

JAK1/2 inhibition with baricitinib in the treatment of autoinflammatory interferonopathies

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BACKGROUND. Monogenic Interferon (IFN)-mediated autoinflammatory diseases present in infancy with systemic inflammation, an IFN-response-gene-signature (IRS), inflammatory organ damage and high mortality. We used the janus kinase (JAK) inhibitor baricitinib with IFN-blocking activity in vitro, to ameliorate disease.

METHODS. Between October 2011 and February 2017, 10 patients with CANDLE (chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperatures), 4 with SAVI (Stimulator of IFN genes (STING)-associated vasculopathy with onset in infancy), and 4 patients with other interferonopathies were enrolled in an Expanded Access Program. Patients underwent dose-escalation, benefit was assessed by reductions in daily disease symptoms and corticosteroid requirement. Quality-of-life, organ inflammation, changes in IFN-induced biomarkers, and safety were longitudinally assessed.

RESULTS. 18 patients were treated for a mean duration of 3.0 years (1.5–4.9 years). The median daily symptom score decreased from 1.3 (IQR 0.93–1.78) to 0.25 (IQR 0.1–0.63) ($P < 0.0001$). In 14 patients receiving steroids at baseline, daily prednisone doses decreased from 0.44 mg/kg/day (IQR 0.31–1.09) to 0.11 mg/kg/day (IQR 0.02–0.24) ($P < 0.01$); 5 of 10 CANDLE patients achieved lasting clinical remission. Quality of life, height and bone mineral density Z-scores significantly improved, and IFN biomarkers decreased. Three [...]

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Abstract

Background. Monogenic Interferon (IFN)-mediated autoinflammatory diseases present in infancy with systemic inflammation, an IFN-response-gene-signature (IRS), inflammatory organ damage and high mortality. We used the janus kinase (JAK) inhibitor baricitinib with IFN-blocking activity in vitro, to ameliorate disease.

Methods. Between October 2011 and February 2017, 10 patients with CANDLE (chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperatures), 4 with SAVI (Stimulator of IFN genes (STING)-associated vasculopathy with onset in infancy), and 4 patients with other interferonopathies were enrolled in an Expanded Access Program. Patients underwent dose-escalation, benefit was assessed by reductions in daily disease symptoms and corticosteroid requirement. Quality-of-life, organ inflammation, changes in IFN-induced biomarkers, and safety were longitudinally assessed.

Results. 18 patients were treated for a mean duration of 3.0 years (1.5-4.9 years). The median daily symptom score decreased from 1.3 (IQR 0.93-1.78) to 0.25 (IQR 0.1-0.63) ($p < 0.0001$). In 14 patients receiving steroids at baseline, daily prednisone doses decreased from 0.44 mg/kg/day (IQR 0.31-1.09) to 0.11 mg/kg/day (IQR 0.02-0.24) ($p < 0.01$); 5 of 10 CANDLE patients achieved lasting clinical remission. Quality of life, height and bone mineral density Z-scores significantly improved, and IFN biomarkers decreased. Three patients discontinued, two with genetically undefined conditions due to lack of efficacy, and one CANDLE patient due to BK viremia and azotemia. The most common adverse events were upper respiratory infections, gastroenteritis, BK viruria and viremia.

Conclusion. On baricitinib treatment, clinical manifestations, inflammatory and IFN biomarkers improved in patients with the monogenic interferonopathies, CANDLE, SAVI and 2 other interferonopathies. Monitoring safety and efficacy is important in benefit-risk assessment.

Trial registration: ClinicalTrials.gov NCT01724580 and NCT02974595.

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Introduction

The IFN-mediated autoinflammatory diseases, CANDLE and SAVI, are Mendelian innate immune-dysregulatory disorders that present early in life with fevers, sterile organ inflammation and a high type-I IFN-response gene signature (IRS) in peripheral blood cells (1, 2), and are part of the spectrum of conditions termed interferonopathies (3). CANDLE is caused by loss-of-function mutations in genes encoding proteasome complexes that regulate protein degradation (4-8). Patients with CANDLE present with fever, neutrophilic panniculitis, lipodystrophy, cytopenias, myositis, and lymphocytic aseptic meningitis. Forty to 80% of patients develop systemic hypertension, metabolic syndrome and hepatic steatosis often in the first decade of life (2). SAVI is caused by gain-of function mutations in the viral sensor, stimulator of interferon (IFN) genes (STING), resulting in constitutive transcription of the potent antiviral cytokine IFN β (9-11). Patients present with cold-induced acral vasculitis resulting in loss of digits, and interstitial lung disease, the latter may be the presenting symptom (2, 9).

Both syndromes respond poorly to biologic disease-modifying antirheumatic drugs (DMARDs) that target pro-inflammatory cytokines, (i.e. IL-1, TNF and IL-6) (8, 9) or to conventional DMARDs. A high IRS is absent in patients with clinically active autoinflammatory diseases who respond to treatment with IL-1 blocking agents (9, 12); together these findings support a potential role for type-I IFN in propagating systemic and organ inflammation and damage, and high mortality (2).

Until recently, treatments that block IFN signaling have not been available. However, the JAK/ Signal Transducers and Activators of Transcription (STAT) pathway constitutes the principal signaling pathway for cytokine and growth factor receptors including the IFN α/β receptor (IFNAR) and the IFN γ receptor (IFNGR) (13, 14). Small molecules that inhibit JAKs, reduce Type-I and Type-II IFN-induced STAT-1 phosphorylation (pSTAT1) in CANDLE and SAVI patients *in vitro* (7, 9), which suggested their potential utility in reducing the IFN signaling and disease manifestations in CANDLE and SAVI patients. In October 2011 we developed an Expanded Access

Program with baricitinib, a selective JAK1 and JAK2 inhibitor (15), to treat patients with CANDLE, SAVI and other presumed interferonopathies. Baricitinib is currently approved for the treatment of moderately to severely active RA in adults (16) in Europe, Japan, and other countries. Data were collected to ensure patient safety and to assess benefit that justified continuation of baricitinib administration.

Results

Clinical manifestations of CANDLE, SAVI and other Interferonopathies

Between October 2011 and October 2016, we treated 18 patients, 10 with genetically confirmed CANDLE, 4 with genetically confirmed SAVI, and 4 patients with other interferonopathies. One patient was later found to have Aicardi Goutières syndrome 5 (AGS5), and one has a novel disease-causing mutation. The baseline demographics and clinical presentations are summarized in Table 1. All patients with CANDLE and SAVI developed disease symptoms in the first 2.5 weeks of life. The mean age at enrollment was 12.5 years (range 1.2-24.1), 72% of patients were below the 3rd percentile for height, and 50% were below the 3rd percentile for weight (Table 1). Fourteen of 18 (78%) patients were on chronic corticosteroid treatment for an average of 5.7 years (1-17yrs) prior to entry into the program, 3 SAVI and one CANDLE patient had failed and discontinued corticosteroids prior to enrollment. All patients had failed between 1 to 6 conventional and/or biologic DMARDs. Most patients had frequent and prolonged hospitalizations prior to enrollment.

Clinical symptoms improve on treatment with baricitinib

All patients underwent dose-escalation until they reached “optimal tolerated treatment doses” (Figure 1A). The median duration in the program at time of analysis was 1023 days or 2.8 years (IQR 842 to 1419.5); patients had been on optimized doses for a median of 897 days or 2.5 years (IQR 639 to 1160 days). (Supplemental Table 1). At the last NIH visit 12/18 (67%) patients fulfilled diary score improvement criteria (80% of CANDLE, 75% of SAVI and 1 of 4 (25%) patients with other interferonopathies). Of the 14 patients on corticosteroids at baseline, 10/14 (71%) fulfilled corticosteroid improvement criteria (Table 2 and Supplemental Table 3). The median diary score decreased from 1.3 (IQR 0.93 to 1.78) at baseline to 0.25 (IQR 0.10 to 0.63), $p < 0.0001$. The median corticosteroid dose dropped from a prednisone equivalent dose of 0.44 (IQR 0.31 to 1.09) mg/kg/day at baseline to 0.11 (IQR 0.02 to 0.24) mg/kg/day $p < 0.005$ (Table

3). All available data were used in a repeated-measures model to assess responses over time. Least-squares means for diary scores and corticosteroid dose decreased from baseline (phase 1), during the baricitinib dose escalation (phase 2) and further when optimal treatment doses were reached (phase 3) and remained stable during the last 90 days of the observation period (phase 4). Corticosteroids were weaned during dose escalation and further on optimal tolerated baricitinib doses ($p < 0.001$ for both respectively, Figure 1B). Patient pain, overall wellbeing and quality of life improved on treatment (Figure 2); 5 (50%) of patients with CANDLE achieved remission with no disease symptoms ($DDS < 0.15$) and normal CRP, despite discontinuation of corticosteroids (Table 2). The CRP was below 5 mg/L in 84.6% of subsequent visits and the IFN scores were normal in 66.7% of visits at last follow-up which encompassed a mean of 654.4 (range 581-822) days after the patients first achieved remission criteria until data analysis, suggesting durable remission (Supplemental Table 4). The clinical responses were most pronounced in patients with CANDLE, in SAVI patients, the vasculitis flares improved but still occurred albeit reduced in duration and severity; no SAVI patient experienced further loss of digits (Figure 3 A-H and Supplemental Figures 2 A,B). The patient with SAVI on corticosteroid treatment at baseline had an initial reduction but increased corticosteroid doses prior to the final visit due to subjective symptoms of respiratory difficulties. In the context of stable PFTs and chest CT, her corticosteroid dose was subsequently weaned to 0.11 mg/kg/day.

Three patients discontinued treatment. Two patients without a genetic diagnosis discontinued after 244 and 98 days of treatment, one due to lack of efficacy and one due to osteonecrosis and an unsatisfactory treatment response. One CANDLE patient, in whom corticosteroids could not be weaned, developed BK viremia and azotemia and was discontinued from treatment (Supplemental Figure 1). The 2 patients with other interferonopathies, who both stayed on treatment, (one patient with AGS 5 and one with a novel disease-causing mutation), had symptom improvements and weaned corticosteroids to < 0.15 mg/kg/day (Supplemental Figure 2C-H).

Prior to baricitinib treatment, growth and physical maturation were delayed with the mean bone age lower by 3.49 ± 3.99 years relative to chronological age (Supplemental Figure 3). On baricitinib, 13 patients with growth potential improved their mean height Z-scores from -4.03 ± 2.64 to -3.19 ± 2.33 ; with “catch up growth” in 9 patients who were able to wean corticosteroids to doses below 0.16 mg/kg/day (Figures 4A, B, Supplemental Table 5 and Supplemental Figure 4A-E). Bone mineral density

increased with a mean Z-scores change from -3.25 ± 1.97 to -2.20 ± 1.36 ($p < 0.005$) (Figure 4A and Supplemental Table 6).

At baseline 6 of 10 patients with CANDLE had metabolic syndrome; 10 patients (7 CANDLE and 3 other patients) had hyperlipidemia; 7 pediatric patients on corticosteroids (C3, C5, C6, C7, S1, O1 and O4) met the Centers for Disease Control and Prevention (CDC) body mass indices (BMIs) criteria for obesity, and 2 patients had hepatic steatosis (one patient with CANDLE (C5) and one with SAVI (S2)). On baricitinib, BMIs improved towards more normal values; in 4 out of 5 underweight patients (C9, C10, S4 and O3), BMI improved in 2 patients (S4 and O3) and normalized in 2 other patients (C9 and C10). In 5 out of 7 obese patients, the BMI dropped to the overweight category (C3, C5, C6, S1, and O4), one patient who was overweight at baseline became obese (S2) (Supplemental Table 7). The median lipid levels (HDL, LDL and triglycerides) increased on baricitinib treatment (Supplemental Table 8). Three patients with CANDLE (C3, C4 and C8) who had hyperlipidemia at baseline developed hepatic steatosis (Supplemental Figure 5A-C) with no improvement in the 2 patients with hepatic steatosis at baseline. In patients with CANDLE, myositis and aldolase improved on baricitinib treatment ($p = 0.06$) (Supplemental Table 9). In the 3 SAVI patients with baseline lung disease, signs of chronic interstitial lung disease, forced vital capacity (FVC), carbon monoxide diffusing capacity (DLCO) and walk distance improved on baricitinib (Supplemental Table 10).

Hematologic and immunologic markers improve on treatment with baricitinib

At baseline, 12 of 18 (67%) patients were anemic, 7 (39%) had lymphopenia, and 4 (22%) had thrombocytopenia. The hemoglobin concentration, absolute lymphocyte count (ALC) and platelet count increased during treatment in patients with cytopenias at baseline ($p \leq 0.05$ for hemoglobin and ALC). In patients with normal cell counts at baseline, hemoglobin and ALC trended non-significantly down (Figure 4C).

At baseline, 60% of patients with CANDLE patients and all patients with SAVI had detectable autoantibodies to endothelial antigens/targets including phospholipids (lupus anticoagulant and anti-cardiolipin antibodies (Ab)), anti-myeloperoxidase and proteinase-3 Ab, and/or to nuclear antigens (ANA, SSA) and DNA, (dsDNA). Autoantibody positivity significantly decreased during treatment ($p = 0.013$) (Supplemental Table 11), while cell subsets and immunoglobulin levels remained stable (Supplemental Figures 6 and 7).

Baricitinib suppresses inflammatory markers including the IFN signature and the serum IFN cytokine IP-10

Among the acute phase reactants (ESR and CRP), the CRP continuously decreased on treatment, the reduction was largest in patients with CANDLE (Table 3, Figures 5A and Supplemental Figure 8C). The ESR did not significantly decrease and remained elevated in most patients (Supplemental Figures 8A,B).

Biomarkers of interferon signaling, serum levels of the chemokine IP-10 and a 25-gene IFN response gene score significantly decreased during treatment with baricitinib (Table 3 and Figure 5B-D. The IFN score normalized in the 5 patients with CANDLE who achieved remission (Figure 5B). The 25-gene IFN score and serum IP-10 levels significantly correlated with each other (Supplemental Figure 9A). Both correlated significantly with daily symptoms ($r=0.26$ and $r=0.37$, $p<0.0001$) and with lower doses of corticosteroids, indicating the ability to taper corticosteroids ($r=0.24$ and $r=0.44$, $p<0.005$ and $p<0.0001$ respectively) (Supplemental Table 12 and Supplemental Figures 9B and C). The IFN biomarkers, IP-10 and 25-gene IFN score correlated better with the ability to wean steroid doses than the acute phase reactants (ESR and CRP). Prior to treatment, diurnal variability of IFN scores obtained in one day was high. The fluctuation correlated with higher morning scores and the daily variability was greatly reduced during baricitinib treatment when overall IFN scores decreased (Supplemental Figure 10A).

We measured IFN- α stimulated STAT-1 phosphorylation to assess type-I IFN receptor responsiveness during baricitinib treatment; the IFN- α stimulation induced STAT-1 phosphorylation was reduced to the lower tertile measured in healthy controls (Supplemental Figure 10B). While patients with CANDLE were hyper-responsive to IFN- α stimulation before treatment with baricitinib (7), most patients with SAVI were maximally STAT-1 phosphorylated and did not respond to IFN- α stimulation (9). On baricitinib, the IFN response of patients with SAVI recovered to levels that were observed in CANDLE patients. Other cytokines that significantly decreased in baricitinib treated patients included MCP-1, GM-CSF, IL-15 and IL-5 (Supplemental Figure 11).

Safety summary

Overall, baricitinib was well tolerated. At the time of safety analysis (June 2017), the mean baricitinib exposure was 3.5 years (range 2.3 to 5.6 years for ongoing patients), representing 63 patient years of exposure. No deaths have been reported during the

program. Two patients (11%) with inadequate responses discontinued treatment due to adverse events. One patient with an undifferentiated interferonopathy (O3) had evidence of osteonecrosis (right femur) 3 days after starting baricitinib and was discontinued due to progression after 14 weeks of baricitinib treatment. He died 18 months after discontinuing baricitinib due to worsening of pre-existing nodular regenerative hyperplasia and portal hypertension complicated by recurrent esophageal variceal hemorrhages, IgA nephropathy and renal insufficiency. One patient with CANDLE (C7) developed azotemia in the context of BK viruria and viremia, and discontinued treatment due to acute kidney injury after 117 weeks. This patient died 4 months later due to exacerbation of CANDLE syndrome, in the context of a respiratory tract infection and interstitial lung disease. Fifteen patients (83%) had at least 1 serious adverse event (SAE) (Supplemental Table 13). In most instances, the SAEs resolved without interrupting baricitinib treatment. Treatment-emergent infections were observed in 16 patients (89%) (Table 4). Upper respiratory tract infections were most frequent. Two patients developed herpes zoster with unilateral lesions restricted to 2-3 contiguous dermatomes. Transient cytopenias developed in the context of infections and intermittent disease exacerbations (Supplemental Table 14-15). An unexpected finding was the development of polyomavirus (BK) viremia in patient C7. While 2 patients had low titer intermittent BK viremia before baricitinib treatment, 8 additional patients developed intermittent BK viremia during baricitinib treatment. In contrast to the first patient who had high titer BK viremia in the context of worsening renal disease, the copy number in the other patients is low and variable, with stable low copy number viremia and stable renal function over time (Supplemental Table 16).

Discussion

We found that treatment with baricitinib improved the signs and symptoms and allowed significant reduction of corticosteroid treatment in patients with CANDLE and SAVI and in two patients with other interferonopathies in a compassionate use program. Of the 10 patients with CANDLE, 5 (50%) patients could permanently discontinue corticosteroid therapy, without return of disease symptoms; their inflammatory markers normalized and they achieved durable inflammatory remission on baricitinib. In patients with SAVI, baricitinib treatment improved flares of vasculitis, prevented the progression of spontaneous amputations, and the development of gangrene. Baricitinib also stabilized interstitial lung disease by preserving pulmonary function indices including DLCO and improved walk distance. Despite these clinical improvements, inflammatory

markers did not normalize in any of the patients with SAVI, and although IFN scores decreased, the absolute levels remained elevated. These findings are consistent with 2 previous reports of a total of 7 patients with SAVI who were treated between 3 and 15 months with the JAK inhibitor ruxolitinib (17, 18).

Among the four patients with other (initially uncharacterized) interferonopathies, two patients, in whom a genetic diagnosis could not be established, did not respond and discontinued treatment. The 2 responders had both severe panniculitis and lipoatrophy. One of the patients had peripheral vasculitis, and at the age of 7 years developed Moya-Moya-like cerebral vasculopathy resulting in vascular occlusion and a stroke. She was later found to be homozygous for a *SAMHD1* deletion which allowed a retrospective diagnosis of later-onset AGS5 (19). The second patient had nodular panniculitis, lipoatrophy and marked systemic inflammation. He was later found to have a novel frameshift mutation in *SAMD9L*, suggesting a possible novel interferonopathy. The 2 responders had higher 25-gene IFN scores at baseline compared to the two non-responders, thus suggesting that a combination of clinical phenotype and grossly elevated IFN scores might be useful in predicting responses to IFN blocking treatments such as JAK1/2 inhibition.

Most patients with CANDLE and SAVI and some other interferonopathies have significant growth and bone maturation delays, and low bone mineral density. The significant improvement in height and bone mineral density Z-scores demonstrates that the baricitinib dosing regimen we developed to optimize disease control, allows for catch up growth and bone production, most prominent in patients who were able to wean corticosteroid doses to less than 0.16mg/kg/day. These observations ease concerns that JAK inhibitors could reduce growth hormone (GH) function through inhibition of GH-receptor induced tyrosine kinase Janus kinase 2 (JAK2) phosphorylation (20, 21). Despite improvement in inflammatory markers, hyperlipidemia and hepatic steatosis did not improve in CANDLE patients. In fact, hepatic steatosis developed in 3 CANDLE patients on baricitinib treatment in the context of moderately decreasing their BMIs from baseline, pointing to a role of proteasome dysfunction in the development of hepatic steatosis that is independent of IFN-mediated inflammation (22).

The abnormal IFN responses *in vivo* were modulated by reducing IFN- α -induced STAT-1 phosphorylation, and downstream IFN targets such as serum IP-10 levels and a 25-gene IFN score after treatment with baricitinib. The reduction in IFN biomarkers correlated with the improvement of clinical signs and symptoms, and with the ability to

wean corticosteroids. Peripheral blood mononuclear cells (PBMCs) of most untreated patients with SAVI show high constitutive STAT-1 phosphorylation and are unresponsive to further IFN- α -stimulation (9). However, decreased constitutive activations and restored type-I IFN receptor responsiveness were observed with baricitinib treatment. Reductions in serum levels of granulocyte-macrophage colony-stimulating factor (GM-CSF) and its downstream mediator, the chemokine, MCP-1 (CCL2) were also observed with baricitinib treatment (23). GM-CSF promotes myeloid differentiation towards inflammatory, M1-like macrophages, and CCL2 promotes monocyte/macrophage recruitment to sites of tissue injury or infection (24-26). GM-CSF, particularly in the context of IFNs, “primes” monocytes to amplified stimulation-induced cytokine and chemokine overproduction that includes the production of IFN biomarkers (27) through epigenetic remodeling (28). The reduction in diurnal variability of the 25-gene interferon score and the modulation of IFN- α -induced STAT-1 phosphorylation in baricitinib-treated patients may be consistent with a reset of the IFN receptor sensitivity through epigenetic remodeling that is mediated by JAK inhibition; a mechanism that was recently suggested in a murine model treated with IFN- α and a JAK inhibitor (29). The impact of JAK inhibition on epigenetic remodeling as a molecular mechanism that may attenuate the “IFN- amplification loop” (1) needs further evaluation.

Overall, the mean drug exposure levels to optimize disease control in the context of acceptable safety profile in our patients were 1.83-fold higher than in patients with rheumatoid arthritis taking 4mg/day of baricitinib (30); the severe disease manifestations and the need to target the type-I IFN signaling are likely reasons for the higher exposures in our patients.

Treatment with baricitinib was overall well tolerated. Consistent with studies in adult rheumatoid arthritis patients, upper respiratory tract infections were the most frequently reported treatment-emergent adverse events, two patients developed herpes zoster (30, 31). However, the observation of viral reactivation with BK virus (BK viremia and viruria) was unique in this patient population. Viral titres in urine and blood remained stable over a 2-year period on treatment. While the clinical significance of measurable BK titers remains uncertain (32-34), viral titres in the blood should be monitored in this vulnerable population.

Although the number of patients enrolled was small, the long duration of treatment (2.3 to 5.6 years and continuing), the durable responses to treatment, and the reduction in inflammatory markers confirm a long-lasting effect of baricitinib treatment.

In summary, our data show that clinical signs and symptoms of patients with CANDLE and SAVI improved with baricitinib treatment. The decrease in systemic and organ specific inflammation in the context of interferon (IFN) biomarker reduction support a causative role for chronic IFN signaling on disease pathogenesis in these patients with type-I IFN-mediated diseases” (1, 35).

Patients and Methods

Patients. Patients with genetically confirmed CANDLE or SAVI, or a suspected undifferentiated interferonopathy who were referred to our center for evaluation and treatment recommendations and who were ≥ 17.5 months of age, weighed ≥ 8.5 kg, and had active clinical disease (CANDLE diary score ≥ 0.5 , or SAVI diary score ≥ 1.0) were eligible. Patients had to receive or previously fail treatment with oral corticosteroids (≥ 0.15 mg/kg/day of prednisone or its equivalent).

Program Design and Treatment. The open-label “Expanded Access Program” (NCT01724580) provides access to baricitinib to eligible patients who are without other satisfactory treatment options. Data presented here, are from patients enrolled at the NIH. Data were collected in a company provided database and at a clinical database at the NIH.

Treatment with the JAK inhibitor baricitinib and dosing adjustments/escalation. At the start of the program, pediatric pharmacokinetic (PK) data were not available and baricitinib was started orally at 100 mcg once daily. As PK and clinical response data became available, the dose escalation scheme for baricitinib was modified (36). Inadequate response, defined as elevated average diary scores and active clinical disease (diary scores ≥ 0.5 (CANDLE diary) or ≥ 1 (SAVI diary) or ongoing clinical symptoms of disease activity), in the absence of signs of drug toxicity (i.e. drop in hemoglobin), allowed increases in daily doses of baricitinib. Dose escalations were initially allowed when pharmacokinetic monitoring (baricitinib peak and trough levels) were within levels observed in adult healthy controls, RA and psoriasis patients (Lilly internal data); further dose increases were approved in amendments and were monitored by PK data. We determined the visit when enrolled patients reached “optimal tolerated dosing” for baricitinib (Supplemental Table 1). Population pharmacokinetic (PopPK) analyses were performed at “Optimized tolerated baricitinib doses” (36). The mean exposures measured as $AUC_{24,SS}$ were 1.83-fold higher than those obtained in adult RA patients receiving oral baricitinib doses at 4 mg once-daily in phase 3 studies. A dosing table has been published (36).

Clinical Benefit Assessment. We collected limited efficacy data on the expanded access protocol (diary scores, corticosteroid doses, medications, safety data) to aid dose

titration and ensure evidence of benefit justifying risk. Additional efficacy data were collected on the Natural History protocol or as part of routine patient care (see Supplemental Methods).

Disease-specific daily symptom score (DDS)

“Primary benefit” was defined as a decrease in the disease-specific daily symptom score (DDS) to <0.5 for patients with CANDLE and other interferonopathies, and to <1.0 for patients with SAVI. The cutoffs corresponded with clinically meaningful responses to treatment. Each patient or caregiver was instructed to complete the diary at approximately the same time every day and to rate the impact of each symptom on the patient. The calculated average score for each symptom was summed up and divided by the number of assessed symptoms (Supplemental Methods). Diary scores were used in the assessment of disease activity, the need for baricitinib dose increases and the initiation of steroid weaning. Diary scores were collected for 2 weeks prior to baricitinib administration and every day throughout the program. All patients completed diaries more than 92% of days except for patient O3 who completed 76.7% of days (Supplemental Table 2).

Reduction in daily corticosteroids:

“Secondary benefit” was assessed for patients on treatment with corticosteroids at enrollment. “Successful reduction” was defined as a *reduction in corticosteroids* to <0.15mg/kg/day of prednisone equivalent or a decrease of at least 50% of the patient’s daily dose at baseline.

Other clinical outcome measures:

We assessed “Clinical remission”, change in disability, quality of life, and patient and physician global assessments. Z-scores for height, weight, body mass index (BMI), bone age, and bone mineral density were calculated at baseline and respective follow-up visits (Supplemental Methods). CANDLE-specific outcomes included assessment of hyperlipidemia and hepatic steatosis (37). SAVI-specific outcomes included assessment of interstitial lung disease by pulmonary function tests (PFTs), 6-minute walk test (6MWT) and yearly chest computed tomography (CT) (Supplemental Methods).

Immunological Evaluation. The immunological evaluations included inflammatory markers, hsCRP and erythrocyte sedimentation rate (ESR), number of detected

autoantibodies, lymphocyte subset panel, immunoglobulin levels and hematologic values. IFN signaling was assessed by a STAT-1 phosphorylation assay, quantification of a 25-IFN-gene score in whole blood (12), and measurement of serum IP-10 and other cytokine levels. (Supplemental Methods).

Safety Assessment. The development of co-morbidities and hospitalizations were documented. The National Institutes of Health Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 was used to categorized abnormal results after enrollment. Vital signs including weight and height, clinical laboratory tests, complete blood cell count with differential, renal and liver function, lipid profile, urinalysis, and other safety assessments were assessed at all protocol visits. BK titers were followed since the discovery of the first case, in June 2015 (Supplemental Methods).

Statistics. *Post-hoc analyses to assess relationships between treatment and clinical outcomes, conventional biomarkers (ESR and C-reactive protein) and IFN-biomarkers.* As this was an expanded access protocol, and the medical conditions treated in this program are rare, we anticipated that not many patients may enroll, therefore, no formal statistical analyses were planned. Instead, data listings were anticipated to summarize the results. Null and alternative hypotheses were not defined prospectively and analyses were not adjusted for multiple comparisons. We assessed the mean change in clinical measures obtained under the compassionate use program, including diary scores, prednisone doses and data collected under the natural history study by comparing baseline (before treatment) and the last NIH visit. We used a two-sided Student t-test for parametric and the Wilcoxon signed-rank test for nonparametric data at a significance level of <0.05. Cutoff date for compassionate use data was October 31, 2016; for natural history outcomes, February 22, 2017 and for safety data, June 5, 2017.

Assessment of dose-response relationships of clinical and biomarker measures. As the dose of baricitinib was titrated to clinical response, we determined the visit when patients were first treated with “optimal tolerated baricitinib doses” (Supplemental Table 1). For each patient, the timeline was divided into 4 study phases and the primary outcome was averaged over each period, resulting in 4 averages per period per patient. *Phase 1* or baseline included the 29 days prior to baricitinib start date and the 1st day of treatment; *Phase 2* or Post-treatment “pre-optimal baricitinib doses” included the dose escalation

period and started with the 2nd day of treatment up to the 1st day of achieving optimal tolerated doses; *Phase 3* or “post-optimal tolerated dose” period, started with the day the “optimal tolerated dose” was achieved up to 90 days before the end of study evaluation; and *Phase 4* included the last 90 days before the last included visit (all patients were on “optimal tolerated” doses) (Supplemental Table 1). To confirm trends in longitudinally collected data (diary scores, prednisone dose and inflammatory and bio-markers), the data were fitted to a repeated-measures model with “phase” as a categorical independent variable. Least-squares means with 95% confidence intervals for each phase were assessed. The Cochran–Mantel–Haenszel test was used to analyze the association between binary outcomes.

Assessment of associations between clinical outcomes, IFN-biomarkers and conventional biomarkers (ESR and C-reactive protein). Linear mixed model with random slope and intercept with an unstructured variance-covariance matrix were used to assess the association of clinical outcome measures (DDS and corticosteroid dose) on biomarkers of IFN signaling (serum IP-10 levels and the 25-gene IFN score. To obtain a correlation between repeated measures, a method based on a reconfiguration of the X- and Y-variables that use of the mixed model via the SAS procedure PROC MIXED applied is was used (38).

Study Approval

The program was approved by institutional review boards at the National Institutes of Health, NIAMS/NIDDK, and NIAID. All patients co-enrolled in the NIH Natural History Protocol of Autoinflammatory Diseases (NCT02974595) under which clinical and biomarker data were assessed. Patients or their parents provided written informed consent. Patients were evaluated at baseline, monthly for the first 12 months, and every 3 months thereafter. The program is currently ongoing.

Authors contributions

GAMS acquired data, oversaw the clinical and regulatory aspects of the study, analyzed data and participated in writing the manuscript. AR, SR, HW, PJH, YB, SS, SML, JAD, DB, DLS, LG, TK, DF, DCC, HK, SD, RAC, LF, BK, MOB, SMP, AB, AS, EWC, JGD, CH, SMH, JF, ASA, SO and PAB acquired and interpreted clinical data, AAJ, AB and MG conducted experiments, acquired and analyzed data, JCR, LRF, KRC, NW, AJL, RJB, KIR, TH, KMB, PK, SRB, MW, HKS and VN acquired and analyzed clinical and

biomarker subspecialty data, MS, AP and JMJ summarized and analyzed the safety data and participated in writing the manuscript, PGW conducted and oversaw the statistical analyses of the study data, WLM and RGM designed the compassionate use program and reviewed and analyzed the data and wrote the manuscript. GAMS and RGM wrote the first draft of the manuscript. All authors reviewed and approved the final version of the manuscript.

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TABLES

Table 1. Baseline Demographics and Clinical Characteristics (n=18)

Characteristic	Value	Characteristic	Value
Demographic		Clinical	
Age at enrollment— yr. mean (min-max)	12.5 (1.2-24.1)	Clinical manifestations — no. (%)	
Age group — no. (%)		Systemic inflammation ^g	18 (100)
0-2 yr	1 (5)	Anemia	12 (67)
3-6 yr	2 (11)	Basal Ganglia calcifications (n=16)	10 (62)
7-10 yr	4 (22)	Height < 3 rd percentile	13 (72)
11-18 yr	5 (28)	Weight < 3 rd percentile	9 (50)
≥18 yr	6 (33)	Bone age — mean (min-max) ^h	
Sex — no. (%)		Delayed bone age (n=13)— yr.	3.5 (0.4-12.3)
Male	12 (67)	CANDLE Specific (n=10) — no. (%)	
Race or ethnic group — no. (%)		Panniculitis induced lipodystrophy	10 (100)
White	16 (89)	Joint contractures	10 (100)
Black	2 (11)	Myositis ^d	8 (80)
Hispanic	4 (22)	Metabolic syndrome ⁱ	6 (60)
Clinical Characteristics (all patients)		Pulmonary Arterial Hypertension	1 (10)
By Genetic Diagnosis — no. (%)		SAVI Specific (n=4) — no. (%)	
CANDLE ^A	10 (55)	Interstitial Lung Disease	4 (100)
SAVI ^B	4 (22)	Cutaneous vasculitis	4 (100)
Other Interferonopathy ^C	4 (22)	Skin ulcers	3 (75)
Immunomodulators prior to baseline ^D — no. (%)	17 (94)	Amputation distal extremities	3 (75)
≥2 Immunomodulators prior to baseline	10 (55)	Other Interferonopathy (n=4) — no. (%)	
Mean use of Immunomodulators prior to baseline	2.7 (0-6)	Panniculitis	4 (100)
Biologics prior to baseline ^E — no. (%)	13 (72)	Lipodystrophy	3 (75)
≥2 biologics prior to baseline	10 (55)	Arterial Hypertension	3 (75)
Mean use of biologics prior to baseline	2.3 (0-6)	Livedo reticularis	2 (50)
Oral corticosteroids ^F — no. (%)	14 (78)	CNS disease/Stroke	1 (25)
Mean exposure to oral corticosteroids - yr	5.7 (1-17)	IgA nephropathy/Non-cirrhotic portal hypertension	1 (25)

^A *PSMB8* (5 homozygous, 1 compound heterozygous), *PSMB4* (1 compound heterozygous), *PSMB4/PSMB9* (2 digenic), and *PSMA3/PSMB8* (1 digenic)

^B All with de novo gain-of-function mutation N154S in *TMEM173*

^C *SAMHD1* (1 homozygous deletion), *SAMD9L* (1 patients), unknown (2 patients, one with a heterozygous *PSMB8* mutation)

^D Azathioprine, Colchicine, Cyclosporine, Cyclophosphamide, Dapsone, Hydroxychloroquine, IVIG, Leflunomide, Methotrexate, Mycophenolate mofetil, Tacrolimus, Thalidomide

^E Adalimumab, Abatacept, Anakinra, Canakinumab, Etanercept, Infliximab, Rituximab, Tocilizumab

^F Prednisone or equivalent

^G CRP, High Sensitivity >3.0mg/L, Erythrocyte Sedimentation Rate (ESR) > 25 mm/hr

^H Bone Age by Greulich and Pyle for 13 patients with open growth plates

^I Documented by MRI bilateral thighs

^J By Ford criteria, Ford et al. Diabetes care 2005; 28, 878-81, all patients with metabolic syndrome had arterial hypertension

Table 2. Primary Benefit assessment

Patient ID	Primary benefit^A (DDS) response	Secondary benefit^B (corticosteroids) response	Remission^C
CANDLE (n=10)	8/10 (80%)	8/9 (89%)	5/10 (50%)
SAVI (n=4)	3/4 (75%)	0/1 (0%)	0/4 (0%)
Other^D (n=4)	1/4 (25%)	2/4 (50%)	0/4 (0%)
All (n=18)	12/18 (67%)	10/14 (71%)	5/18 (28%)

^A Diary score reduction criteria are a mean daily diary score of <0.5 for CANDLE and other IFNopathy, or <1 for SAVI.

^B Prednisone reduction criteria is at least 50% decrease from baseline or < 0.15mg/kg/day.

^C Remission Criteria: mean diary score < 0.15, no prednisone, and a CRP < 5 mg/L.

^D 2 patients (pts.) (O1 and O3) discontinued after 244 and 98 days on the program.

TABLE 3. Change of Measure of Disease Activity and Improvement from Baseline^A

Measure	Open-Label Treatment		P value
	Pre-baricitinib ^B	Post-baricitinib	
Primary measures of clinical response (n=18)			
Global diary score ^C			<0.0001
Median	1.30	0.25	
Interquartile range	0.93 - 1.78	0.10 - 0.63	
Dose of prednisone or prednisone equivalent dose (mg/kg/day) ^D			<0.005
Median	0.44	0.11	
Interquartile range	0.31 - 1.09	0.02 - 0.24	
Other measures of clinical response (n=16)			
CHAQ score ^E			<0.05
Median	1.69	1.13	
Interquartile range	0.53 - 2.09	0.03 - 1.75	
Visual-analogue scale for pain (mm) ^F			<0.01
Median	38.50	4	
Interquartile range	11.75 - 75.75	0.25 - 27.50	
Parent's / Patient's global assessment (mm) ^F			ns
Median	48	26	
Interquartile range	18 - 55	1 - 36	
Physician's global assessment (mm) ^F			<0.001
Median	90	2.50	
Interquartile range	55.50 - 96.25	0.75 - 5.50	
Height (cm) ^G	110.0 ± 26.16	126.52 ± 26.22	<0.001
Weight (kg)	33.78 ± 20.15	43.24 ± 21.59	<0.001
Measures of laboratory responses (n=18)			
C-reactive protein (mg/L)			ns
Median	15.9	2.9	
Interquartile range	4.25 - 51.8	1.2 - 16.4	
Erythrocyte sedimentation rate (mm/hr)			ns
Median	53	37	
Interquartile range	16.25 - 71.75	10.5 - 74	
IFN biomarker responses (n=18)			
25-gene IFN score			<0.01
Median	417.5	113.3	
Interquartile range	216.8 - 735.1	18.5 - 288.8	
Serum IP-10 levels			<0.005
Median	9196.7	1857.6	
Interquartile range	2814.7 - 13299.4	868.7 - 4587.2	

^A cut off for data analysis is October 31st 2016

^B All except 3 subjects (C1, C9 and S2) completed the CHAQ, VAS for pain and parent's/patient's global assessment at baseline or within 10 months prior to the first dose of baricitinib. Subject C1 completed the questionnaires 32 months prior to enrollment. Subjects C9 and S2, 9 months and 2 months after the first dose of baricitinib respectively.

^C Median daily scores of five symptoms for CANDLE and CANDLE-like patients (fever, rash, musculoskeletal pain, headache and fatigue) were evaluated daily with the use of a scale that ranged from 0 (no symptoms) to 4 (severe symptoms). The maximal daily score measured was 4; the minimal score was 0. Median daily scores of six symptoms for SAVI patients (fever, rash, musculoskeletal pain, fatigue, respiratory symptoms, ulcers/ischemic lesions) were evaluated daily with the use of a scale that ranged from 0 (no symptoms) to 4 (severe symptoms). The maximal daily score measured was 4; the minimal score was 0.

^D Values are for 14 patients who were receiving corticosteroids at study entry.

^E Childhood Health Assessment Questionnaire (CHAQ), a standardized test for the assessment of disability, range from 0 to 3, with higher scores indicating more severe impairment. Data for subjects discontinued from the study not included

(O1 and O3).

^F A visual-analogue scale (VAS) was used in which a value of 100 mm indicates the worst possible measure for the condition assessed by the test. Data for subjects discontinued from the study not included (O1 and O3).

^G Values are for 13 patients with open growth plates only.

Table 4. Treatment-emergent Adverse Events (infections)^A

Adverse Event	CANDLE Syndrome, n=10	CANDLE-related Conditions, n=4	SAVI, n=4	Total, N=18
	n (%)	n (%)	n (%)	n (%)
Patients with infections	10 (100.0)	2 (50.0)	4 (100.0)	16 (88.9)
Respiratory Tract Infections	10 (100.0)	2 (50.0)	3 (75.0)	15 (83.3)
Upper respiratory tract infection - no identified organism	10 (100.0)	2 (50.0)	2 (50.0)	14 (77.8)
Pneumonia ^B	4 (40.0)	0	2 (50.0)	6 (33.3)
Upper respiratory tract infection - with identified organism ^C	4 (40.0)	1 (25.0)	0	5 (27.8)
Sinusitis	3 (30.0)	1 (25.0)	1 (25.0)	5 (27.8)
Otitis media	4 (40.0)	1 (25.0)	0	5 (27.8)
Pharyngitis	2 (20.0)	1 (25.0)	0	3 (16.7)
BK Viremia	6 (60.0)	1 (25.0)	2 (50.0)	9 (50.0)
Gastrointestinal Infections^D	6 (60.0)	2 (50.0)	1 (25.0)	9 (50.0)
Urinary Tract and Genital Infections^E	4 (40.0)	1 (25.0)	1 (25.0)	6 (33.3)
Skin, Nail, and Oral Infections	6 (60.0)	2 (50.0)	4 (100.0)	12 (66.7)
Bacterial Infections ^F	3 (30.0)	2 (50.0)	3 (75.0)	8 (44.4)
Other Infections ^G	2 (20.0)	0	3 (75.0)	5 (27.8)
Fungal Infections ^H	3 (30.0)	1 (25.0)	0	4 (22.2)
Viral Infections ^I	2 (20.0)	0	1 (25.0)	3 (16.7)
Herpes zoster	2 (20.0)	0	0	2 (11.1)
Eye Infections^J	3 (30.0)	0	0	3 (16.7)
Device Related Infections^K	3 (30.0)	0	0	3 (16.7)
Bacteremia and Osteomyelitis^L	0	0	2 (50.0)	2 (11.1)

^A Treatment-emergent infections are defined as those events that were new or that worsened after starting baricitinib treatment. Patients with multiple occurrences of a specific event are counted once for the event. Patients with multiple events within a grouped term are counted once for the group term.

^B Pneumonia includes: pneumonia, bacterial pneumonia, pneumocystis jirovecii pneumonia, and hemophilus bacteremia in context of pneumocystis jirovecii pneumonia.

^C Upper respiratory tract infection – with identified organism includes: influenza, corona virus infection, parainfluenza virus infection, respiratory syncytial virus infection, and rhinovirus infection

^D Gastroenteritis includes: gastroenteritis, viral gastroenteritis, gastrointestinal viral infection, clostridium difficile infection, and rotavirus infection

^E Urinary Tract and Genital Infections include: urinary tract infection, urosepsis and pyelonephritis, epididymitis, and vulvovaginitis

^F Bacterial infections include: paronychia, staphylococcal infection, cellulitis, folliculitis

^G Other infections include: wound infection, tooth abscess, tooth infection, skin infection, pustular rash

^H Fungal infections include: oral candidiasis, skin candida, fungal skin infection, and tinea pedis

^I Viral infections include: molluscum contagiosum, herpes simplex, and viral rash

^J Eye infections includes: conjunctivitis, hordeolum and viral conjunctivitis.

^K Device related infections includes: device related and stoma site infection.

^L Bacteremia and osteomyelitis includes: localized infection and osteomyelitis

Abbreviations: n=number of patients

FIGURES:

Figure 1.

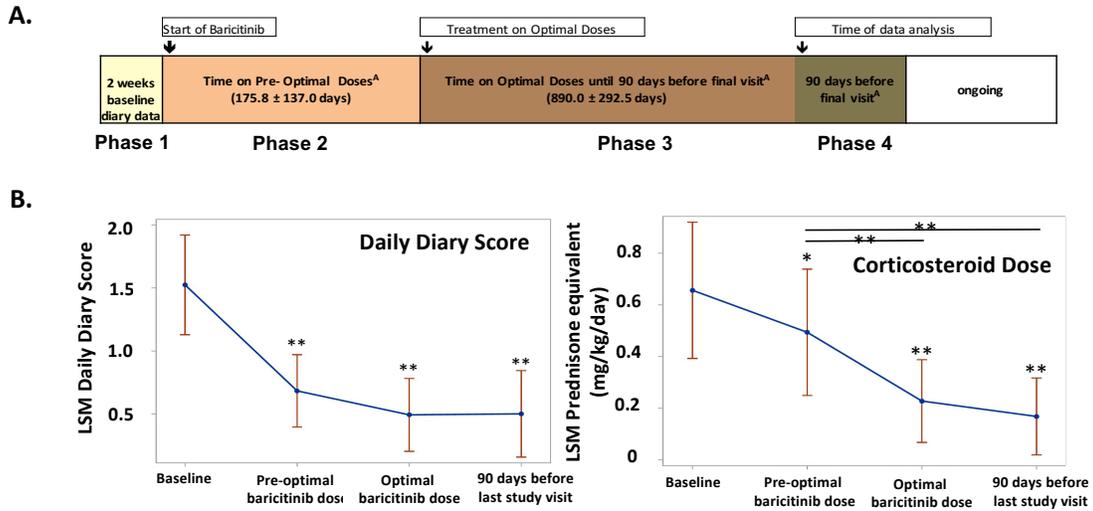


Figure 1. Expanded Access Program Overview and Effect of baricitinib treatment on clinical outcomes (A) Expanded Access Program overview: Phase 1 - time before first baricitinib dose. Phase 2 – period of dose escalation including the time between the first baricitinib dose and achievement of optimal dose regimen. Phase 3 - time on optimal baricitinib doses excluding the last 90 days prior to the final visit. Phase 4 - 90 days prior to final visit included for primary data analysis (daily diaries, steroid doses and biomarkers of IFN signaling). The program is ongoing. ^A Number of days in each phase are reported as Means ± SD. For Phase 2 and Phase 3, patients O1 and O3 were not included in the calculation. Both patients discontinued treatment due to lack of efficacy and or osteonecrosis after only 77 and 56 days on optimal doses respectively. **(B)** Effect of baricitinib treatment on clinical outcomes: To confirm trends in longitudinally collected data (diary scores, corticosteroid dose were fitted to a repeated-measures model with “phase” as a categorical independent variable. Least-squares means with 95% confidence intervals for each phase are assessed.

** Denotes unadjusted *p-values* < 0.001.

Figure 2.

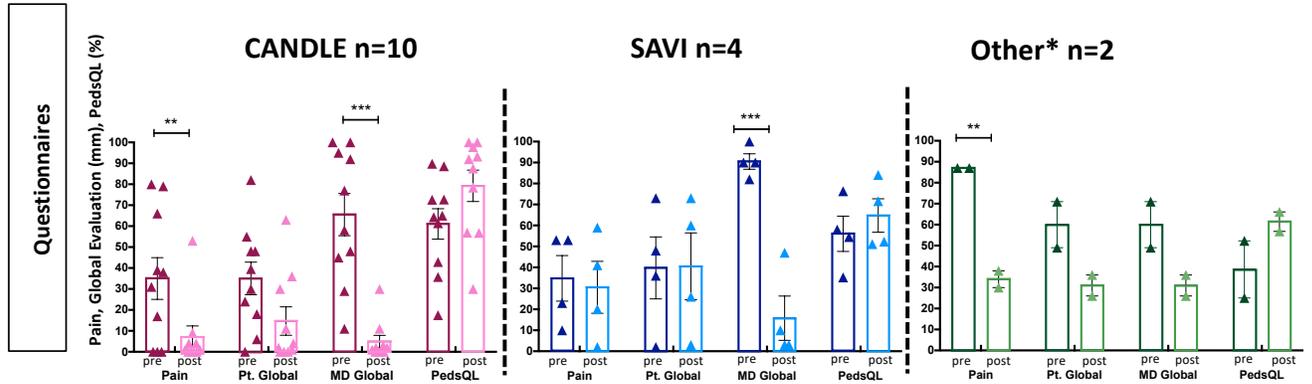


Figure 2. Self-reported assessments, and physician's global by disease subgroup. Parent or patient overall assessment of pain, health (Pt. Global), and physician assessment (MD Global) were assessed using a visual-analogue scale in which a value of 100 mm indicates the worst possible measure for the condition assessed by the test. Quality of life (PedsQL) was measured using a standardized age matched test that ranges from 0% to 100% with higher percentages indicating improvement. Data are presented by disease with CANDLE in red, SAVI in blue and other interferonopathies in green. Only the 2 patients who stayed on study are shown. Darker shades indicate pre-treatment and lighter shades last included visit on baricitinib treatment. ** denotes unadjusted *p*-value < 0.05, *** denotes unadjusted *p*-value < 0.001

Figure 3.



Figure 3. Improvement in clinical disease manifestations in patients with CANDLE and SAVI treated with baricitinib. (A-D) Two CANDLE patients who achieved remission criteria (C2 and C10 respectively) are shown. Pre-treatment images of face show typical distribution of facial panniculitis with periorbital swelling and erythema, and lipodystrophy affecting temporal regions, and areas above and below the zygomatic bone. Lip swelling is also evident. Post-treatment images show complete resolution of areas of panniculitis on face and neck. **(E, F)** Images of 2 of 4 SAVI patients are shown. Images of the lower leg of SAVI patient (S3) show extensive eschar formation overlying infested non-healing ulcers on the left lower leg; Post-treatment the ulcers healed with complete re-epithelialization. **(G, H)** Images of right palmar surface of hand from SAVI patient (S4) indicate chronic cutaneous vasculitis that resulted in partial amputation of the 2nd and 3rd fingers and complete loss of the 4th and 5th fingers. On baricitinib treatment significant improvement in cutaneous vasculitis resulted in preservation of fingers without further tissue loss.

Figure 4.

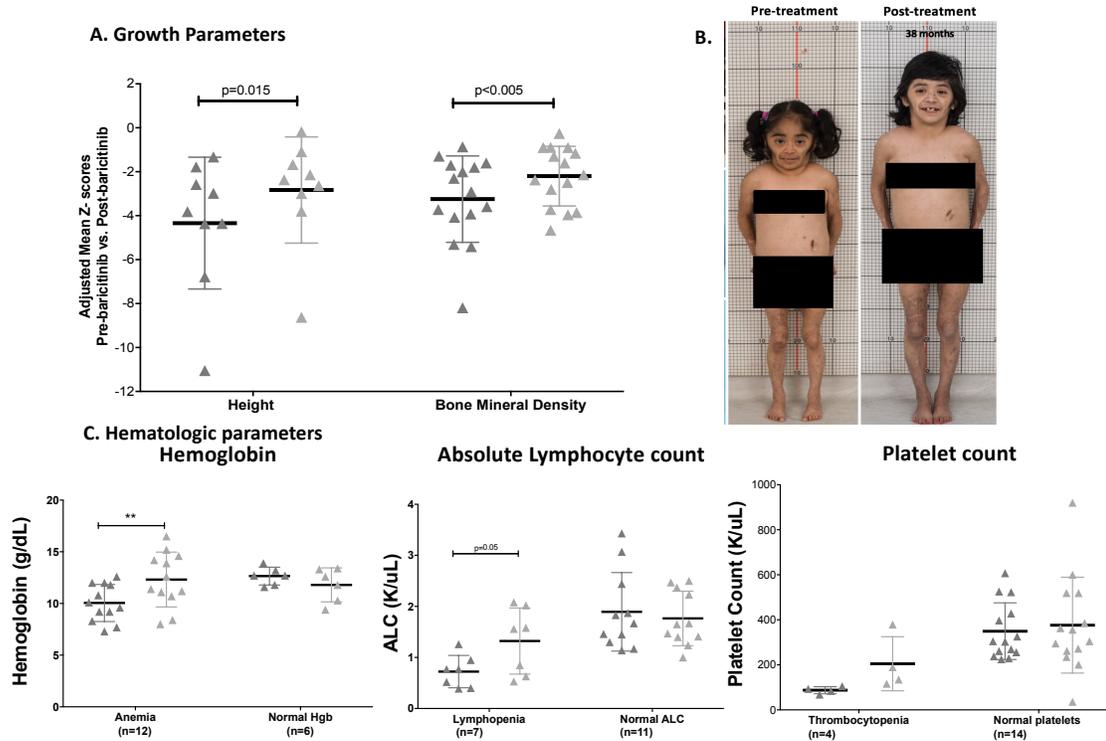


Figure 4. Improvement in longitudinal growth and hematologic parameters. (A) Clinical significant improvement in the height Z-scores and percentiles of patients with growth potential (n=13) was seen, when comparing pre-baricitinib to last visit on baricitinib data. Mean height Z-scores improved from -4.03 ± 2.64 to -3.19 ± 2.33 ; with “catch up growth” observed in 9 patients, their improvement translates into a mean height percentile increase from the 1.4th percentile to 7.2th percentile. **(B)** CANDLE patient (C8) with stunted growth since 2 years of age, and a severe delay in bone age (chronological age 14.3 years vs. bone age 2 years). Within 30 months of treatment, linear height increased from 90 cm to 106.8 cm and bone age improved from 2 years to 7.8 years. **(C)** Signs of bone marrow immunosuppression have improved in all patients but 2 (C1 and O3) with increases of platelets, absolute lymphocyte counts and hemoglobin. Patient C1 continues with persistent lymphopenia (ALC 0.5), patient O3 (discontinued from the program due to poor response and osteonecrosis) had a lower hemoglobin and platelet count at the time of his last visit. This patient had multiple comorbidities including upper gastrointestinal bleeding, esophageal varices, IgA nephropathy and idiopathic thrombocytopenia.

** Denotes unadjusted *p* value < 0.05

Figure 5.

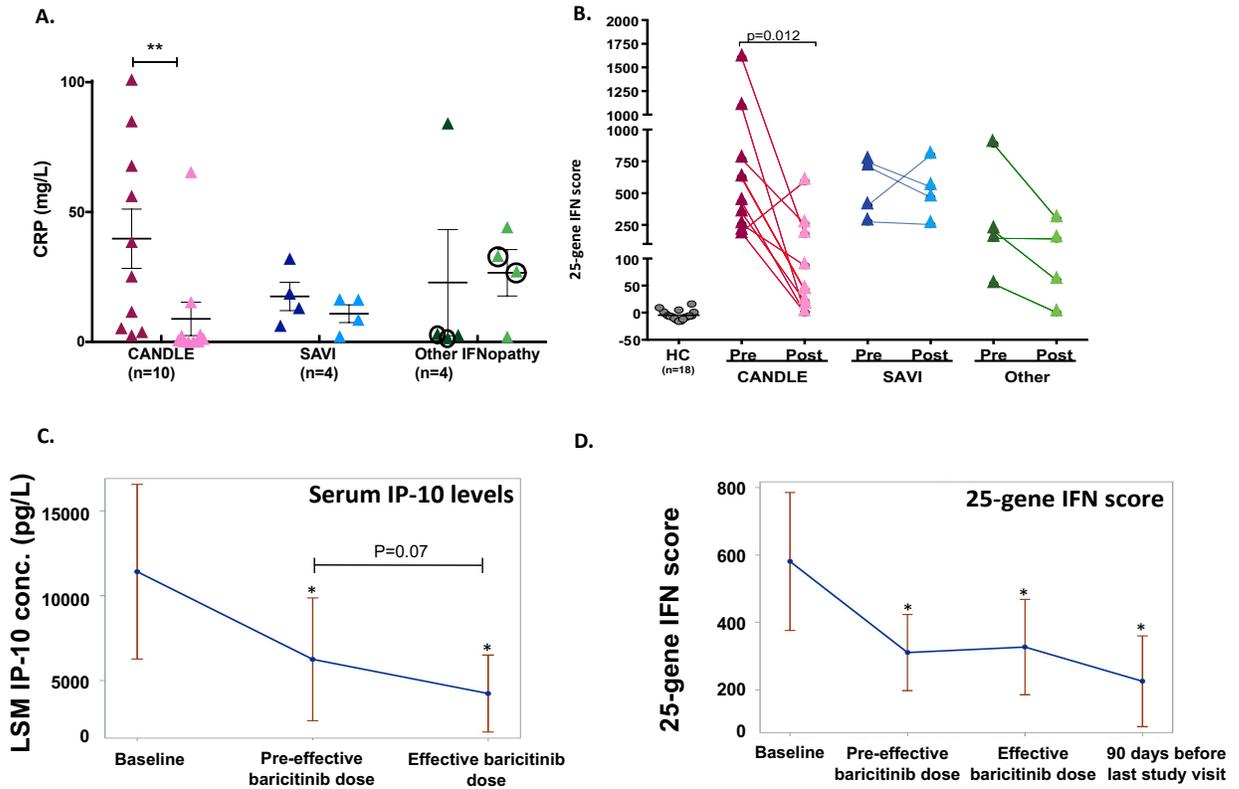


Figure 5. Assessment of conventional inflammatory parameters (C-reactive protein (CRP)) and the IFN biomarkers (serum IP-10 levels and 25-gene-IFN score) on baricitinib. (A) The CRP dropped most significantly in CANDLE patients with 5 of 10 normalizing their CRP. Patients O2 and O4 with “other interferonopathies” who stayed in the program had improvement in CRP. The 2 patients who discontinued from the program and due to lack of efficacy had no improvement and are circled. ** Denotes unadjusted *p* value < 0.05, graph represents means and standard deviations. **(B)** The 25-gene-IFN-response-gene signature (IRS) was graphed with baseline score and the IFN score obtained at the last included visit only. Colors indicate data by disease with CANDLE red, SAVI blue and other interferonopathies green. The IFN score normalized in 5 of 10 CANDLE patients who achieved remission criteria. **(C, D)** Longitudinally assessed serum IP-10 levels and 25-gene IFN score measurements were fitted to a repeated-measures model with “treatment phase” as a categorical independent variable. Least-squares means of serum IP-10 and 25-gene IFN score with 95% confidence intervals for each phase are graphed. * Denotes unadjusted *p*-values < 0.05