N-ter STAT1- APC
pSTAT1- PerCP Cy5.5
E)
STAT1-PBMC
Relative Expression
8
6
4
2
0
P2
Control
IFNγ - + + + +
Ruxo(μM) - - 0.1 0.5 1
F)
HC
P2
IFNα (100ng/ml) - + + - + +
Ruxolitinib (1μM) - - + - - +
STAT1
pSTAT1
β-Actin
100 KDa
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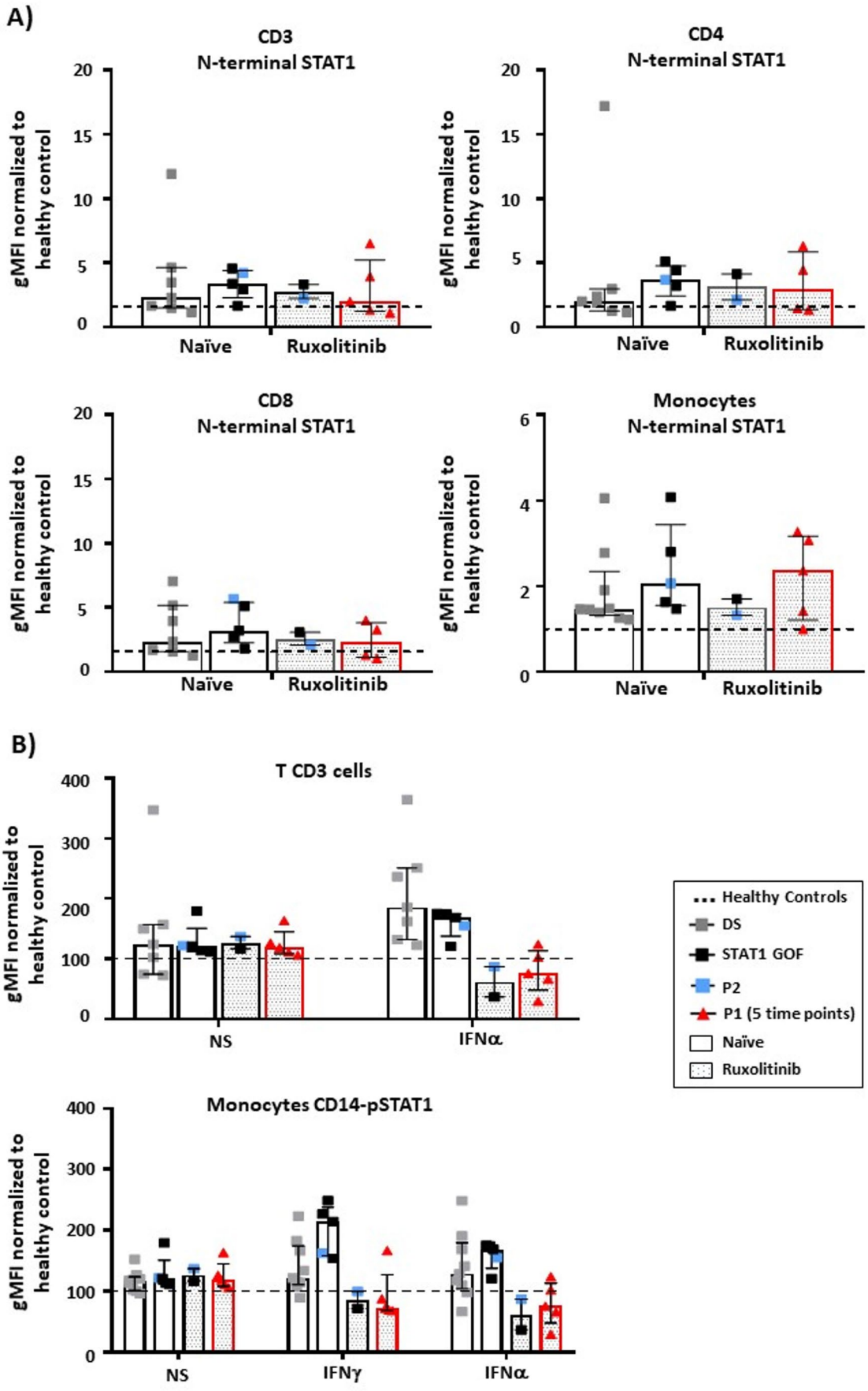
A)
CD3
N-terminal STAT1
CD4
N-terminal STAT1
CD8
N-terminal STAT1
Monocytes
N-terminal STAT1
gMFI normalized to healthy control
Naïve
Ruxolitinib
B)
T CD3 cells
NS
IFNα
Monocytes CD14-pSTAT1
IFNγ
Healthy Controls
DS
STAT1 GOF
P2
P1 (5 time points)
Naïve
Ruxolitinib

Fig. 3 STAT1 and pSTAT1 levels measured by flow cytometry. (**A**) Geometric mean fluorescence intensity (gMFI) of STAT1 in resting $CD3^+$, $CD4^+$ and $CD8^+$ T cells and $CD14^+$ monocytes of healthy controls (white circles), DS (gray squares), STAT1 GOF (black or blue squares) and P1 (red triangle). Standard or dotted background in bars indicate the naïve or under ruxolitinib status of individuals. Dotted line represents normalized healthy control values. (**B**) Levels of pSTAT1 in unstimulated (NS) or IFNγ/IFNα stimulated monocytes of healthy controls (white circles), DS (gray squares), STAT1 GOF (black or blue squares) and P1 (red triangle). Each gray and black square represents an individual DS or STAT1 GOF while red triangles represent different time-points of P1 [18 months (September 2019), 24 months (March 2020), 36 (March 2021), 60 (March 2023), 70 months (January 2024)]. Black lines associate the patients with their respective healthy controls. Median and interquartile ranges are represented. Dotted line represents normalized healthy controls values

Persistent Inflammatory Profile in P1 Despite Ruxolitinib Therapy on the Cytokine Level

To further evaluate P1´s inflammatory status, a comprehensive analysis of cytokine levels was conducted on serum samples collected at 36- and 60-months post-treatment. Whilst individuals with DS exhibited increased IL-6 levels; this was not observed in STAT1 GOF patients. Interestingly, in contrast to P2 and GOF5, P1 demonstrated a persistent elevation in TFN-α, IL-1Ra and IL-6 levels under ruxolitinib treatment, suggesting an unresolved proinflammatory cytokine production state despite JAKi therapy (Fig. 4).

Discussion

Down syndrome (DS) is associated with an increased incidence of recurrent respiratory tract infections [2, 7, 34–36]. Additionally, pulmonary hypertension is also frequently observed in DS, often because of congenital heart disease [37, 38]. In this study, we describe the case of a 5-years-old patient with DS (P1) suffering from recurrent pneumonia, chronic mucocutaneous candidiasis (CMC) and secondary pulmonary hypertension.

Validation of a Novel *STAT1* Variant and Response to Ruxolitinib

Because chronic mucocutaneous candidiasis (CMC) is uncommon in DS and is primarily associated with pathogenic variants in STAT1, STAT3, AIRE, or IL17 genes we first performed a genetic analysis in P1, which revealed a novel heterozygous STAT1 P326S variant inherited from her mother (P2).

In silico prediction tools and functional assays of the variant suggested a gain-of-function (GOF) effect, with elevated STAT1 and phosphorylated STAT1 (pSTAT1) levels upon IFNγ stimulation [32]. While earlier studies attributed GOF variants in STAT1 to impaired dephosphorylation [22, 39–41], increased total STAT1 protein levels have been recently proposed as an alternative mechanism [24]. Ex vivo experiments demonstrated that ruxolitinib effectively modulates IFN responses in P2 cells at clinically relevant plasma concentrations (0.1–0.5 µM), which can be achieved with oral 5–10 mg doses every 12 h [22–24, 42].

Clinical Response to Ruxolitinib

Ruxolitinib therapy (0.4 mg/kg/day) was administered to P1, resulting in the resolution of CMC within a period of 3 months. Furthermore, there was a significant improvement, in her lung pathology, characterized by a substantial decrease in the incidence of pneumonia and associated complications. Before treatment, P1 required 30 non-elective hospital admissions over a 2-year period, primarily due to respiratory infections. Following a period of 7 years on ruxolitinib, no further non-elective hospital admissions were recorded, supporting a profound and sustained clinical benefit. P2 demonstrated comparable improvement under ruxolitinib therapy. Her CMC resolved within a period of 4 months at a dosage of 0.3 mg/kg/day. JAKi treatment was well tolerated in both patients, with no significant adverse effects, which is consistent with existing data on JAKi treatment in patients harboring STAT1 GOF variants [25–28]. This is the first report of a documented case of ruxolitinib treatment in a patient with concurrent DS and STAT1 GOF.

Interferon Signaling and Gene Expression

Given the hyperactive IFN signaling in DS and STAT1 GOF, we hypothesized that their coexistence in a unique patient would exacerbate immune dysregulation when compared to isolated cases of each condition. We therefore included individuals with DS who exhibited infectious and/or autoimmune manifestations, untreated patients with STAT1 GOF variants, and a patient with a STAT1 GOF variant receiving ruxolitinib (GOF5). In DS, IFN hyperactivity is believed to be linked to the presence of additional copies of IFN receptor genes, located on chromosome 21, leading to elevated IFNAR1/IFNAR2 expression [8, 9]. IFNAR2 expression was markedly higher in P1 than in HC and P2, while the remaining IFN receptor subunits were not appreciably altered. Prior work has proposed that increased IFNAR2 alone can amplify type I IFN–induced pSTAT1 signaling [43] Notably, ruxolitinib did not normalize IFNAR2 levels in P1, consistent with a previous DS case in which tofacitinib reduced the IFN score but did not restore IFNAR expression [15]. To explore whether this IFNAR overexpression together with effects of the GOF mutation in STAT1 could result in an additional amplification of IFN response in P1,

Table 2. Individual Z-scores from interferon signature analysis

GENES	PATIENTS											
	W/O RUXOLITINIB TREATMENT									W/ RUXOLITINIB TREATMENT		
	DS-1	DS-2	DS-3	DS-5	DS-6	DS-8	DS-9	DS-11	P2	P2 (1 m)	GOF5 (36 m)	P1 (36 m)
CXCL10	3,16	3,36	1,81	10,11	0,02	0,24	0,84	2,38	6,62	0,54	6,23	2,15
DDX60	0,17	0,04	-0,41	0,66	-0,12	0,94	6,21	1,09	2,87	0,52	0,4	3,85
EPSTI1	2,00	0,35	0,32	5,00	1,11	3,02	8,40	2,23	3,04	0,69	3,48	2,23
GBP1	0,47	0,46	-0,27	4,15	0,17	4,32	1,30	1,22	6,92	2,06	8,77	2,30
HERC5	-0,08	-0,58	-0,50	0,61	-0,68	0,22	3,88	0,91	2,56	0,43	-0,36	2,36
HERC6	0,91	0,42	-0,30	0,14	-0,32	0,72	5,49	2,18	1,74	0,37	-0,68	2,73
IFI27	2,92	0,06	1,16	7,85	0,06	1,65	75,10	1,68	0,31	-0,32	0,1	2,97
IFI44	0,44	-0,12	0,23	1,54	-0,14	0,73	6,74	1,25	3,12	0,75	-0,1	3,43
IFI44L	0,39	-0,38	-0,15	0,96	-0,42	0,10	6,77	0,75	2,35	0,13	-0,43	3,27
IFI6	-0,16	-0,32	-0,25	1,37	-0,71	-0,08	2,34	0,18	2,47	0,18	-0,74	1,63
IFIT1	-0,04	-0,35	-0,11	1,46	-0,50	0,34	5,39	0,54	3,01	0,63	-0,35	1,80
IFIT2	-0,28	0,02	-1,00	2,03	-0,83	2,40	9,64	-0,26	4,58	1,56	1,16	2,87
IFIT3	-0,37	-0,36	-0,56	1,30	-0,86	0,59	3,84	0,15	3,32	0,66	0,34	1,67
IFIT5	0,53	-0,2	-0,44	2,14	-0,64	1,00	6,06	1,55	5,11	2,20	1,43	4,12
ISG15	-0,11	-0,29	-0,41	1,08	-0,39	-0,30	5,01	0,61	1,67	-0,22	-0,17	1,42
LAMP3	1,52	0,69	-0,15	-0,29	-0,05	0,60	3,48	1,27	3,62	1,07	1,23	1,31
LY6E	0,94	-0,27	0,20	2,24	-0,18	-0,06	10,25	1,40	1,15	-0,26	-0,33	2,55
MX1	1,73	0	1,10	3,06	0,23	1,12	9,58	2,54	2,10	0,2	-0,89	5,60
OAS1	0,54	0,24	0,05	3,50	-0,81	-0,69	8,79	1,05	3,29	0,39	0,85	4,71
OAS2	1,77	1,15	-0,21	2,04	-0,52	-0,14	12,57	2,85	5,08	1,22	0,7	6,75
OAS3	0,55	0,31	-0,04	2,47	-0,20	-0,01	7,97	1,08	3,05	0,12	0,24	4,52
OASL	-0,12	-0,4	-0,87	0,10	-1,02	-0,95	3,06	0,86	3,72	1,38	-0,11	1,24
RSAD2	0,15	-0,13	-0,33	1,24	-0,38	0,22	6,94	0,57	2,63	0,18	-0,31	2,24
RTP4	-0,32	0	-0,35	0,28	-1,10	0,55	2,74	0,26	0,90	-0,83	0,92	-0,33
SIGLEC1	0,84	0	-0,05	2,49	-0,14	-0,22	13,47	1,11	1,43	-0,18	-0,6	5,98
SOCS1	0,23	0,37	-0,51	-0,52	-0,86	0,65	0,38	-1,39	1,36	-1,92	-1,06	-0,56
SPATS2L	-0,24	-0,37	3,30	0,58	-0,68	-0,61	3,20	2,07	-0,05	-1,31	-1,22	2,05
USP18	1,97	-0,18	0,28	1,14	0,26	0,12	7,61	1,84	1,41	-0,04	-0,63	2,55
CXCL9	4,44	1,25	1,15	17,00	2,35	1,12	1,23	2,51	3,36	0,39	10,21	2,48
STAT1	-0,01	-0,75	-0,85	2,46	-0,21	2,40	0,88	-0,01	3,51	0,57	4,86	0,93
28IRG*	0,46	-0,06	-0,18	1,42	-0,39	0,29	6,13	1,10	2,75	0,28	-0,48	2,46
6IRG*	0,27	-0,21	-0,13	1,35	-0,39	0,16	6,85	0,68	2,01	-0,03	-0,53	2,60

**Z-score exceeding 1.73 for the 28-IRG set and 1.96 for the 6-IRG indicates an upregulation of type I IFN-response genes*

we interrogated the IFN-JAK-STAT pathway comparing to DS and STAT1 GOF patients. As expected, individuals with DS and treatment-naïve STAT1 GOF patients had elevated basal STAT1 and increased pSTAT1 levels upon IFNα and IFNγ stimulations [8, 22, 24]. Whilst ruxolitinib normalized pSTAT1 responses in P1, P2, and GOF5, total STAT1 levels remained augmented. Type I IFN signaling normalization in P2 can be attributed to ruxolitinib, however, the case of P1 appears to be more complex.

Prior studies have suggested that individuals with DS exhibit baseline activation of the type I IFN pathway and elevated pSTAT1 levels, resulting in a refractory state in which cells fail to respond to subsequent type I IFN stimulation [43]. In contrast, in the present study, DS cells displayed normal basal pSTAT1 levels, with elevations observed only after IFNα stimulation. We assessed type I IFN signature scores, recently implemented to improve the diagnostic evaluation, monitoring of disease activity, and assessment of treatment responses in patients with type I interferonopathies [30]. As demonstrated in previous studies, JAK inhibition has been shown to reduce ISG expression in both DS and STAT1 GOF patients [15, 44]. Consequently, the IFN signature was found to be within normal parameters in both P2 and GOF5 subjects under treatment with ruxolitinib. However, complete normalization could not be confirmed due to the lack of pre-treatment samples for comparison and the persistence of altered expression in some IFN-related genes, such as *CXCL10*, *CXCL9*, and *STAT1*. P1 exhibited elevated expression of 22 out of 32 analyzed ISGs, suggesting sustained IFN dysregulation. Notably, *USP18*, a negative IFNAR regulator was overexpressed, which may have resulted in a reduction in IFNα responsiveness [6, 43]. However, this did not correlate with an increased viral susceptibility in P1. Interpretation of IFN signature results may be confounded by therapies such as corticosteroids, which suppress ISG expression, or by active infections, which can transiently elevate it. Nevertheless, IFN signature assessment remains a useful tool to capture a patient's overall interferon status [45].

Complex Immune Dysregulation and Therapeutic Considerations

IFN hyperactivity has been linked to a proinflammatory profile, characterized by elevated IL-6 and TNFα in both DS [15, 46] and STAT1 GOF [47]. As expected, P1 exhibited increased serum IL-6 and TNFα levels. It has been demonstrated that IL-6 can activate pSTAT1 independently of IFN signaling, suggesting a potential pathogenic role. Consequently, IL-6 inhibitors may represent a promising adjunctive therapeutic approach [48–50]. A potential interplay between pSTAT1-mediated pathways and TNFα signaling has also been described but further studies are required to determine whether direct TNFα inhibition could modulate STAT1 activation or vice versa [51]. P1 also exhibited increased IL-10 and IL-1Ra, suggesting a compensatory anti-inflammatory response to STAT1 hyperactivation, an aspect not yet studied in the context of *STAT1* GOF [52]. Similarly, *Malle et al.*. identified a subset of DS individuals with elevated IL-10, though its biological impact remains to be elucidated [46]. Overall, these findings underscore the complexity of immune dysregulation in this dual pathology and highlight the potential role of targeted therapeutic strategies beyond JAKi monotherapy.

Limitations of Ruxolitinib and Need for Additional Therapeutic Strategies

While the efficacy of ruxolitinib in reducing clinical symptoms and pSTAT1 activation has been evident, its inability to fully normalize the IFN signature or resolve inflammation suggests that adjunctive therapeutic strategies may be needed. Controlling this subclinical inflammation is probably relevant as it has been hypothesized that chronic inflammation, which is associated with immunosenescence [53], may contribute to long-term immune dysfunction and neurodegeneration [54] potentially accelerating Alzheimer-like disease, which is well described in DS [55].

Of note, longitudinal assessments of P1 revealed progressive lymphopenia affecting all subsets, a known but yet poorly understood feature of STAT1 GOF [21, 56].

Despite the promising clinical outcomes, certain limitations of the study must be acknowledged. The lack of measurements of plasma ruxolitinib levels limits insights into its pharmacokinetics and correlation with clinical responses. Furthermore, biomarkers such as pSTAT1 and total STAT1 levels, IP-10 or IFN signature scores do not always correlate with clinical improvement, indicating the need for more reliable biomarkers to monitor disease progression and treatment response in the DS and *STAT1* GOF settings [44, 56, 57]. Additionally, the absence of baseline molecular data from P1 prior to treatment prevents a full evaluation of the effect of JAK inhibition in this particular patient.

Conclusions

Comprehensive immunological evaluation is essential in individuals with DS and immune dysregulation, as it may uncover previously undiagnosed genetic diseases and provide data to guide targeted treatment strategies. In our unique case of coexistence of DS and STAT1 GOF we observed amplified IFN signaling abnormalities, further complicating the case management. While ruxolitinib proved effective in reducing

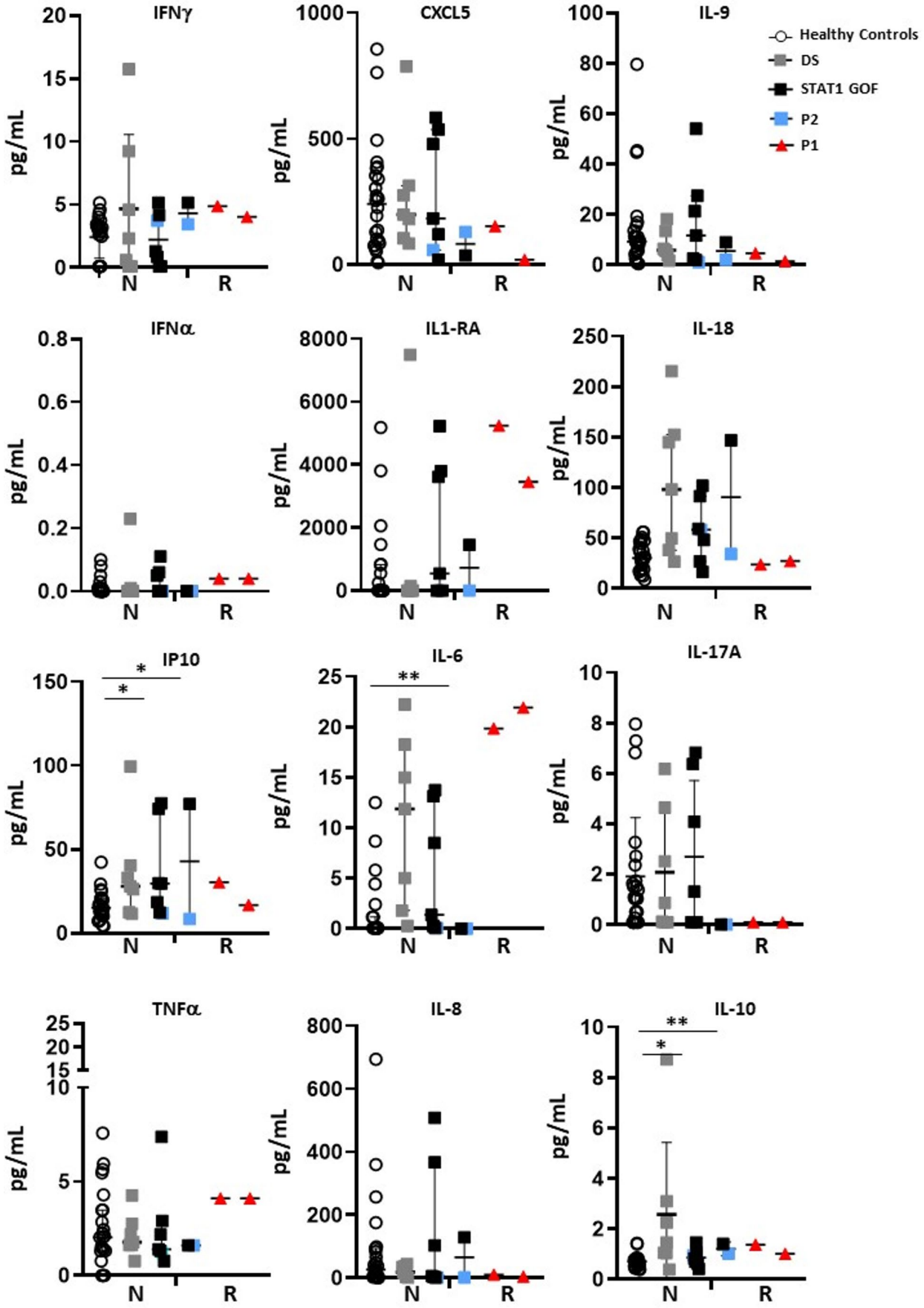
IFNγ
CXCL5
IL-9
Healthy Controls
DS
STAT1 GOF
P2
P1
IFNα
IL1-RA
IL-18
IP10
IL-6
IL-17A
TNFα
IL-8
IL-10
pg/mL
N
R
*
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Fig. 4 Serum cytokine levels. Quantification of cytokines (pg/ml) found in healthy controls (white circles), DS (gray squares), STAT1 GOF (black or blue squares) and P1 (red triangle). Median and interquartile ranges are represented. Two time points after ruxolitinib initiation is included: 30 and 60 months post-ruxolitinib initiation. *p-values lower than 0.05 were considered statistically significant

pSTAT1 activation and controlling CMC and recurrent respiratory infections, it did not fully normalize inflammation, highlighting the potential need for additional therapeutic interventions. Future studies should explore alternative therapies, refine biomarker-based monitoring, and optimize dosing strategies to improve outcomes in IFN-driven disorders.

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Author Contributions Peter Olbrich and Olaf Neth contributed to the conception of the work and revision and approval of the final manuscript. Pilar Blanco-Lobo supervised the experiment performance and wrote the manuscript. Paula Gilabert Prieto, Beatriz de Felipe, Paloma Guisado Hernández, Ana Ortiz-Ramírez and David Moreno-Fuentes performed the experiments included in the final manuscript. Beatriz de Felipe organized sample shipping and processed all samples. Nicolas Valerdiz Menendez performed immunohistochemistry tests. All of them have revised and approved the final manuscript. Anna Mensa-Vilaró and [Juan I Aróstegui](https:/pubmed.ncbi.nlm.nih.gov/?sort=date&size=20&term=Ar%C3%B3stegui+JI&cauthor_id=36049972) determined and analysed the type I interferon signature, have revised and approved the final manuscript. Natalia Palmou, Valle Velasco Gonzalez, Ángela Deyà Martinez, Jan Ramakers, José Ivorra Cortés, Elisa Cordero, Cristina Roca, Mirella Gaboli, Immaculada Guillen and José Manuel Lucena contributed to the diagnosis, monitoring and recruitment of patients included in the final work. They have revised and approved the final manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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