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Transcriptional differences between *JAK2*-V617F and wild-type bone marrow cells in myeloproliferative neoplasm patients

Debra Van Egeren[1,2,3], **Baransel Kamaz**[4], **Shichen Liu**[1], **Maximilian Nguyen**[1,2], **Christopher R. Reilly**[5], **Maria Kalyva**[6], **Daniel J. DeAngelo**[5], **Ilene Galinsky**[5], **Martha Wadleigh**[5], **Eric S. Winer**[5], **Marlise R. Luskin**[5], **Richard M. Stone**[5], **Jacqueline S. Garcia**[5], **Gabriela S. Hobbs**[7], **Franziska Michor**[1,8,9], **Isidro Cortes-Ciriano**[6], **Ann Mullally**[4,5,8], **Sahand Hormoz**[1,2,8]

[1]Department of Data Science, Dana-Farber Cancer Institute, Boston, MA, USA

[2]Department of Systems Biology, Harvard Medical School, Boston, MA, USA

[3]Stem Cell Program, Boston Children's Hospital, Boston, MA, USA

[4]Division of Hematology, Brigham and Women's Hospital, Boston, MA, USA

[5]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, USA

[6]European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI), Hinxton, UK

[7]Leukemia Center, Massachusetts General Hospital, Boston, MA, USA

[8]Broad Institute of MIT and Harvard, Cambridge, MA, USA

[9]Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, MA, USA; Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA; The Center for Cancer Evolution, Dana-Farber Cancer Institute, Boston, MA, USA; and The Ludwig Center at Harvard, Boston, MA, USA

Abstract

The JAK2-V617F mutation is the most common cause of myeloproliferative neoplasms. While experiments have shown that this gain-of-function mutation is associated with myeloid blood cell expansion and increased production of white cells, red cells and platelets, the transcriptional consequences of the JAK2-V617F mutation in different cellular compartments of the bone marrow have not yet been fully elucidated. To study the direct effects of JAK2-V617F on bone marrow cells in myeloproliferative neoplasm patients, we performed joint single-cell RNA sequencing and JAK2 genotyping on CD34+ enriched cells from 8 patients with newly diagnosed essential thrombocythemia or polycythemia vera. We found that the JAK2-V617F mutation increases the expression of interferon-response genes (e.g., HLAs) and the leptin receptor in hematopoietic progenitor cells. Furthermore, we sequenced a population of CD34-bone marrow monocytes and

Corresponding authors: Ann Mullally (Ann_Mullally@dfci.harvard.edu), Isidro Cortes-Ciriano (icortes@ebi.ac.uk), and Sahand Hormoz (sahand_hormoz@hms.harvard.edu).

found the JAK2 mutation increased expression of intermediate monocyte genes and the fibrocyte-associated surface protein SLAMF7 in these cells.

Keywords

myeloproliferative neoplasm; single-cell sequencing; JAK2-V617F

INTRODUCTION

The *JAK2*-V617F mutation is a somatic mutation found in the majority of patients with myeloproliferative neoplasms (MPNs)[1]. The mutation causes constitutive JAK-STAT pathway activation in hematopoietic stem and progenitor cells (HSPCs), leading to overproduction of red blood cells, platelets, white blood cells, and/or bone marrow fibrosis. Previous work characterizing *JAK2*-mutant mouse models[2,3] and MPN patient samples[4] have shown that *JAK2*-V617F increases the fitness of hematopoietic stem cells (HSCs) and promotes megakaryocyte-erythroid differentiation. However, it is unclear what molecular mechanisms connect JAK-STAT pathway activation with the observed changes in differentiation and cell division. Furthermore, it is unclear which cell types within the bone marrow are directly affected by the mutation and which cells are affected by cell-non-autonomous factors. Previous studies have shown that *JAK2*-V617F HSPCs have increased JAK-STAT signaling compared to their wild-type (WT) counterparts in the same MPN patient[5,6]. However, these studies generally focused on the mutation's effects on HSCs and megakaryocyte erythrocyte progenitors (MEPs).

Here, we used joint scRNA-seq and *JAK2* genotyping of bone marrow from polycythemia vera (PV) and essential thrombocythemia (ET) patients to determine the impact of the *JAK2*-V617F mutation in an unbiased way on multiple bone marrow cell types. In addition to affecting MEPs, we show that the *JAK2*-V617F mutation directly affects bone marrow monocytes, changing their surface phenotype and increasing expression of *SLAMF7*, a marker associated with fibrocyte differentiation.

MATERIALS AND METHODS

Experimental procedures

Cell isolation, scRNA-seq with specific amplification of *JAK2*, and preprocessing and cell type identification using marker genes were performed using the same methods previously described[4]. All patients were newly diagnosed and treatment-naive at the time of sampling and harbored the *JAK2*-V617F mutation in their peripheral blood. Bone marrow samples from four healthy donors were also collected and scRNA-seq was performed without *JAK2* amplification using the same protocol as used on the MPN patients.

scRNA-seq data analysis

Preprocessing of the scRNA-seq data and identification of cells with mutant or wild-type *JAK2* amplicon transcripts was performed as previously published[4]. Differential expression analysis comparing cells with and without the *JAK2*-V617F mutation was performed in

scanpy using the Wilcoxon rank sum test. All raw p-values were combined between patients using Fisher's method and adjusted for multiple comparisons using the Benjamini-Hochberg method. Gene set enrichment analysis was performed using GSEApy to find enriched KEGG biological processes and ChEA/ENCODE transcription factor target groups.

After integrating and clustering data from all patients as previously published, classical, intermediate, and nonclassical subsets were identified in the monocyte population by Louvain clustering the monocyte population only. We used the expression levels of marker genes (classical: *CD14*, nonclassical: *CD16*, intermediate: *CD74*, *CD64*, *HLA-DRA*)[7] to manually assign each cluster to a subtype.

Calling somatic mutations in the scRNA-seq data

In the whole-genome sequencing (WGS) data, we identified somatic mutations that only occurred in the *JAK2*-V617F cells (220 mutations in ET 1, 398 in ET 2). Then, we detected these mutations in the scRNA-seq data by PCR amplification and sequencing (e.g., for a point mutation in *UPF1* in patient ET 1) or by calling mutations in the raw 10X transcriptome reads. To call the mutations in scRNAseq data, we extracted all reads mapping to each position mutated in the WGS data using Pysam[8]. Only reads with unambiguous cellular and molecular barcode sequences were considered. Subsequently, we classified reads as mutant or wild type depending on whether the reads contained the mutant or wild-type allele, respectively, requiring a minimum base quality of 30. Mutations that generated false positive mutant calls in any of the 36 10X Chromium single-cell RNAseq data sets from bone marrow and peripheral blood samples from healthy individuals were considered unreliable and discarded.

Bone marrow monocyte flow cytometry

Flow cytometry of bone marrow monocytes from three patients (ET 4, PV 1, and PV 3) and three healthy donors was performed to validate some of the results of the monocyte differential expression analysis. SLAMF7 cell surface staining was done on CD14+ cells as previously published[9].

RESULTS AND DISCUSSION

We analyzed joint single-cell *JAK2* genotyping and scRNA-seq data from CD34-enriched bone marrow samples from 4 ET and 4 PV patients (Supplementary Fig. 1). Six of these patients were sequenced in a previous study[4]. Two patients (PV 2 and PV 3) also had *TET2* mutations, and one patient (PV 1) had a low-frequency *EZH2* mutation detected in peripheral blood by a clinical NGS assay[10].

Bone marrow cells with the *JAK2*-V617F mutation were intermixed with the cells with WT transcripts when plotted together (Fig. 1A–C), suggesting that the mutation does not disrupt the overall structure of the differentiation hierarchy. However, as we reported previously[4], we found that cells with the *JAK2*-V617F transcript detected were more likely to be megakaryocyte or erythroid progenitors than those with WT *JAK2* transcripts (Fig. 1A–C), suggesting that *JAK2*-V617F induces a megakaryocyte-erythroid fate bias. We also found a substantial population of CD14+ bone marrow cells with the *JAK2* mutation which

do not express *CD34* and therefore likely represent monocytes (Fig. 1A–C, Supplementary Fig. 1).

We compared the transcriptomic profiles of *JAK2*-V617F bone marrow cells to those from WT cells to determine how the mutation changes gene expression in individual cells in MPN patients. To increase the power of our differential expression analysis, we used the published single cell WGS data[4] for ET 1 and ET 2 to identify and detect somatic mutations other than *JAK2*-V617F that uniquely marked the *JAK2*-mutant clonal population in each of these patients (Fig. 1D, Supplementary Table 1). By leveraging the WGS data, we were able to increase the number of cells that could be assigned as either *JAK2*-mutant or *JAK2*-WT by more than two-fold for ET 1 and ET 2.

Using the expanded set of genotyped cells, we identified genes that were differentially expressed between *JAK2*-mutant and *JAK2*-WT bone marrow cells in ET and PV patients (Fig. 1E–F). While we found few or no significantly differentially expressed genes in HSCs or GMPs (Supplementary File 1), MHC class I antigen presentation genes (e.g., HLAs, B2M) were consistently upregulated in *JAK2*-V617F MEPs, erythroid progenitors, and monocytes in both ET and PV patients. Interferon signaling through STAT1 has been previously associated with increased MHC I expression[11], suggesting that the observed upregulation of these genes in MPN could be caused by increased JAK-STAT activity. MHC I presentation of T-cell antigens, including *JAK2*-V617F itself[12], could induce an adaptive immune response against MPN cells, and PD-L1-mediated immune escape through reduced T-cell activation was previously reported in *JAK2*-mutant MPN[13]. Upregulation of these inflammation-associated genes in JAK2-mutant MEPs could also contribute to platelet activation and thromboinflammation[14].

We also observed that *JAK2*-V617F bone marrow monocytes had increased expression of proinflammatory and interferon response genes in both ET and PV patients (Fig. 1E–F). These genes are enriched for IRF and STAT targets (Supplementary Fig. 2), suggesting that their upregulation may be due to direct effects of constitutive JAK2 activation. Finally, in *JAK2*-V617F MEPs, we also noted increased expression of the leptin receptor (Fig. 1E), which has been reported to be a marker for long-term engrafting HSCs in mice[15].

We further characterized the transcriptional phenotype of the bone marrow CD34-CD14+ cluster and found that these cells express monocyte genes (Fig. 2A). Using expression of monocyte subtype markers[7], we identified classical, intermediate, and nonclassical monocyte subsets in our scRNA-seq data and found that *JAK2*-V617F monocytes were more likely than WT cells to have an intermediate monocyte phenotype (Fig. 2B–D). Furthermore, MPN patients had a higher fraction of intermediate monocytes overall than healthy controls (Supplementary Fig. 3). Intermediate monocytes have been shown to express high levels of antigen presentation genes, secrete both pro- and anti-inflammatory cytokines, and play a role in many infectious and autoimmune conditions[7]. Previous work showed that patients with *JAK2*-V617F myelofibrosis have more intermediate monocytes than healthy donors and exhibit dysregulation of cytokine production[16]. This abnormal phenotype is partially reversed by ruxolitinib treatment, suggesting that JAK-STAT signaling contributes to monocyte dysregulation[16]. Our single cell data suggests that *JAK2*-V617F acts directly

on monocytes to change their phenotype, since we found that individual cells harboring the mutation are more likely to be intermediate monocytes.

Another way monocytes may contribute to MPN pathogenesis is by differentiating into fibrocytes and contributing to bone marrow fibrosis. In *JAK2*-V617F myelofibrosis patients, CD14+ monocytes have been previously shown to preferentially differentiate into fibrocytes in vitro and express elevated levels of the cell surface marker SLAMF7[9]. After combining our monocyte scRNA-seq data from both ET and PV patients, we found that *SLAMF7* is significantly more highly expressed in *JAK2*-V617F monocytes than WT monocytes (Fig. 2E), although this difference could be due to the higher intermediate monocyte fraction in *JAK2*-mutant monocytes.

Flow cytometry revealed that ET and PV patients have a higher fraction of SLAMF7+ bone marrow monocytes than healthy donors (Fig. 2F–G), suggesting that SLAMF7+ monocytes may play a pathogenic role even in MPN subtypes not defined by bone marrow fibrosis. Consistent with this, none of the patients in our study had evidence of significant reticulin fibrosis in the bone marrow (four patients had MF grade 0, one patient (ET 4) had MF grade 0 –1 and three patients (ET 2, PV 1, and PV 3) had MF grade 1; Supplementary Fig. 1A). Inhibition of SLAMF7 with the monoclonal antibody drug elotuzumab has been shown to suppress fibrocyte differentiation and prevent progression in *in vitro* and *in vivo* models of myelofibrosis[9]. Our results also suggest that presence of *JAK2*-mutant monocytes could be investigated as an early biomarker of myelofibrosis risk in ET and PV patients.

In summary, we found that the *JAK2*-V617F mutation increases the expression of STAT signaling targets (e.g., antigen presentation and other pro-inflammatory genes) in HSPCs as well as monocytes. Our results suggest that the *JAK2* mutation could lead to a pathogenic pro-inflammatory, pro-fibrotic phenotype in bone marrow monocytes, and that this population should be further investigated to determine what role they play in the clinical manifestations of ET and PV and in progression to myelofibrosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

Joint scRNA-seq and *JAK2* genotyping in myeloproliferative neoplasm (MPN) patients.

- *JAK2*-V617F hematopoietic progenitors express interferon response genes more highly.
- Monocytes with *JAK2*-V617F have a pro-inflammatory, intermediate monocyte phenotype.
- *JAK2*-V617F monocytes express SLAMF7, which is associated with fibrosis in MPNs.

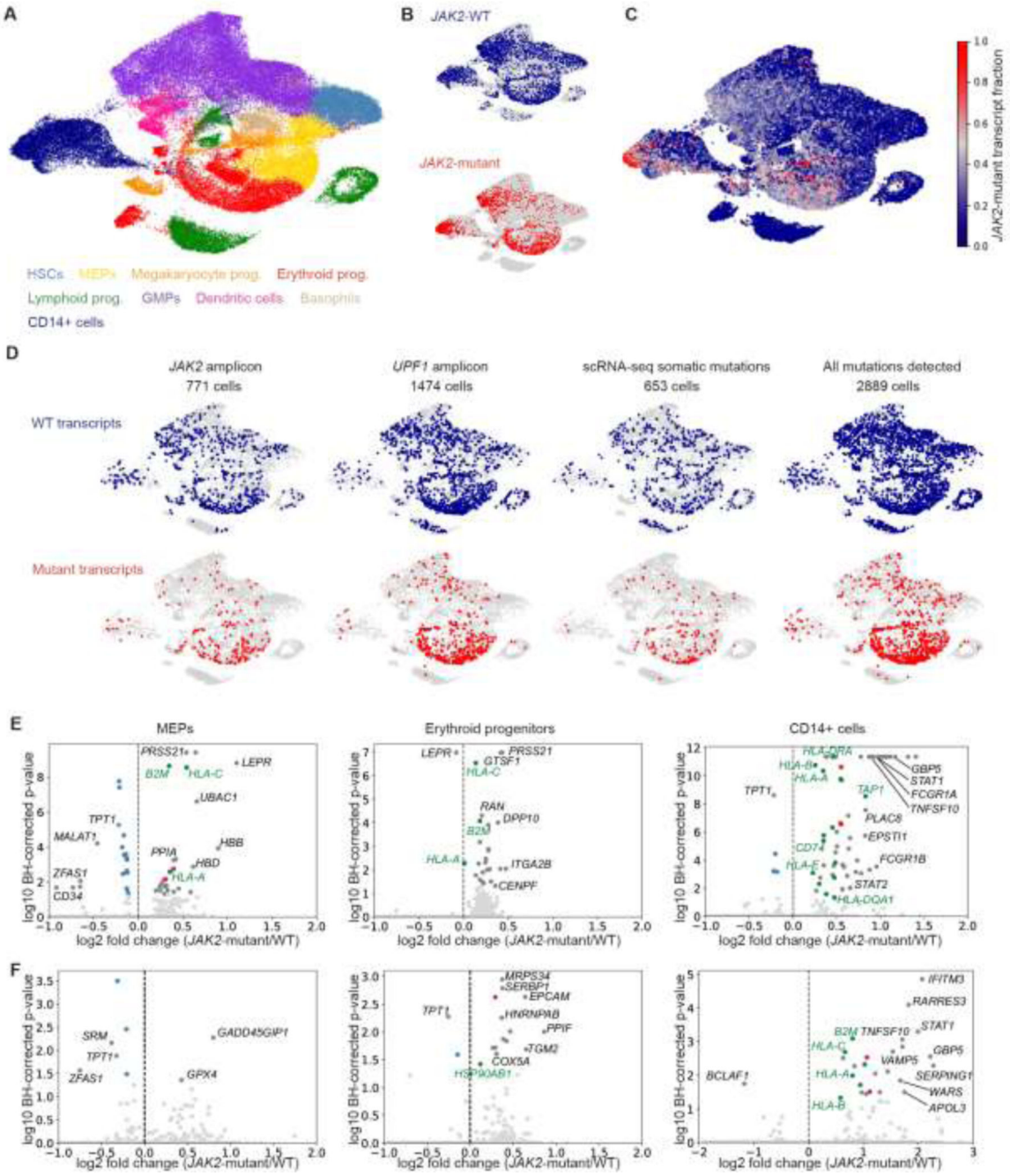

Figure 1. *JAK2*-V617F megakaryocyte and erythroid progenitors have higher expression of pro-inflammatory and antigen presentation genes.

A. UMAP of scRNA-seq data from bone marrow from 8 MPN patients, colored by cell type classifications. **B.** *JAK2*-WT (blue) and *JAK2*-mutant (red) transcripts detected in single cells in MPN patient bone marrow. **C.** Smoothed *JAK2*-V617F transcript fraction for all patients combined. **D.** Detection of mutations associated with *JAK2*-V617F in patient ET 1. Additional mutations were called using targeted amplification of loci identified from WGS (e.g., *UPF1*) and by directly identifying somatic mutations in the scRNA-seq data. **E-F.**

Volcano plots showing differential expression analysis results from comparing cells with mutant transcripts to cells with WT transcripts within the MEP, erythroid progenitor, and CD14+ compartments for ET patients (**E**) and PV patients (**F**). Ribosomal genes, antigen presentation genes, and proteasomal genes are colored in blue, green, and red, respectively.

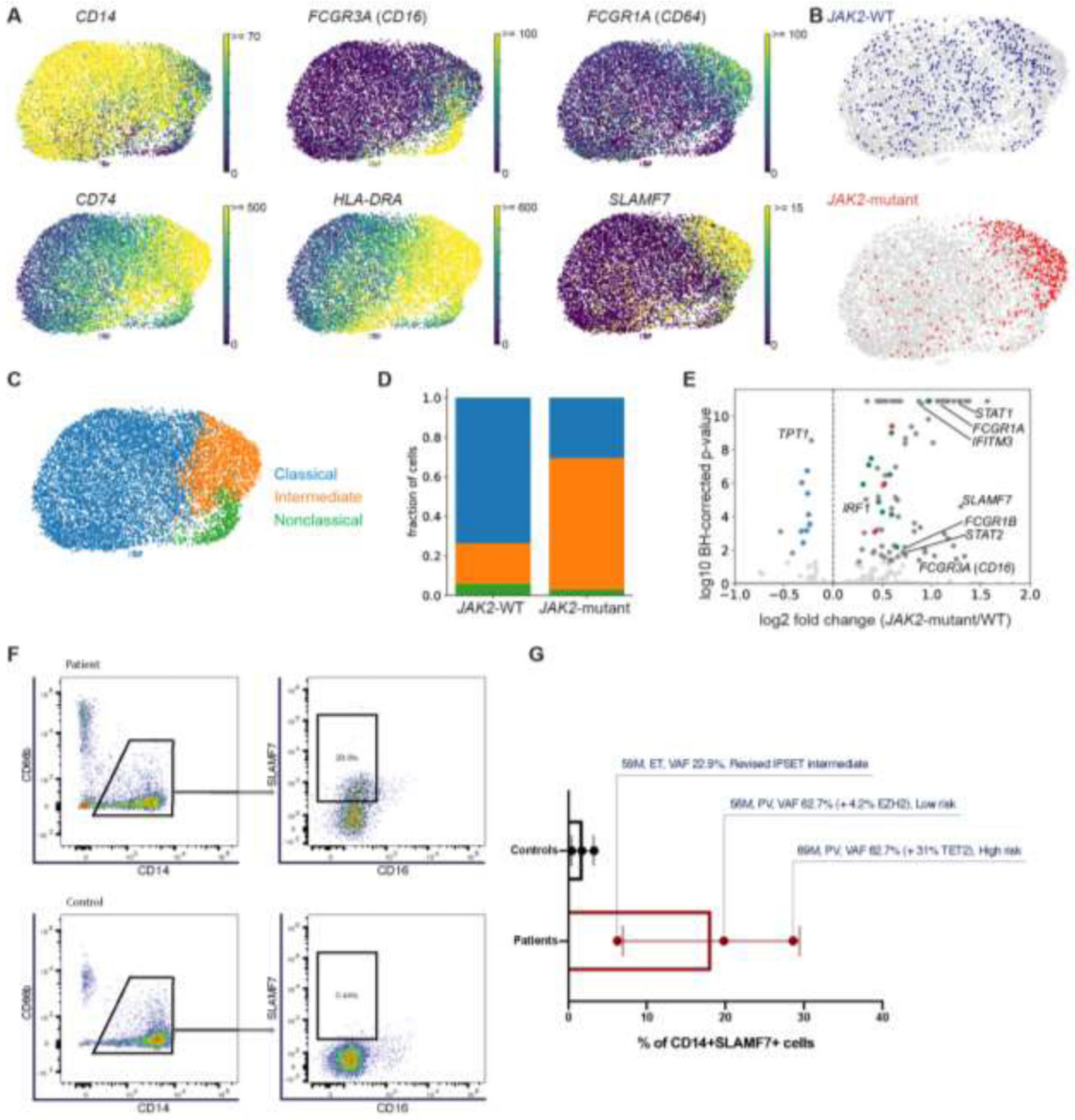

Figure 2. *JAK2*-V617F induces *SLAMF7* expression and an intermediate monocyte phenotype in MPN patients.

A. Marker gene expression UMAPs of the bone marrow monocyte compartment measured by scRNA-seq in 8 MPN patients. **B.** *JAK2*-WT (blue) and *JAK2*-V617F (red) transcripts detected in monocytes. **C.** UMAP of scRNA-seq data from monocytes colored by transcriptionally defined monocyte subset classifications. **D.** Fraction of CD14+ cells with a *JAK2*-WT or *JAK2*-mutant transcript detected by scRNA-seq that are classical, intermediate, or nonclassical monocytes. The monocyte subset definitions and color scheme are the

same as in **C**. **E.** Differential expression analysis of monocytes from all 8 MPN patients, comparing cells with at least one mutant transcript detected to cells with a WT transcript detected. Ribosomal genes, antigen presentation genes, and proteasomal genes are colored in blue, green, and red, respectively. **F-G.** Flow cytometry and SLAMF7 staining of CD14+ cells from three MPN patients and three healthy controls. Gating scheme to identify SLAMF7 is shown in **F**, and the proportion of CD14+ cells expressing SLAMF7 is shown in **G**.