An N-of-1 Trial of Itacitinib for a Patient with Aplastic Anemia Associated with a Gain-of-Function Variant in STAT1

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Abstract

An 18-year-old man presented with aplastic anemia, and exome sequencing identified a germline gain-of-function variant in the gene STAT1. Treatment with itacitinib, an investigational selective Janus Kinase 1 (JAK1) inhibitor, resulted in prompt recovery of hematopoiesis. An exhausted CD8+ T cell population and myeloid populations enriched for an interferon-γ signature correlated with disease activity. Patient bone marrow sections displayed increased phospho-STAT1 staining, as did other idiopathic aplastic anemia cases, suggesting a shared pathophysiologic mechanism. This study describes the success and mechanism of a molecularly targeted therapy with potential implications for the treatment of aplastic anemias and other autoimmune disorders.

Introduction

An 18-year-old man presented with 3 weeks of pallor, weakness, and dyspnea. On exam, the patient was pale but well appearing with a BMI of 17. He had buccal aphthous ulcers and thrush on the soft palate. His peripheral blood laboratory values were notable for pancytopenia with a hemoglobin level of 3.9 g/dL (reference range 12-16) without signs of hemolysis, a white blood cell count nadir of 350 cells/µl (reference range 4,500-11,000), an absolute neutrophil count nadir of 210 cells/µl (reference range 1,800-7,700), and a platelet count of 118,000/µl (reference range 140,000-430,000). He was transfused red blood cells, and a bone marrow biopsy demonstrated hypocellularity (20-30% cellularity) with a myeloid predominance, absence of erythroid precursors, and decreased megakaryocytes. An infectious and rheumatologic workup was unrevealing (Fig. S1). Testing for paroxysmal nocturnal hemoglobinuria, Fanconi anemia, and telomere shortening were negative. Bone marrow panel mutational testing, karyotypic analysis, and T-cell receptor spectrotyping were all within normal limits. Though platelets were relatively preserved, based on criteria of peripheral neutrophils <500/mL, reticulocytes < 1%, and bone marrow hypocellularity, a diagnosis of severe aplastic anemia was made.1
His past medical history was notable for recurrent severe oral aphthous ulcers since childhood, for which he took prednisone 10mg during flares. Given his thrush, we prescribed nystatin oral suspension which he took as needed. His paternal grandmother, father, and other family members suffered similar oral ulcers since youth, and construction of a pedigree suggested an autosomal dominant pattern of inheritance (Fig. 1A). His father also suffered from thrush, but additional family history beyond these clinical features was unable to be obtained. No other family members were known to have aplastic anemia.

**Results**

**Molecular Evaluation**

Aplastic anemia is a rare disorder characterized by pancytopenia due to bone marrow failure. It can be caused by direct damage (e.g. toxins or radiation), rare genetic syndromes, infection, or, most commonly, idiopathic autoimmune T cell attack of hematopoietic cells.\(^2\) Standard-of-care therapy includes allogeneic stem cell transplantation or intensive immunosuppression.

Given the autosomal dominant inheritance pattern of the patient’s aphthous lesions and unclear etiology of his aplastic anemia, we performed exome sequencing of both patient and father. A heterozygous variant (c.800C>T, p.Ala267Val) in the coiled-coil domain of \(STAT1\) was identified in both the patient and his father and confirmed by Sanger sequencing (Fig. 1B).

\(STAT1\) is a transcription factor downstream of JAK signaling, a pathway critical to hematopoiesis, immunity, and development.\(^3\) More than 50 cytokines and growth factors, including interferons, signal through this pathway by binding to cognate receptors and effecting dimerization. Dimerization activates receptor-bound JAKs which then phosphorylate and activate STATs. STATs form either homo- or hetero-dimers, translocate into the nucleus, bind specific DNA sequences, and activate discrete transcriptional programs.

Gain of function (GOF) variants in \(STAT1\) cause an autosomal dominant syndrome with a spectrum of autoimmune features and near complete penetrance of mucocutaneous candidiasis.\(^4,5\) More than 400
patients with more than 100 different STAT1 GOF variants have been reported.\textsuperscript{4,6,7} Aplastic anemia has been described, as have numerous other autoimmune cytopenias, and severe aphthous stomatitis is frequent.\textsuperscript{4,6,8,9} GOF variants lead to hyper-phosphorylation of STAT1 in response to stimulation, increasing STAT1-dependent transcription.\textsuperscript{7,10,11} The p.Ala267Val variant identified in our patient is absent from large population databases, but has been identified in more than 10 individuals in multiple families with chronic mucocutaneous candidiasis, and shown to have a GOF effect.\textsuperscript{6,12} We thus considered the p.Ala267Val variant as causal for our patient’s recurrent aphthous ulcers, thrush, and aplastic anemia.

Itacitinib Trial

As STAT1 activation is heavily dependent on JAK signaling, the JAK inhibitors ruxolitinib and tofacitinib have been trialed in STAT1 GOF patients. Successful and unsuccessful cases have been reported for the treatment of alopecia, diabetes, thrush, fungal infections, and autoimmune cytopenias.\textsuperscript{8,13–18} Ruxolitinib and tofacitinib inhibit both JAK1 and JAK2 with the potential for anemia and thrombocytopenia, since JAK2 is downstream of the erythropoietin and thrombopoietin receptors. In considering a JAK inhibitor, we thus sought to avoid JAK2 inhibition.

The investigational drug itacitinib is a potent JAK1 inhibitor with an IC\textsubscript{50} of 3.2 nM and selectivity for JAK1.\textsuperscript{19} Itacitinib has been studied in myeloproliferative neoplasms, graft-versus-host disease, and autoimmune disorders.

The patient remained dependent on red blood cell transfusions, and he was thrice admitted to the hospital for febrile neutropenia. Preparations were underway for hematopoietic stem cell transplantation when, based on genotyping results, we enrolled the patient in a single patient expanded use trial of itacitinib (\url{https://clinicaltrials.gov/ct2/show/NCT03906318}). On day 169 after presentation, he received his weekly transfusion and initiated itacitinib 300mg daily. The following week, and for all subsequent visits after the initiation of itacitinib, laboratory values demonstrated resolution of neutropenia and anemia, and the patient became red blood cell transfusion independent (Fig. 1C). A subsequent bone marrow biopsy demonstrated a return of trilineage hematopoiesis.
Increased STAT1 signaling as measured by phospho-STAT1 (pSTAT1) staining, which had been present at time of diagnosis, resolved after treatment (Fig. 1D). The patient’s thrush and oral ulcers persisted but at significantly decreased frequency and severity. Clinically, fatigue and weight loss resolved, and he returned to schooling. He completed 20 months of itacitinib therapy without any adverse events before electing to self-discontinue. Aplastic anemia has not recurred.

**Immunological Profiling**

To understand the cellular mechanisms involved in the pathogenesis and resolution of this patient’s aplastic anemia, we performed comprehensive immunophenotyping. To confirm STAT1 GOF activity, we used phospho-CyTOF to measure phospho-STAT1 in monocytes after stimulation with interferon-γ at timepoints pre- and post-itacitinib treatment. At all timepoints, the patient’s monocytes had higher levels of pSTAT1 than healthy control. Post-itacitinib, pSTAT1 levels decreased, though not to the level of healthy control (Fig. 2A).

The largest studies of STAT1 GOF patients have demonstrated increased frequencies of T_H1 and decreased frequencies of T_H17 T cells. Increased signaling through STAT1 biases T cell differentiation into the T_H1 phenotype, which is characterized by secretion of interferon-γ. T_H1 differentiation comes at the expense of STAT3-mediated T_H17 differentiation, which is characterized by secretion of IL-17A and cytokines critical for immunity to mucocutaneous Candida. It is hypothesized that JAK inhibition may restore the balance between T_H1 and T_H17 differentiation by dampening STAT1 signaling.

Interrogating the T_H1 and T_H17 pathways, we compared plasma cytokine levels pre- and post-treatment with itacitinib. This identified significant decreases in the T_H1 cytokines interferon-γ and IL-12p40 following therapy, but no significant shifts in the T_H17 cytokines IL-17A or IL-17F (Fig. 2B). Of note, elevated serum interferon-γ has been described in aplastic anemia.

Examining CD4+ T cells by post-stimulation intracellular cytokine staining, we found a near absence of IL-17A-producing CD4+ T cells (T_H17) in the patient compared to healthy controls; this did not
change with treatment (Fig. S2B). Frequencies of interferon-γ-producing CD4+ T cells (T_{H1}) did not differ between healthy controls, the patient pre-itacitinib, or the patient post-itacitinib (Fig. 2C). Thus, we did not observe evidence of itacitinib restoring a balance between STAT1-mediated T_{H1} and STAT3-mediated T_{H17} frequencies. Moreover, while we observed other peripheral blood mononuclear cells (PBMC) abnormalities including an absence of T_{reg} cells, elevated frequencies of memory CD4+ and CD8+ T cells, and reduced frequencies of T follicular helper (T_{FH}) cells compared to healthy controls (Fig. S2A), none of these frequencies changed significantly with itacitinib treatment.

Surprisingly, the patient’s pre-itacitinib CD8+ T cells produced significantly less interferon-γ than healthy controls in vitro (Fig. 2D). After treatment with itacitinib, CD8+ interferon-γ production increased but not to the level of healthy controls. Pre-itacitinib memory CD8+ T cells also had a striking increase in expression of the activation marker Programmed Death 1 (PD-1), which decreased with itacitinib treatment (Fig. 2E). Thus PD-1+ CD8+ T cells—which are defective in their ability to secrete interferon-γ upon stimulation in vitro—closely correlate with aplastic anemia disease activity.

Taken together, these findings suggest a model whereby during aplastic anemia, the patient’s CD8+ T cells are chronically activated against hematopoietic progenitors in vivo leading to an exhausted phenotype characterized by impaired ability to secrete interferon-γ in vitro and increased PD-1 expression. By inhibiting JAK1-driven activation, itacitinib relieved CD8+ T cell exhaustion. Consistent with our findings, elevated frequencies of activated CD8+ T cells have been described in aplastic anemia.23 We did observe the seemingly paradoxical finding of increased serum interferon-γ (Fig. 2B) yet decreased interferon-γ secretion by CD8+ T cells in vitro (Fig. 2D). One interpretation of these results is that these CD8+ T cells are chronically secreting interferon-gamma in vivo, yet their exhausted phenotype reduces their capacity to secrete interferon-gamma in vitro upon stimulation.24 To explore the in vivo unstimulated cytokine effector function of these cells, we performed single-cell transcriptional analyses.
Single-Cell Gene Expression Analysis

To understand the transcriptional programs governing our patient's aplastic anemia, we performed single-cell transcriptional sequencing (scRNA-Seq), comparing PBMCs from healthy controls and our patient at timepoints before and after itacitinib. Unsupervised clustering demonstrated that healthy, pre-itacitinib, and post-itacitinib cells represent distinct clusters. Post-itacitinib samples clustered between healthy and pre-itacitinib samples, suggesting progression from disease towards the healthy state (Fig. 3A).

We then separately subclustered T cell-containing and myeloid populations for further analysis (Fig. S3). T cell-containing cells from the patient and healthy controls subclustered into a CD8+ population, NK population, and mixed CD4+ and CD8+ memory and naïve populations. Given the activated but dysfunctional state of our patient’s CD8+ T cells, we scored all CD8+ T cells for cytotoxicity, cytokine effector function, and exhaustion (Table S1). Each of these scores were elevated in the patient’s pre-itacitinib cells compared to healthy controls, confirming their cytotoxic and exhausted phenotype (Fig. 3B). After treatment, exhaustion and cytokine effector scores decreased. Our finding of increased transcriptional cytokine effector scores in the patient’s pre-itacitinib CD8+ T cells supports the hypothesis that these cells secrete higher levels of cytokines such as interferon-\(\gamma\) in vivo, despite their reduced capacity to secrete interferon-\(\gamma\) in vitro upon stimulation.

Subclustering of myeloid populations identified five populations (Fig. 3C). Of note, “activated CD14+ monocytes” and “C1Q+ monocytes” were found virtually exclusively in the patient’s and not in the healthy controls’ cells. Both of these subclusters expressed STAT1 activation-induced genes such as the complement genes C1QC, C1QB, C1QA or the interferon-inducible genes FAM26F or GBP1, respectively (Fig. S3). Type I interferons (\(\alpha\), \(\beta\), and others) and Type II interferon (only \(\gamma\)) induce overlapping but distinct transcriptional signatures. Since monocytes express high levels of the interferon-\(\gamma\) receptor, we scored each myeloid cell to assess for interferon exposure (Table S1). Both Type I and II interferon scores in pre-treatment monocytes were higher than healthy control or post-
itacitinib monocytes. Within pre-itacitinib monocytes, Type II scores were higher than Type I, suggesting a primarily interferon-γ induced state ($p<10^{-10}$, Wilcoxon rank-sum).

**Single-Cell Epigenetic Analysis**

To understand the epigenetic effects of the STAT1 GOF variant and itacitinib treatment, we performed single-cell Assay for Transposase-Accessible Chromatin with Sequencing (scATAC-seq). Within effector CD8+ T cells, we saw increased accessibility at the PD-1 locus, consistent with our observation of increased PD-1 protein expression ([Fig. 2E](#)), and this accessibility decreased after treatment ([Fig. 3D and S4](#)).

Genome-wide, STAT1 motif accessibility pre-itacitinib was increased when compared to healthy controls or post-itacitinib samples ([Fig. 3E](#)). These studies suggest that changes in accessible chromatin correlate with STAT1-mediated autoimmunity and can be reversed with itacitinib.

**pSTAT1 Bone Marrow Immunostaining**

Immunostaining our patient’s bone marrow sections, we found increased pSTAT1 staining pre-itacitinib, and this STAT1 activity resolved following itacitinib therapy ([Fig. 1D](#)). To determine whether similar STAT1 dysregulation exists in patients with idiopathic (non-STAT1-mutated) aplastic anemia, we analyzed bone marrow sections from other aplastic anemia patients. Compared to healthy donor marrow in which no staining was detected, three of four marrows exhibited positive pSTAT1 staining ([Fig. 4](#)). This raises the exciting possibility that STAT1 activation is a feature of a subset of aplastic anemias for which pSTAT1 is a potential biomarker.

**Discussion**

Here we present a man with a history of oral ulcers inherited in an autosomal dominant pattern who developed aplastic anemia. Exome sequencing identified a pathogenic GOF mutation in **STAT1**. Molecular inhibition of JAK1, a kinase upstream of STAT1 activation, resulted in resolution of the patient’s aplastic anemia.
Longitudinal immunophenotyping of samples unperturbed by confounding immunosuppressive therapy revealed an expanded, activated, cytolytic, and exhausted memory CD8+ T cell population that correlated with disease activity, as did plasma levels of interferon-γ. After itacitinib treatment, these abnormalities improved, with parallels to mouse and human models of idiopathic aplastic anemia.\textsuperscript{23,25}

To our knowledge, this represents the first report of a targeted therapy for the treatment of autoimmune aplastic anemia. This case also marks the first report of itacitinib for use in a primary immunodeficiency. Given the high frequency of cytopenias in primary immunodeficiencies, itacitinib's JAK1 selectivity may be of particular clinical benefit.\textsuperscript{26,27}

Establishing the causality of a therapy in a single-patient trial is challenging, but the co-incident timing of itacitinib with near immediate hematopoietic recovery after 6 months of transfusion-dependence, as well as the low expected rate of spontaneous recovery in aplastic anemia,\textsuperscript{2} are evidence in favor of a therapeutic effect of itacitinib in this case.

While STAT1 GOF is a rare condition, aplastic anemia is more common. Aplastic anemia can be triggered by a spectrum of host and environmental factors,\textsuperscript{2} but our patient's immunophenotypic similarities to other cases of aplastic anemia raise the possibility of a shared downstream pathophysiology for which JAK inhibitor therapy could be effective. Additionally, our finding of increased bone marrow pSTAT1 staining in a majority of aplastic anemia cases may be a useful biomarker to identify candidate patients. We hope these results spur larger trials to answer these questions, particularly given the relative tolerability of JAK inhibitors compared to current standard treatment modalities including hematopoietic stem cell transplantation or medical immunosuppression.
Online Methods

Please see separate attachment for methods section.
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Author Contributions

J.M.R. conceived the study and designed the experiments with D.B.S. J.M.R., T.H., J.M.P., C.A.L., L.S.L., and L.R.M. performed the experiments and analyzed the data. C.A.T. and H.L.R. performed the exome sequencing. B.B., A.K.S., Y.B.C., S.M.F., and D.B.S supported the studies and reviewed the manuscript.

Competing Interests

Y.B.C. reports consulting fees from Incyte. All other authors declare no competing interests.
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