Methods

Institutional Review Board Protocols

Sample collection, genetic sequencing, and bone marrow pathology were approved by our institutional review board (IRB), protocols #2017P000108, #2014P000460/BWH, 2020P001858. Itacitinib treatment was also approved by our IRB protocol #18-326, www.clinicaltrials.gov NCT03906318, and the U.S. Food and Drug Administration (F.D.A.).

Panel Mutation Genotyping

Panel mutation genotyping of bone marrow specimens was performed using SNaPshot as previously described[1].

Exome Sequencing

Peripheral blood DNA extraction, library preparation, exome capture, and sequencing were performed by the Genomics Platform of the Broad Institute of Harvard and MIT (Cambridge, MA). Sequencing covered 96% of exome targets with 20X coverage or higher. Reads were aligned to the human reference sequence (GRCh37) using the Burrows-Wheeler Aligner (BWA), and variant calls were made using the Genomic Analysis Tool Kit (GATK). Variants were filtered for: (1) variants classified as disease causing mutations in public databases that have a minor allele frequency <5.0% in the Genome Aggregation Database (gnomAD, http://gnomadexac.broadinstitute.org/); (2) nonsense, frameshift, and +/-1,2 splice-site variants in disease-associated genes with a minor allele frequency ≤1.0% in gnomAD; and, (3) variants with a minor allele frequency ≤1.0% in gnomAD in a phenotype-driven gene list.

Sanger Sequencing

Clinical confirmation of the STAT1 variant was performed by Sanger sequencing with the following primer sequences. Forward primer: CCTCCACAAACTCTCTTGCC. Reverse primer: TTAAACCCTTGTAAATCATCTGAA.

Cryopreservation of Peripheral Blood Mononuclear Cells

Samples were aliquoted into 50ml falcon tubes and centrifuged at 500g for 15 min. Plasma was pipetted off and aliquoted/saved. Buffy coat with 5mL PBS was layered onto 5mL of Ficoll in a fresh 15mL Falcon tube. Tubes were spun for 25min at 400g. Buffy coat was washed in PBS, resuspended in RPMI media and added in equal volume to 20% DMSO and 80% FBS, and cryopreserved in liquid nitrogen for subsequent analysis via flow cytometry and single cell genomics.

Statistics

Statistical differences in flow cytometry cell subset frequencies and cytokine measurements were assessed by unpaired *t*-test. scRNA-Seq and scATAC-Seq statistical differences were calculated using a Wilcoxon rank-sum test.

Single-cell RNA-Sequencing

Single-cell mRNA sequencing was performed using the Seq-Well platform[2]. Ten samples were analyzed: 4 samples from 4 distinct healthy controls, 3 samples from the patient pre-itacitinib treatment, and 3 samples from the patient post-itacitinib treatment. Each patient sample was from a separate blood draw separated by at least 1 week. For each sample, 20,000 cells were applied to a Seq-Well device pre-loaded with mRNA capture beads, as previously described. Following cell loading, devices were reversibly sealed, cells were lysed, and mRNA was hybridized to bead-bound capture oligos. Beads were removed from arrays and reverse transcription was performed along with randomly templated second-strand synthesis. Following PCR amplification, sequencing libraries were prepared using the Illumina Nextera system, and

sequencing was performed on an Illumina NextSeq using 75 cycle v2 sequencing kits. Demultiplexed sequencing reads were aligned to the hg19 assembly of the human genome using DropseqTools v2.0.

Single-cell mRNA-sequencing data was analyzed using Seurat v3.0. UMI counts were normalized to total UMIs in each cell and represented as transcripts-per-10,000 (TP10K). We then took log(1+TP10K), scaled, and centered for input into PCA. PCA was performed on the top 3,000 variable genes. Downstream PCA dimensions were determined using translated Poisson distributions (R package *intrinsicDimension*). The reduced PCA cell embeddings matrix were corrected for array-specific effects using Harmony (R package *Harmony*)[3]. The batch-corrected embeddings were utilized to generate a shared nearest-neighbor graph for clustering. Cells were then clustered with the walktrap algorithm (R package *igraph*, steps = 4). UMAP visualizations were generated on both original and batch-corrected PCA embeddings. Gene list scores were generated for each cell by averaging the expression of all genes in the list and then subtracting the average expression of 30 control genes matched by expression level bin per gene in the gene list[4]. Differentially-expressed genes were identified using the Wilcoxon Rank-Sum test implemented in R package *presto.*

Single-cell Assay for Transposase-Accessible Chromatin with Sequencing

Single-cell Assay for Transposase-Accessible Chromatin with Sequencing (scATAC-seq) libraries were prepared using 10x Genomics and processed with CellRanger-ATAC v1.2. A healthy control PBMC library (10k v1) was downloaded from www.10xgenomics.com and was used as a reference. For all libraries, cells were defined as high-quality barcodes that captured at least 1,000 unique nuclear fragments and had a percent reads in peaks exceeding 40%. Dimensionality reduction, clustering, and cell state label transfer was performed using Seurat v3.0 and Signac using default parameters[5]. Transcription factor scoring was performed using

chromVAR with default parameters using a combination of all libraries to compute background peaks[6,7,8].

PBMCs from the patient at 3 separate time points before treatment were used as pre-treatment samples. PBMCs from the patient at 2 separate time points after treatment were used as post-treatment samples. All samples were separated in time by at least 1 week. To compare to healthy control PBMCs, we utilized a public dataset of 10,000 PBMCs from a healthy donor[9].

Phospho-STAT1 Bone Marrow Staining

Immunohistochemistry on bone marrow specimens was performed on Ventana Discovery ULTRA (Cat No. 750-601). Phospho-STAT1 antibody (Cell Signaling Technology; Cat No. 9167) was used at 1:600 dilution for 28 minutes. Epitope retrieval was performed with CC2 solution for 32 minutes.

Flow Cytometry Immunophenotyping

Peripheral blood mononuclear cells were thawed. Flow cytometry was performed using a BD FACSAria III, collected using BD FACSDiva v8, and analyzed using FlowJo v10. Antibodies and volumes used are listed below.

Antibody	Fluorochrome	Laser	Detector	Volume in uL	Supplier	Catalog Number
PD1	BUV395	355	395	5	BD	745619
CXCR3	BV421	405	421	2.5	BioLegend	353716
viability	DAPI	355	461		BD	564907
CD45RA	FITC	488	530	2.5	eBioscience	11-0458-42
CCR6	PE	561	585	5	BD	551773
CXCR5	AF 647	640	670	2.5	BD	558113
CD127	PerCP Cy5.5	488	695	2.5	BD	560551
CD8	Alexa 700	640	720	1.5	Biolegend	300920
CD4	APC Cy7	640	780	2.5	BD	341095
CD25	PE-Cy7	405	710	3.5	BD	557741

Flow Cytometry Intracellular Cytokine Staining

Peripheral blood mononuclear cells were thawed. Flow cytometry was performed using a BD FACSAria III, collected using BD FACSDiva v8, and analyzed using FlowJo v10. Antibodies and volumes used are listed below.

Antibody	Fluorochrome	Laser	Vendor	Catalog Number	Volume in uL
IL17	APC	633	biolegend	512333	5
IFNg	Pacblue	405	BioLegend	506525	5
CD3	PerCP	488	BioLegend	300427	5
CD4	PE	488	biolegend	300508	5
CD8	BUV395	355	BD	563795	5
CD45RO	PE/Cy7	488	BD	560608	5
CD45RA	FITC	488	BD	561882	10

Phosphoflow CyTOF

This assay was performed by the Human Immune Monitoring Center at Stanford University. PBMC were thawed in warm media and stimulated by adding 50 ul (25ug) of interferon-γ (BD Biosciences, 554617) or left unstimulated and incubated at 37°C for 15 minutes. Cells were then fixed with paraformaldehyde, washed twice with CyFACS buffer (PBS supplemented with 2% BSA, 2 mM EDTA, and 0.1% sodium azide) and stained for 30 min at room temperature with 20 mL of surface antibody cocktail. Cells were permeabilized with 100% methanol, washed with CyFACS buffer, and resuspended in intracellular antibody cocktail in CyFACS for 30 minutes before washing twice in CyFACS. Cells were resuspended in 100 mL iridium-containing DNA intercalator (1:2000 dilution in 2% PFA in PBS) and incubated at room temperature for 20 min. Cells were washed and acquired on CyTOF. Data analysis was performed using FlowJo v10 by gating on intact cells based on the iridium isotopes from the intercalator, then on singlets by Ir191 vs cell length followed by cell subset-specific gating. Monocytes were gated based on DNA content for live singlets and CD14 expression.

Plasma Cytokine Levels

This assay was performed by the Human Immune Monitoring Center at Stanford University. Luminex magnetic bead human 62-plex Procarta kits were purchased from eBiosciences/Affymetrix/Thermo Fisher, Santa Clara, California, USA, and used according to the manufacturer's recommendations. Plates were read using a Luminex 200.

Figures

The JAK/STAT schematic (**Fig. S1D**) was created using www.Biorender.com.

Methods References

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