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# Interferon-alpha2 treatment of patients with polycythemia vera and related neoplasms favorably impacts deregulation of oxidative stress genes and antioxidative defense mechanisms
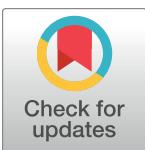
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## Abstract

Chronic inflammation is considered a major driving force for clonal expansion and evolution in the Philadelphia-negative myeloproliferative neoplasms, which include essential thrombocythemia, polycythemia vera and primary myelofibrosis (MPNs). One of the key mutation drivers is the *JAK2V617F* mutation, which has been shown to induce the generation of reactive oxygen species (ROS). Using whole blood gene expression profiling, deregulation of several oxidative stress and anti-oxidative defense genes has been identified in MPNs, including significant downregulation of *TP53*, the *NFE2L2* or *NRF2* genes. These genes have a major role for maintaining genomic stability, regulation of the oxidative stress response and in modulating migration or retention of hematopoietic stem cells. Therefore, their deregulation might give rise to increasing genomic instability, increased chronic inflammation and disease progression with egress of hematopoietic stem cells from the bone marrow to seed in the spleen, liver and elsewhere. Interferon-alpha2 (rIFNα) is increasingly being recognized as the drug of choice for the treatment of patients with MPNs. Herein, we report the first gene expression profiling study on the impact of rIFNα upon oxidative stress and antioxidative defense genes in patients with MPNs (n = 33), showing that rIFNα downregulates several upregulated oxidative stress genes and upregulates downregulated antioxidative defense genes. Treatment with rIFNα induced upregulation of 19 genes in ET and 29 genes in PV including *CXCR4* and *TP53*. In conclusion, this rIFNα- mediated dampening of genotoxic damage to hematopoietic cells may ultimately diminish the risk of additional mutations and accordingly clonal evolution and disease progression towards myelofibrotic and leukemic transformation.

## Introduction

The Philadelphia-negative myeloproliferative neoplasms (MPNs) include essential thrombo-cythemia (ET), polycythemia vera (PV) and primary myelofibrosis (PMF), which in the large majority are acquired hematopoietic stem cell diseases, arising and driven by somatic stem cell mutations (*JAK2V617F*, *CALR*, *MPL*). Additional mutations are key determinants for clonal expansion and evolution [1, 2]. Chronic inflammation is today considered a major driving force for clonal expansion and evolution in the biological continuum from the early cancer stages (ET, PV) towards the advanced myelofibrosis stage with bone marrow failure, huge splenomegaly and ultimately leukemic transformation [3–27]. The *JAK2V617F* mutation has been shown to generate reactive oxygen species (ROS) [28]. Both the *JAK2V617F* mutation and ROS associate with an increased risk of thrombosis [29–33], extracellular neutrophil trap formation (NETosis) [32] ischemic heart disease [34–39], and cancer as well [34, 40, 41]. Most recently, the *CALR* mutation has also been shown to be involved in the development of a chronic inflammatory state, cells harboring the *CALR* mutation exhibiting cell-autonomous activation of the IL-6 pathway [42].

By whole blood transcriptional profiling, we have previously identified a massive deregula-tion of several oxidative stress and antioxidative defense genes in patients with MPNs [43]. Amongst the genes significantly downregulated are *TP53* and the *NFE2L2* or *NRF2* gene, the latter having a key role in the regulation of the oxidative stress response and in modulating both migration and retention of hematopoietic stem cells (HSCs). During MPN-disease pro-gression, the HSC pool is steadily expanding with the egress of CD34+cell from stem cell niches into the circulation. In addition to *NRF2*, several other genes are involved in this pro-cess, including *CXCR4,* which is also significantly downregulated in MPNs [43].

Interferon-alpha2 (rIFNα) is increasingly been recognized as a very efficacious and promis-ing treatment modality in the treatment of MPNs [44–60]. Taken into account that chronic inflammation with ROS accumulation has a major role in the pathogenesis of MPNs [3–28], ultimately implying the induction of an altered redox balance of pivotal significance for stem cell mobilization [31, 43], we herein report the first study containing novel information on the impact of rIFNα upon oxidative stress and antioxidative defense genes, showing that rIFNα downregulates several upregulated oxidative stress genes and upregulates downregulated anti-oxidative defense genes.

## Materials and methods

Nineteen patients with ET, 41 patients with PV, and 9 patients with PMF (data set 1), and 8 patients with ET, 21 patients with PV, and 4 patients with PMF (data set 2) as well as 21 control subjects participated in the study. Patients from data set 2 received monotherapy with rIFNα, in the large majority in a dosage ranging from 45 μg x 1 sc/week to 90 μg x 1 sc/week. When patients initiated IFN-monotherapy, hydroxyurea (n = 18) or anagrelide (n = 6) were discon-tinued. Patient characteristics are presented in Tables 1 and 2, which have been published pre-viously [43]. The study was approved by the regional ethics committee and was performed in accordance with the declaration of Helsinki. Informed written consent was obtained from all subjects before participation.

In data set 1 and 2, gene expression profiles from patients with ET, PV or PMF were com-pared with 21 control subjects. In data set 2, gene expression profiling of patients with ET, PV and PMF was performed at baseline and after 3 months of treatment with rIFNα. The effect of rIFNα on transcriptional changes in patients from data set 2 was tested using gene expression profiling of 38.500 genes. Whole blood was collected in Paxgene tubes (Preanalytix, Hom-brechtikon, Switzerland), stored at room temperature for 24 h, then at -20˚C for minimum

**Table 1. Patient characteristics.** Data set 1.

| | No | Gender | Age | Disease duration (months) | JAK2 V617F | JAK2 V617F | Therapy | Thrombosis (+/-) |
|---|---|---|---|---|---|---|---|---|
| | | (m/f) | (years) | | (+/-) | (%) | | |
| ET | 19 | 9/10 | 60 (35–87) | 40 (15–278) | 9/10 | 23 (1–55) | HU = 10 | 9/10 |
| | | | | | | | rIFNα = 3 | |
| | | | | | | | ANA = 5 | |
| | | | | | | | BU = 1 | |
| PV | 41 | 21/20 | 69 (35–85) | 39 (2–171) | 40/1 | 37 (28–48) | HU = 26 | 19/22 |
| | | | | | | | rIFNα = 5 | |
| | | | | | | | ANA = 1 | |
| | | | | | | | BU = 6 | |
| | | | | | | | None = 3 | |
| PMF | 9 | 3/6 | 68 (53–74) | 31 (11–204) | 2/7 | 59 | HU = 1 | 1/8 |
| | | | | | | | None = 8 | |

Age: Median and range; Disease duration: Median and range; *JAK2V617F* %: median and 95% confidence interval; HU = hydroxyurea; rIFNα = interferon-alpha2; ANA = anagrelide; BU = busulfan.

one day, and finally transferred to a -80˚C freezer. The Paxgene Blood RNA kit (Qiagen, Franklin Lakes, NJ, USA) was used to extract total RNA from each sample. The quantity and quality of RNA were tested with NanoDrop spectrophotometer ND-8000 (NanoDrop Technologies) and Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA), respectively. Three hundred ng of purified total RNA were converted to biotin-labeled aRNA using the Message-AmpTM III RNA amplification kit (Ambion, Austin, TX). Amplified RNA was hybridized to Affymetrix HG-U133 2.0 Plus microarrays and scanned with the Affymetrix GeneChip Scanner 3000-7G (Affymetrix, Santa Clara, CA).

The R statistical software [61] and the "robust multi-array average method" (RMA) [62] from the Bioconductor package "affy" were applied to perform background correction, normalization, and gene expression index calculation of probe intensities. Only perfect match probes were used for data analysis. Differences in gene expression between patients and controls (data set 1 and 2) as well as in patients before and after 3 months of treatment with rIFNα (data set 2) were calculated using the regularized t-test from the Bioconductor package "limma" for unpaired and paired data, respectively. Data set 1 has been performed and analyzed as a hypothesis generating study, therefore we applied an FDR value<0.05 to control for

**Table 2. Patient characteristics at baseline.** Data set 2.

| | No | Gender | Age | JAK2 V617F | JAK2 V617F | Therapy at baseline |
|---|---|---|---|---|---|---|
| | | (m/f) | (years) | (+/-) | (%) | |
| ET | 8 | 4/4 | 57 (45–66) | 6/2 | 14 (1–48) | HU = 5 |
| | | | | | | None = 3 |
| PV | 21 | 10/11 | 62 (26–69) | 21/0 | 20 (10–79) | HU = 14 |
| | | | | | | None = 5 |
| | | | | | | ANA = 2 |
| PMF | 4 | 2/2 | 68 (55–73) | 4/0 | 30 (6–92) | None = 4 |

Age: Median and range; *JAK2V617F* %: median and 95% confidence interval; HU = hydroxyurea; ANA = anagrelide.

multiple hypothesis testing. Data set 2 was performed later and hypotheses generated in data set 1 were tested in data set 2. Thus, a p value <0.05 was applied as a cut off for significance in data set 2. Data are available from Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo; accession no. GSE57793).

## Results

Single gene analysis of 148 genes found to be included in previous studies focusing on deregulation of oxidative stress and antioxidative defense genes in various diseases were chosen for further analysis. Since our previous study of gene expression profiling of oxidative stress genes in patients with MPNs compared to control subjects [43], 6 genes have been added to the panel of oxidative stress and antioxidative defense genes, totaling 154 genes. In response to treatment with rIFNα, 19 genes were upregulated in ET including *CXCR4* and *TP53*, and 11 genes were downregulated including *FOXO3*, *PRDX2*, and *PRDX6* (Table 3).

In patients with PV, *ATOX1*, *CXCR4*, *SEPP1*, and *TP53* were among the 29 upregulated genes, and *FOXO3* and *PRDX2* were among the 14 downregulated genes (Table 4).

In response to treatment with rIFNα, two genes were upregulated in PMF which were *CYBB* and *MSRA*, and nine genes were downregulated including *PRDX2*, and *FOXO3* (Table 5) (All P<0.05).

The Top 10 significantly up- or downregulated genes in ET or PV during treatment with rIFNα are shown in Figs 1 and 2, respectively, and the significantly up- or downregulated genes in PMF during rIFNα therapy are shown in Fig 3.

In a previous microarray study (data set 1) [43], we have shown significant downregulation of genes associated with oxidative stress and antioxidative defense including *ATM*, *CYBA*, *NRF2*, *SIRT2*, and *TTN*, and significant upregulation of *AKR1B1*, *CCND1*, *DEFB122*, *GPX8* and *PTGS1* in patients with ET, PV, and PMF compared to controls. These genes were not significantly deregulated during treatment with rIFNα in any of the disease entities. In ET and PV, *CXCR4* and *TP53* were significantly downregulated at baseline and significantly upregulated during treatment with rIFNα. In PV and PMF, *FOXO3* and *GCLC* were significantly upregulated at baseline and significantly downregulated during treatment with rIFNα. In addition, *PRDX2* was significantly upregulated at baseline and significantly downregulated during treatment with rIFNα in all three disease entities. Fold changes and p-values for all 154 oxidative stress and antioxidative defense genes at baseline and during treatment with rIFNα are presented in S1 Table.

## Discussion

In recent years, several studies have substantiated that rIFNα is highly efficacious in the treatment of patients with ET, PV and early hyperproliferative myelofibrosis, implying a normalization of elevated blood cell counts within weeks, a decrease in the *JAK2V617F* allele burden within the first year in most patients [45–60], and in a subset of patients even induction of minimal residual disease as defined by low-burden *JAK2V617F* (< 1%) in concert with normalization of the bone marrow after long-term treatment (> 5 years) [63–67]. The mechanisms of action of rIFNα are pleitropic including upregulation of downregulated HLA-genes [68] and boosting of virtually all immune cells [69–71]. Furthermore, rIFNα has been shown to exhaust and/or deplete malignant stem cells by inducing changes in the cell cycle and apoptosis [50, 51, 57]. Adding these mechanisms, we herein report that rIFNα has a major impact upon deregulated oxidative stress genes and antioxidative defense genes, implying a gene signature that in essence corresponds to decreased oxidative stress and enhancement of antioxidative defense genes. We have previously reported significant deregulation of several oxidative

**Table 3. The significantly most up- or downregulated genes in patients with ET during treatment with rIFNα (data set 2).** In addition, data from the same patients compared to controls (data set 2) and another cohort (data set 1) are presented.

| | Data set 1 | | Data set 2 | | Data set 2 | |
|---|---|---|---|---|---|---|
| | Ctrl vs baseline | | Ctrl vs baseline | | Baseline vs rIFNα | |
| **Upregulated genes** | ET | | ET | | ET | |
| *Symbol* | *FC* | *FDR* | *FC* | *Pvalue* | *FC* | *Pvalue* |
| MSRA | -1,2 | 0,1 | 2,6 | 3,7E-09 | 1,9 | 0,0002 |
| DEFA4 | 1,5 | 0,2 | 1,8 | 0,04 | 1,7 | 0,02 |
| DEFA1 | 1,1 | 0,8 | 1,6 | 0,09 | 1,7 | 0,047 |
| PRDX4 | -1,02 | 0,9 | 1,3 | 0,09 | 1,3 | 0,004 |
| PRPS2 | -1,1 | 0,3 | 1,1 | 0,2 | 1,3 | 0,01 |
| DEFT1P | 1,1 | 0,4 | 1,2 | 0,006 | 1,2 | 0,04 |
| AKR1A1 | -1,1 | 0,2 | 1,3 | 0,005 | 1,2 | 0,02 |
| HSPA1A | -1,8 | 0,002 | 1,2 | 0,02 | 1,2 | 0,02 |
| TP53 | -1,4 | 0,0003 | -1,2 | 0,009 | 1,2 | 0,006 |
| GLRX | -1,0 | 0,8 | 1,1 | 0,3 | 1,2 | 0,01 |
| PRDX1 | -1,2 | 0,02 | 1,02 | 0,9 | 1,2 | 0,04 |
| DEFB106A | 1,1 | 0,2 | 1,1 | 0,4 | 1,2 | 0,04 |
| MPO | 1,3 | 0,005 | 1,2 | 0,009 | 1,2 | 0,02 |
| SELS | -1,4 | 0,0006 | -1,03 | 0,8 | 1,2 | 0,04 |
| PFKP | -1,1 | 2,0E-01 | 1,1 | 0,1 | 1,2 | 0,04 |
| CXCR4 | -2,1 | 3,7E-09 | -1,1 | 0,2 | 1,2 | 0,04 |
| MGST2 | -1,0 | 0,9 | 1,03 | 0,7 | 1,2 | 0,04 |
| PFKM | -1,1 | 1,7E-01 | 1,3 | 5,3E-06 | 1,1 | 0,01 |
| ALDOA | -1,3 | 0,002 | 1,1 | 0,2 | 1,1 | 0,03 |
| **Downregulated genes** | ET | | ET | | ET | |
| *Symbol* | *FC* | *FDR* | *FC* | *Pvalue* | *FC* | *Pvalue* |
| IPCEF1 | -1,1 | 0,2 | 1,03 | 0,9 | -1,9 | 0,001 |
| PRDX2 | 1,6 | 0,01 | 1,01 | 1,0 | -1,6 | 0,008 |
| CAT | -2,4 | 1,3E-08 | -2,0 | 1,6E-06 | -1,5 | 8,8E-05 |
| FOXO3 | 1,2 | 0,2 | 1,5 | 0,02 | -1,5 | 0,0007 |
| CSDE1 | -1,5 | 1,3E-05 | -1,4 | 5,3E-08 | -1,3 | 0,0007 |
| SOD2 | -1,8 | 0,003 | -1,9 | 0,005 | -1,3 | 0,03 |
| NQO2 | -1,2 | 3,0E-01 | -1,5 | 0,046 | -1,2 | 0,003 |
| PRDX6 | 1,3 | 0,03 | 1,3 | 0,03 | -1,2 | 0,02 |
| GSTZ1 | 1,2 | 4,2E-04 | -1,4 | 1,8E-06 | -1,2 | 0,008 |
| SRXN1 | 1,1 | 0,6 | -1,1 | 0,6 | -1,2 | 0,01 |
| PXDN | 1,1 | 0,1 | -1,1 | 0,02 | -1,1 | 0,03 |

Ctrl: control; rIFNα: interferon-alpha2; ET: essential thrombocythemia; FC: fold change; FDR: false discovery rate.

**Table 4. The significantly most up- or downregulated genes in patients with PV during treatment with rIFNα (data set 2).** In addition, data from the same patients compared to controls (data set 2) and another cohort (data set 1) are presented.

| | Data set 1 | | Data set 2 | | Data set 2 | |
|---|---|---|---|---|---|---|
| | Ctrl vs baseline | | Ctrl vs baseline | | Baseline vs rIFNα | |
| **Upregulated genes** | PV | | PV | | PV | |
| *Symbol* | *FC* | *FDR* | *FC* | *Pvalue* | *FC* | *Pvalue* |
| MSRA | 1,1 | 0,3 | 1,5 | 0,0002 | 2,2 | 1,2E-08 |

*(Continued)*

**Table 4.** (Continued)

| | Data set 1 | | Data set 2 | | Data set 2 | |
|---|---|---|---|---|---|---|
| | Ctrl vs baseline | | Ctrl vs baseline | | Baseline vs rIFNα | |
| DEFA4 | 1,6 | 0,06 | 1,3 | 0,4 | 1,5 | 0,04 |
| DEFA1 | 1,2 | 0,5 | 1,01 | 1,0 | 1,5 | 0,04 |
| TP53 | -1,6 | 3,6E-06 | -1,6 | 2,5E-06 | 1,3 | 1,3E-06 |
| PRDX4 | -1,1 | 0,4 | -1,1 | 0,2 | 1,3 | 0,0004 |
| CYBB | -1,6 | 7,3E-06 | -1,4 | 0,0001 | 1,3 | 0,0003 |
| MGST3 | -1,1 | 0,3 | 1,3 | 0,02 | 1,3 | 0,02 |
| PRDX1 | -1,2 | 0,003 | -1,2 | 0,02 | 1,2 | 0,0007 |
| ATOX1 | 1,3 | 5,2E-06 | 1,2 | 7,0E-05 | 1,2 | 2,1E-05 |
| PFKP | -1,2 | 6,8E-03 | -1,1 | 0,06 | 1,2 | 0,003 |
| HSPA1A | -1,2 | 0,2 | 1,1 | 0,2 | 1,2 | 0,001 |
| CXCR4 | -2,0 | 1,1E-11 | -1,1 | 0,3 | 1,2 | 0,007 |
| MPO | 1,2 | 0,006 | -1,2 | 0,002 | 1,2 | 1,7E-05 |
| GLRX2 | -1,0 | 1 | -1,1 | 0,09 | 1,2 | 0,007 |
| PRPS2 | 1,0 | 0,9 | -1,1 | 0,5 | 1,2 | 0,03 |
| AKR1A1 | -1,1 | 0,1 | -1,02 | 0,7 | 1,2 | 0,0002 |
| GSTM4 | -1,2 | 0,01 | -1,3 | 0,003 | 1,2 | 0,003 |
| SEPP1 | 1,3 | 0,0001 | 1,2 | 0,0004 | 1,2 | 0,009 |
| GSR | 1,1 | 0,2 | -1,1 | 0,03 | 1,2 | 0,002 |
| GLRX | 1,2 | 0,1 | -1,1 | 0,2 | 1,2 | 0,004 |
| NCF1 | -1,1 | 0,2 | 1,1 | 0,2 | 1,2 | 0,01 |
| SELS | -1,4 | 5,2E-06 | -1,2 | 0,006 | 1,1 | 0,02 |
| GTF2I | -1,6 | 1,2E-07 | -1,04 | 0,5 | 1,1 | 0,002 |
| NUDT1 | 1,0 | 4,6E-01 | -1,01 | 0,8 | 1,1 | 0,004 |
| DGKK | -1,03 | 0,5 | -1,1 | 0,07 | 1,1 | 0,005 |
| MGST2 | -1 | 0,9 | -1,02 | 0,7 | 1,1 | 0,02 |
| MGST1 | 1,05 | 0,4 | -1,05 | 0,2 | 1,1 | 0,01 |
| PFKM | -1,1 | 2,8E-01 | 1,01 | 0,8 | 1,1 | 0,02 |
| TXNRD2 | -1,1 | 0,03 | -1 | 0,9 | 1,1 | 0,04 |
| **Downregulated genes** | **PV** | | **PV** | | **PV** | |
| *Symbol* | *FC* | *FDR* | *FC* | *Pvalue* | *FC* | *Pvalue* |
| FOXO3 | 1,6 | 6,3E-05 | 1,6 | 6,1E-05 | -1,5 | 2,3E-06 |
| NQO2 | 1,2 | 0,2 | 1,5 | 0,008 | -1,5 | 1,8E-05 |
| CAT | -1,9 | 6,4E-06 | -1,1 | 0,5 | -1,4 | 0,0002 |
| PRDX2 | 1,7 | 0,0004 | 1,4 | 0,006 | -1,4 | 0,0009 |
| GSTZ1 | 1,2 | 4,8E-05 | -1,1 | 0,006 | -1,3 | 4,3E-06 |
| IPCEF1 | -1,05 | 0,5 | 1,4 | 0,1 | -1,3 | 0,01 |
| SOD2 | -1,6 | 0,004 | -1,5 | 0,008 | -1,3 | 6,8E-05 |
| CSDE1 | -1,3 | 4,8E-05 | -1,1 | 0,1 | -1,2 | 0,002 |
| GCLC | 1,4 | 0,008 | 1,4 | 0,008 | -1,2 | 0,002 |
| TALDO1 | -1,1 | 1,5E-01 | 1,2 | 0,02 | -1,2 | 0,004 |
| SRXN1 | 1,2 | 0,05 | 1,1 | 0,1 | -1,2 | 0,0003 |
| TKT | -1,6 | 2,0E-07 | -1,1 | 0,4 | -1,2 | 0,02 |
| PREX1 | -1,4 | 0,001 | -1,1 | 0,1 | -1,1 | 0,03 |
| SQSTM1 | 1,2 | 4,6E-06 | 1,1 | 0,0002 | -1,1 | 0,005 |

Ctrl: control; rIFNα: interferon-alpha2; PV: polycythemia vera; FC: fold change; FDR: false discovery rate.

**Table 5. The significantly most up- or downregulated genes in patients with PMF during treatment with rIFNα (data set 2).** In addition, data from the same patients compared to controls (data set 2) and another cohort (data set 1) are presented.

| | Data set 1 | | Data set 2 | | Data set 2 | |
|---|---|---|---|---|---|---|
| | Ctrl vs baseline | | Ctrl vs baseline | | Baseline vs rIFNα | |
| **Upregulated genes** | **PMF** | | **PMF** | | **PMF** | |
| *Symbol* | *FC* | *FDR* | *FC* | *Pvalue* | *FC* | *Pvalue* |
| MSRA | 1,2 | 0,1 | 1,4 | 0,006 | 1,8 | 4,9E-05 |
| CYBB | -1,2 | 0,2 | -1,2 | 0,2 | 1,2 | 0,04 |
| **Downregulated genes** | **PMF** | | **PMF** | | **PMF** | |
| *Symbol* | *FC* | *FDR* | *FC* | *Pvalue* | *FC* | *Pvalue* |
| FOXO3 | 1,7 | 0,008 | 1,9 | 0,0005 | -1,7 | 0,003 |
| PRDX2 | 4,4 | 9,1E-06 | 3,2 | 2,4E-06 | -1,7 | 0,0008 |
| SOD2 | -2,1 | 0,006 | -1,6 | 0,1 | -1,5 | 0,009 |
| GSTZ1 | 1,4 | 2,9E-05 | -1,2 | 0,02 | -1,3 | 0,01 |
| GCLC | 2,4 | 0,0002 | 2 | 0,0006 | -1,3 | 0,03 |
| CSDE1 | -1,1 | 0,1 | -1,02 | 0,7 | -1,3 | 0,03 |
| SRXN1 | 1,2 | 0,3 | 1,4 | 0,01 | -1,3 | 0,01 |
| PDLIM1 | 1,4 | 0,1 | 1,5 | 0,008 | -1,3 | 0,046 |
| TALDO1 | -1,2 | 0,07 | 1,1 | 0,4 | -1,2 | 0,046 |

Ctrl: control; rIFNα: interferon-alpha2; PMF: primary myelofibrosis; FC: fold change; FDR: false discovery rate.

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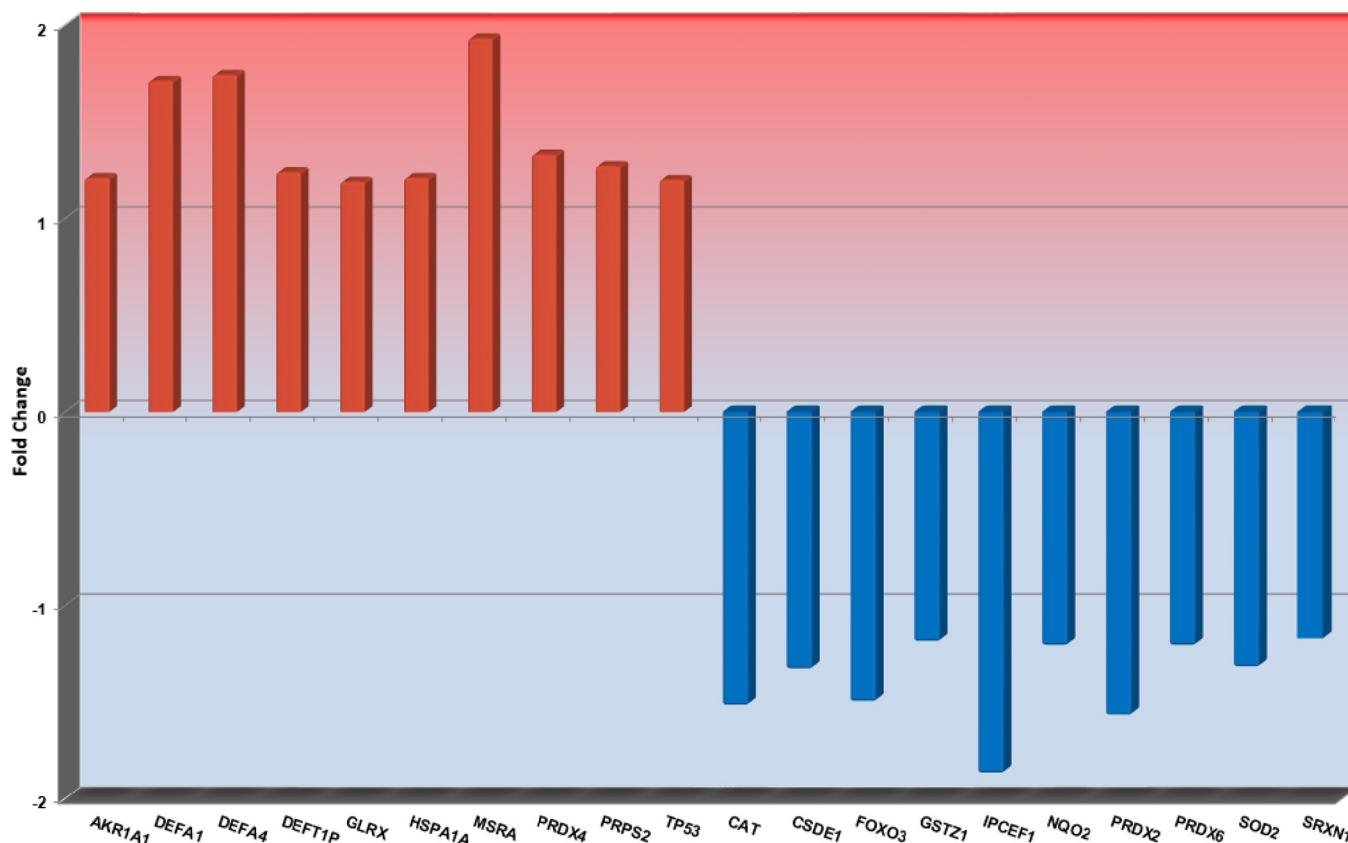


**Fig 1. The Top10 most up- or downregulated genes in patients with ET during rIFNα therapy.** Fold changes for each gene are shown on the y-axis, and gene names are shown on the x-axis.

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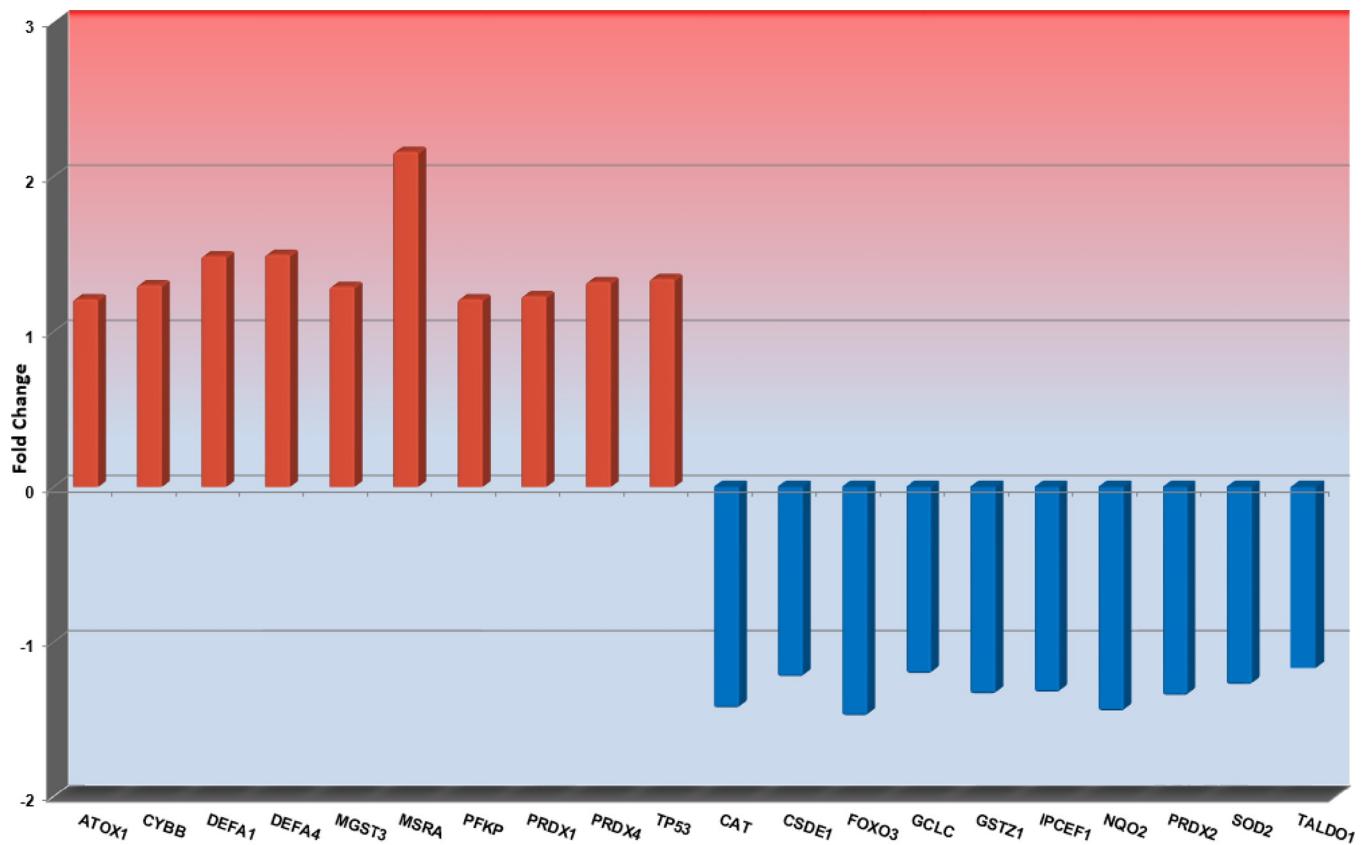
**Fig 2. The Top10 most up- or downregulated genes in patients with PV during rIFNα therapy.** Fold changes for each gene are shown on the y-axis, and gene names are shown on the x-axis.

https://doi.org/10.1371/journal.pone.0270669.g002

stress and antioxidative defense genes in patients with MPNs compared to control subjects [43]. In regard to *TP53*—being significantly downregulated before rIFNα treatment–this gene was significantly upregulated in ET and PV and no longer deregulated in PMF during rIFNα treatment. Downregulation of *TP53* implies genomic instability due to an increased burden of oxidative stress upon the genome, which accordingly is reduced by rIFNα treatment. Although our genome profile of upregulation of *TP53* strongly argues for enhancement of genomic stability during rIFNα-treatment, this has to be confirmed in protein (e.g. proteomics) and functionality studies, confirming that the function of P53 as a tumor suppressor protein is indeed being improved during rIFNα-treatment of patients with MPNs. *NRF2* is a master regulator of the antioxidant response and has a major role for normal stem cell function [72]. Furthermore, *NRF2* also has a protective role in chronic inflammatory diseases by attenuating the inflammatory state, e.g. in rheumatoid arthritis and atherosclerosis [73]. Thus, our findings of a change in the *NRF2* gene expression from being significantly downregulated before rIFNα exposure to not being deregulated during treatment with rIFNα may imply an improvement in the antioxidative defense mechanisms against increased oxidative stress and accumulation of ROS. These effects may not only dampen the chronic inflammatory drive on the malignant clone, but also potentially enhance the efficacy of immune cells, which are known to be malfunctioning in MPNs [74–76] and negatively impacted by ROS [77, 78]. Since ROS has been shown to decrease interferon production [79], the impact of rIFNα in regulating deregulated oxidative stress and antioxidative defense genes may actually also improve the defense against infections.
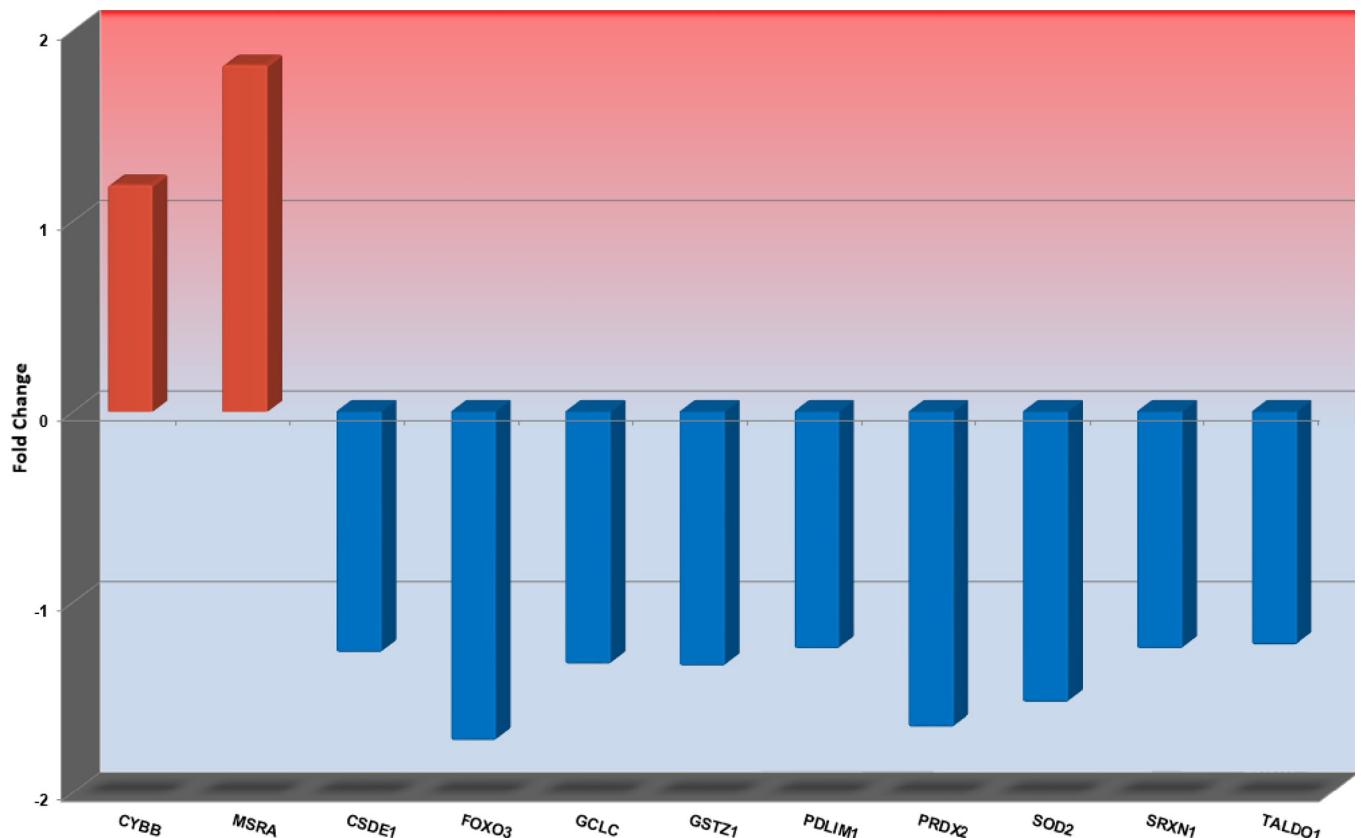
**Fig 3. The Top10 most up- or downregulated genes in patients with PMF during rIFNα therapy.** Fold changes for each gene are shown on the y-axis, and gene names are shown on the x-axis.

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This is not trivial, since recent studies have documented MPN patients to have an increased morbidity and mortality due to infections [80, 81], which accordingly might not only be explained by impaired functionality of immune cells (e.g. T-cell exhaustion) [77] but also by defective interferon production, mediated by excessive ROS accumulation.

The *CXCR4* gene was significantly downregulated at baseline in ET and PV patients and became significantly upregulated during treatment with rIFNα. *CXCR4* has an important role for homing and retention of hematopoietic stem cells (HSC) [82] and for maintaining HSC quiescence as well [83]. We and others have previously reported *CXCR4* to be significantly downregulated in MPNs, in particular in myelofibrosis [43], consistent with previous studies displaying downregulation of *CXCR4* in CD34+ cells [84]. Accordingly, upregulation of the *CXCR4* gene during rIFNα treatment might prohibit egress of CD34 + positive stem cells from bone marrow niches and accordingly development of extramedullary hematopoiesis in the spleen and liver. Indeed, by significantly upregulating Nrf2 and CXCR4, rIFNα may improve the balance between stem cell quiescence and proliferation, self-renewal and differentiation, and also significantly influencing homing and retention of HSCs in the bone marrow niche. Since excessive ROS accumulation and oxidative stress may also contribute to aberrant DNA methylation [85], which has been reported in patients with MPNs [86], including hypermethylation of the CXCR4 promoter in CD34+ cells in PMF patients, it is intriguing to consider, if rIFNα might actually normalize aberrant DNA methylation in MPNs–a research topic to be pursued in the future.

*PRDX2* (Peroxiredoxin 2) is an antioxidant enzyme that is involved in the scavenging of $H_2O_2$ and ROS, thereby protecting cells from oxidative stress [87]. The *PRDX2* gene has been found to be upregulated in several cancers, and most recently, *PRDX2* has been included in a 6-gene anti-oxidant signature that effectively predicts the prognosis of patients with kidney cancer [88]. Highly interesting, in our patients the *PRDX2* gene was significantly upregulated at baseline and treatment with rIFNα was followed by a significant downregulation of *PRDX2* in all MPNs.

In conclusion, our findings of a major impact of rIFNα upon several oxidative stress genes and antioxidative defense genes may imply an rIFNα mediated dampening of genotoxic damage to hematopoietic cells and stromal cells as well, thereby ultimately diminishing the risk of additional mutations that might drive the malignant clone towards myelofibrotic and leukemic transformation.

Having for the first time demonstrated rIFNα as a potent ROS modifying agent, our study has opened the avenue for further transcriptional studies to explore in depth the impact of stem-cell targeting therapy with rIFNα upon the epigenome, genome and proteome landscapes in MPNs. These studies should also include patients treated with hydroxyurea and combination therapies with anti-inflammatory agents, such as statins and JAK1-2 inhibitors. Such efforts may unravel novel important insights into the impact of such therapies on aberrant ROS-associated pathways to be targeted in the future [89, 90] and accordingly novel insights into the synergistic crosstalk between inflammation, oxidative stress, and genomic alterations [23].

## Supporting information

**S1 Table. The significantly most up- or downregulated genes in patients with ET, PV or PMF during treatment with rIFNα (data set 2).** In addition, data from the same patients compared to controls (data set 2) and another cohort (data set 1) are presented. Ctrl: control; rIFNα: interferon-alpha2; ET: essential thrombocythemia; PV: polycythemia vera; PMF: primary myelofibrosis; FC: fold change; FDR: false discovery rate.
(XLSX)

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## References

1. Campbell PJ, Green AR. Mechanisms of disease: the myeloproliferative disorders. N Engl J Med 2006; 355(23): 2452–66.

2. Spivak JL. Myeloproliferative Neoplasms. N Engl J Med. 2017; 376(22):2168–2181. https://doi.org/10.1056/NEJMra1406186 PMID: 28564565

3. Hermouet S, Vilaine M. The JAK2 46/1 haplotype: a marker of inappropriate myelomonocytic response to cytokine stimulation, leading to increased risk of inflammation, myeloid neoplasm, and impaired defense against infection? Haematologica 2011; 96(11): 1575–9. https://doi.org/10.3324/haematol.2011.055392 PMID: 22058280

4. Barbui T, Carobbio A, Finazzi G, Vannucchi AM, Barosi G, Antonioli E, et al. Inflammation and thrombosis in essential thrombocythemia and polycythemia vera: different role of C-reactive protein and pentraxin3. Haematologica 2011; 96(2): 315–8. https://doi.org/10.3324/haematol.2010.031070 PMID: 21173097

5. Hasselbalch HC. Perspectives on chronic inflammation in essential thrombocythemia, polycythemia vera, and myelofibrosis: is chronic inflammation a trigger and driver of clonal evolution and development of accelerated atherosclerosis and second cancer? Blood 2012; 119(14): 3219–25. https://doi.org/10.1182/blood-2011-11-394775 PMID: 22318201

6. Hasselbalch HC. Chronic Inflammation as a Promoter of Mutagenesis in Essential Thrombocythemia, Polycythemia Vera and Myelofibrosis. A Human Inflammation Model for Cancer Development? Leuk Res 2013; 37(2): 214–20. https://doi.org/10.1016/j.leukres.2012.10.020 PMID: 23174192

7. Hasselbalch HC. The role of cytokines in the initiation and progression of myelofibrosis. Cytokine Growth Factor Rev 2013; 24(2): 133–45. https://doi.org/10.1016/j.cytogfr.2013.01.004 PMID: 23415024

8. Hermouet S, Hasselbalch HC, Čokić V. Mediators of Inflammation in Myeloproliferative Neoplasms: State of the Art. Mediators Inflamm. 2015; 2015:964613. https://doi.org/10.1155/2015/964613 PMID: 26681841

9. Hermouet S, Bigot-Corbel E, Gardie B. Pathogenesis of Myeloproliferative Neoplasms: Role and Mechanisms of Chronic Inflammation. Mediators Inflamm 2015:145293. https://doi.org/10.1155/2015/145293 PMID: 26538820

10. Fleischman AG. Inflammation as a Driver of Clonal Evolution in Myeloproliferative Neoplasm. Mediators Inflamm. 2015; 2015:606819. https://doi.org/10.1155/2015/606819 PMID: 26538830

11. Geyer HL, Dueck AC, Scherber RM, Mesa RA. Impact of Inflammation on Myeloproliferative Neoplasm Symptom Development. Mediators Inflamm. 2015; 2015:284706. https://doi.org/10.1155/2015/284706 PMID: 26538823

12. Hermouet S. Pathogenesis of myeloproliferative neoplasms: More than mutations. Exp Hematol. 2015 Dec; 43(12):993–4. https://doi.org/10.1016/j.exphem.2015.08.014 PMID: 26453965

13. Koschmieder S, Mughal TI, Hasselbalch HC, Barosi G, Valent P, Kiladjian J-J, et al. Myeloproliferative neoplasms and inflammation.: whether to target the malignant clone or the inflammatory process or both. Leukemia 2016; 30:1018–1024. https://doi.org/10.1038/leu.2016.12 PMID: 26854026

14. Lussana F, Rambaldi A. Inflammation and myeloproliferative neoplasms. J Autoimmun. 2017 Dec; 85:58–63. https://doi.org/10.1016/j.jaut.2017.06.010 PMID: 28669446

15. Andersen M, Sajid Z, Pedersen RK, Gudmand-Hoeyer J, Ellervik C, Skov V, et al. Mathematical modelling as a proof of concept for MPNs as a human inflammation model for cancer development. PLoS One. 2017 Aug; 12(8):e0183620. https://doi.org/10.1371/journal.pone.0183620 PMID: 28859112; PMCID: PMC5578482.

16. Lussana F, Carobbio A, Salmoiraghi S, Guglielmelli P, Vannucchi AM, Bottazzi B, et al. Driver mutations (JAK2V617F, MPLW515L/K or CALR), pentraxin-3 and C-reactive protein in essential thrombocythemia and polycythemia vera. J Hematol Oncol. 2017 Feb 22; 10(1):54. https://doi.org/10.1186/s13045-017-0425-z PMID: 28228104; PMCID: PMC5322581.

17. Barosi G, Massa M, Campanelli R, Fois G, Catarsi P, Viarengo G, et al. Primary Myelofibrosis: Older Age and High JAK2V617F Allele Burden are Associated With Elevated Plasma High-Sensitivity C-reactive Protein Levels and a Phenotype of Progressive Disease. Leuk Res 2017; 60:18–23. https://doi.org/10.1016/j.leukres.2017.06.004 PMID: 28622624

18. Craver BM, El Alaoui K, Scherber RM, Fleischman AG. The Critical Role of Inflammation in the Pathogenesis and Progression of Myeloid Malignancies. Cancers (Basel). 2018 Apr 3; 10(4):104. https://doi.org/10.3390/cancers10040104 PMID: 29614027; PMCID: PMC5923359.

19. Mendez Luque LF, Blackmon AL, Ramanathan G, Fleischman AG. Key Role of Inflammation in Myeloproliferative Neoplasms: Instigator of Disease Initiation, Progression. and Symptoms. Curr Hematol Malig Rep. 2019 Jun; 14(3):145–153. https://doi.org/10.1007/s11899-019-00508-w PMID: 31119475; PMCID: PMC7746200.

20. Chatain N, Koschmieder S, Jost E. Role of Inflammatory Factors during Disease Pathogenesis and Stem Cell Transplantation in Myeloproliferative Neoplasms. Cancers (Basel). 2020 Aug 12; 12(8):2250. https://doi.org/10.3390/cancers12082250 PMID: 32806517; PMCID: PMC7463735.

21. Ramanathan G, Hoover BM, Fleischman AG. Impact of Host, Lifestyle and Environmental Factors in the Pathogenesis of MPN. Cancers (Basel). 2020 Jul 24; 12(8):2038. https://doi.org/10.3390/cancers12082038 PMID: 32722135; PMCID: PMC7463688.

22. Longhitano L, Li Volti G, Giallongo C, Spampinato M, Barbagallo I, Di Rosa M, et al. The Role of Inflammation and Inflammasome in Myeloproliferative Disease. J Clin Med. 2020 Jul 22; 9(8):2334. https://doi.org/10.3390/jcm9082334 PMID: 32707883

23. Allegra A, Pioggia G, Tonacci A, Casciaro M, Musolino C, Gangemi S. Synergic Crosstalk between Inflammation, Oxidative Stress, and Genomic Alterations in BCR-ABL-Negative Myeloproliferative Neoplasm. Antioxidants (Basel). 2020 Oct 23; 9(11):1037. https://doi.org/10.3390/antiox9111037 PMID: 33114087

24. Koschmieder S, Chatain N. Role of inflammation in the biology of myeloproliferative neoplasms. Blood Rev. 2020 Jul; 42:100711. https://doi.org/10.1016/j.blre.2020.100711 PMID: 32505517

25. Ramanathan G, Fleischman AG. The Microenvironment in Myeloproliferative Neoplasms. Hematol Oncol Clin North Am. 2021 Apr; 35(2):205–216. https://doi.org/10.1016/j.hoc.2020.11.003 PMID: 33641864

26. Di Battista V, Bochicchio MT, Giordano G, Napolitano M, Lucchesi A. Genetics and Pathogenetic Role of Inflammasomes in Philadelphia Negative Chronic Myeloproliferative Neoplasms: A Narrative Review. Int J Mol Sci. 2021 Jan 8; 22(2):561. https://doi.org/10.3390/ijms22020561 PMID: 33429941

27. Fisher DAC, Fowles JS, Zhou A, Oh ST. Inflammatory Pathophysiology as a Contributor to Myeloproliferative Neoplasms. Front Immunol. 2021 Jun 1; 12:683401. https://doi.org/10.3389/fimmu.2021.683401 PMID: 34140953

28. Marty C, Lacout C, Droin N, Le Couédic JP, Ribrag V, Solary E, et al. A role for reactive oxygen species in JAK2 V617F myeloproliferative neoplasm progression. Leukemia. 2013 Nov; 27(11):2187–95. https://doi.org/10.1038/leu.2013.102 PMID: 23558526

29. Hasselbalch HC, Elvers M, Schafer AI. The pathobiology of thrombosis, microvascular disease, and hemorrhage in the myeloproliferative neoplasms. Blood. 2021 Apr 22; 137(16):2152–2160. https://doi.org/10.1182/blood.2020008109 PMID: 33649757

30. Moliterno AR, Ginzburg YZ, Hoffman R. Clinical insights into the origins of thrombosis in myeloproliferative neoplasms. Blood. 2021 Mar 4; 137(9):1145–1153. https://doi.org/10.1182/blood.2020008043 PMID: 33237986

31. Bjørn ME, Hasselbalch HC. The Role of Reactive Oxygen Species in Myelofibrosis and Related Neoplasms. Mediators Inflamm. 2015; 2015:648090. https://doi.org/10.1155/2015/648090 Epub 2015 Oct 11. PMID: 26538833

32. Wolach O, Sellar RS, Martinod K, Cherpokova D, McConkey M, Chappell RJ, et al. Increased neutrophil extracellular trap formation promotes thrombosis in myeloproliferative neoplasms. Sci Transl Med 2018;10. https://doi.org/10.1126/scitranslmed.aan8292 PMID: 29643232

33. Sørensen AL, Hasselbalch HC, Bjørn ME, et al. Elevated levels of oxidized nucleosides in individuals with the JAK2V617F mutation from a general population study. Redox Biol. 2021; 41:101895. https://doi.org/10.1016/j.redox.2021.101895 PMID: 33621788

34. Nielsen C, Birgens HS, Nordestgaard BG, Kjaer L, Bojesen SE. The JAK2 V617F somatic mutation, mortality and cancer risk in the general population. Haematologica. 2011; 96(3):450–3. https://doi.org/10.3324/haematol.2010.033191 PMID: 21160067

35. Dubois-Deruy E, Peugnet V, Turkieh A, Pinet F. Oxidative Stress in Cardiovascular Diseases. Antioxidants (Basel). 2020 Sep 14; 9(9):864. https://doi.org/10.3390/antiox9090864 PMID: 32937950

36. Scioli MG, Storti G, D'Amico F, Rodríguez Guzmán R, Centofanti F, Doldo E, et al. Oxidative Stress and New Pathogenetic Mechanisms in Endothelial Dysfunction: Potential Diagnostic Biomarkers and Therapeutic Targets. J Clin Med. 2020 Jun 25; 9(6):1995. https://doi.org/10.3390/jcm9061995 PMID: 32630452

37. Climent M, Viggiani G, Chen YW, Coulis G, Castaldi A. MicroRNA and ROS Crosstalk in Cardiac and Pulmonary Diseases. Int J Mol Sci. 2020 Jun 19; 21(12):4370. https://doi.org/10.3390/ijms21124370 PMID: 32575472

38. Xiao X, Yang C, Qu SL, Shao YD, Zhou CY, Chao R, et al. S100 proteins in atherosclerosis. Clin Chim Acta. 2020 Mar; 502:293–304. https://doi.org/10.1016/j.cca.2019.11.019 PMID: 31794767

39. Sabbatino F, Conti V, Liguori L, Polcaro G, Corbi G, Manzo V, et al. Molecules and Mechanisms to Overcome Oxidative Stress Inducing Cardiovascular Disease in Cancer Patients. Life (Basel). 2021 Jan 30; 11(2):105. https://doi.org/10.3390/life11020105 PMID: 33573162

40. Hayes JD, Dinkova-Kostova AT, Tew KD. Oxidative Stress in Cancer. Cancer Cell. 2020; 38(2):167–197. https://doi.org/10.1016/j.ccell.2020.06.001 PMID: 32649885

41. Nakamura H, Takada K. Reactive oxygen species in cancer: Current findings and future directions. Cancer Sci. 2021; 112(10):3945–395. https://doi.org/10.1111/cas.15068 PMID: 34286881

42. Balliu M, Calabresi L, Bartalucci N, Romagnoli S, Maggi L, Manfredini R, et al. Activated IL-6 signaling contributes to the pathogenesis of, and is a novel therapeutic target for, CALR-mutated MPNs. Blood Adv. 2021; 5(8):2184–2195. https://doi.org/10.1182/bloodadvances.2020003291 PMID: 33890979

43. Hasselbalch HC, Thomassen M, Riley CH, Kjær L, Larsen TS, Jensen MK, et al. Whole blood transcriptional profiling reveals deregulation of oxidative and antioxidative defence genes in myelofibrosis and related neoplasms. Potential implications of downregulation of Nrf2 for genomic instability and disease progression. PLoS One 2014, 9, 1–9. https://doi.org/10.1371/journal.pone.0112786 PMID: 25397683

44. Silver RT. Long-term effects of the treatment of polycythemia vera with recombinant interferon-alpha. Cancer 2006; 107:451–458. https://doi.org/10.1002/cncr.22026 PMID: 16804923

45. Kiladjian JJ, Cassinat B, Turlure P, Cambier N, Roussel M, Bellucci S, et al. High molecular response rate of polycythemia vera patients treated with pegylated interferon alpha-2a. Blood 2006; 108:2037–2040. https://doi.org/10.1182/blood-2006-03-009860 PMID: 16709929

46. Kiladjian JJ, Cassinat B, Chevret S, Turlure P, Cambier N, Roussel M et al. Pegylated interferon-alpha-2a induces complete haematological and molecular responses with low toxicity in polycythemia vera. Blood 2008; 112(8):3065–3072. https://doi.org/10.1182/blood-2008-03-143537 PMID: 18650451

47. Kiladjian JJ, Chomienne C, Fenaux P. Interferon-alpha therapy in bcr-abl-negative myeloproliferative neoplasms. Leukemia. 2008; 22(11):1990–8. https://doi.org/10.1038/leu.2008.280 PMID: 18843285

48. Kiladjian JJ, Mesa RA, Hoffman R. The renaissance of interferon therapy for the treatment of myeloid malignancies. Blood. 2011; 117(18):4706–15. https://doi.org/10.1182/blood-2010-08-258772 PMID: 21389325

49. Silver RT, Kiladjian JJ, Hasselbalch HC. Interferon and the treatment of polycythemia vera, essential thrombocythemia and myelofibrosis. Expert Rev Hematol. 2013; 6(1):49–58. https://doi.org/10.1586/ehm.12.69 PMID: 23373780

50. Hasan S, Lacout C, Marty C, Cuingnet M, Solary E, Vainchenker W, et al. JAK2V617F expression in mice amplifies early hematopoietic cells and gives them a competitive advantage that is hampered by IFNα. Blood. 2013; 122(8):1464–1477. https://doi.org/10.1182/blood-2013-04-498956 PMID: 23863895

51. Lane SW, Mullaly A. Jak2V617F myeloproliferative neoplasm stem cells and interferon-alpha. Oncotarget. 2013; 4:500–501. https://doi.org/10.18632/oncotarget.986 PMID: 23660238

52. Kiladjian JJ, Giraudier S, Cassinat B. Interferon-alpha for the therapy of myeloproliferative neoplasms: targeting the malignant clone. Leukemia. 2016 Apr; 30(4):776–81. https://doi.org/10.1038/leu.2015.326 PMID: 26601783

53. Bjørn ME, Hasselbalch HC. Minimal residual disease or cure in MPNs? Rationales and perspectives on combination therapy with interferon-alpha2 and ruxolitinib. Expert Rev Hematol. 2017 May; 10(5):393–40. https://doi.org/10.1080/17474086.2017.1284583 PMID: 28402197

54. Hasselbalch HC, Holmström MO. Perspectives on interferon-alpha in the treatment of polycythemia vera and related myeloproliferative neoplasms: minimal residual disease and cure? Semin Immunopathol 2019 Jan; 41(1):5–19. https://doi.org/10.1007/s00281-018-0700-2 PMID: 30203226

55. Kiladjian JJ, Barbui T. From leeches to interferon: should cytoreduction be prescribed for all patients with polycythemia vera? Leukemia. 2020 Nov; 34(11):2837–2839. https://doi.org/10.1038/s41375-020-0984-9 PMID: 32678292

56. How J, Hobbs G. Use of Interferon Alpha in the Treatment of Myeloproliferative Neoplasms: Perspectives and Review of the Literature. Cancers 2020; 12, 1954.

57. Austin RJ, Straube J, Bruedigam C, Pali G, Jacquelin S, Vu T, et al. Distinct effects of ruxolitinib and interferon-alpha on murine JAK2V617F myeloproliferative neoplasm hematopoietic stem cell populations. Leukemia. 2020; 34(4):1075–1089. https://doi.org/10.1038/s41375-019-0638-y PMID: 31732720

58. Abu-Zeinah G, Krichevsky S, Cruz T, Hoberman G, Jaber D, Savage N, et al. Interferon-alpha for treating polycythemia vera yields improved myelofibrosis-free and overall survival. Leukemia. 2021 Mar 2. https://doi.org/10.1038/s41375-021-01183-8 PMID: 33654206

59. Barbui T, Vannucchi AM, De Stefano V, Masciulli A, Carobbio A, Ferrari A. Ropeginterferon alfa-2b versus phlebotomy in low-risk patients with polycythaemia vera (Low-PV study): a multicentre, randomised phase 2 trial. Lancet Haematol. 2021; 8(3):e175–e184. Erratum in: Lancet Haematol. 2021 Mar;8(3): e170. https://doi.org/10.1016/S2352-3026(20)30373-2 PMID: 33476571

60. Hasselbalch HC, Silver RT. New perspectives of interferon-alpha2 and inflammation in treating Philadelphia-negative chronic myeloproliferative neoplasms. HemaSphere 2021 Nov 18; 5(12):e645. https://doi.org/10.1097/HS9.0000000000000645 PMID: 34805764

61. Ihaka R, Gentleman R. R: A language for data analysis and graphics. J. Comput. Graph. Stat. 1996, 5, 299–314.

62. Irizarry RA, Hobbs B, Beazer-barclay YD, Antonellis KJ, Scherf UWE, Speed TP. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics 2003, 4, 249–264. https://doi.org/10.1093/biostatistics/4.2.249 PMID: 12925520

63. Larsen TS, Pallisgaard N, Moller MB, Andersen MT, Møller MB, Hasselbalch HC. Sustained major molecular response on interferon alpha-2b in two patients with polycythemia vera. Ann Hematol. 2008; 87:847–850. https://doi.org/10.1007/s00277-008-0498-4 PMID: 18481066

64. Larsen TS, Møller MB, de Stricker K, Nørgaard P, Samuelsson J, Marcher C, et al. Minimal residual disease and normalization of the bone marrow after long-term treatment with alpha interferon2b in polycythemia vera. A report on molecular response patterns in seven patients in sustained complete hematological remission. Hematology. 2009; 14:331–334. https://doi.org/10.1179/102453309X12473408860587 PMID: 19941739

65. Quintás-Cardama A, Kantarjian H, Manshouri T, Luthra R, Estrov Z, Pierce S, et al. Pegylated interferon alpha-2a yields high rates of hematologic and molecular response in patients with advanced essential thrombocythemia and polycythemia vera. J Clin Oncol. 2009; 27:5418–5424. https://doi.org/10.1200/JCO.2009.23.6075 PMID: 19826111

66. Larsen TS, Iversen KF, Hansen E, Mathiasen AB, Marcher C, Frederiksen M, et al. Long term molecular responses in a cohort of Danish patients with essential thrombocythemia, polycythemia vera and myelofibrosis treated with recombinant interferon alpha. Leuk Res. 2013; 37:1041–5. https://doi.org/10.1016/j.leukres.2013.06.012 PMID: 23827351

67. Utke Rank C, Weis Bjerrum O, Larsen TS, Kjær K, de Stricker K, Riley CH, et al. Minimal residual disease after long-term interferon-alpha2 treatment: a report on hematological, molecular and histomorphological response patterns in 10 patients with essential thrombocythemia and polycythemia vera. Leuk Lymphoma. 2016; 57:348–354. https://doi.org/10.3109/10428194.2015.1049171 PMID: 25956046

68. Skov V, Riley CH, Thomassen M, Kjær L, Stauffer Larsen T, Bjerrum OW, et al. The impact of interferon-alpha2 on HLA genes in patients with polycythemia vera and related neoplasms. Leuk Lymphoma. 2017 Aug; 58(8):1914–1921. https://doi.org/10.1080/10428194.2016.1262032 PMID: 27911124

69. Riley CH, Jensen MK, Brimnes MK, Hasselbalch HC, Bjerrum OW, Straten PT, et al. Increase in circulating CD4⁺CD25⁺Foxp3⁺ T cells in patients with Philadelphia-negative chronic myeloproliferative neoplasms during treatment with IFN-α. Blood. 2011; 118:2170–3. https://doi.org/10.1182/blood-2011-03-340992 PMID: 21708889

70. Riley CH, Hansen M, Brimnes MK, Hasselbalch HC, Bjerrum OW, Straten PT, et al. Expansion of circulating CD56bright natural killer cells in patients with JAK2-positive chronic myeloproliferative neoplasms during treatment with interferon-α. Eur J Haematol. 2015; 94:227–234. https://doi.org/10.1111/ejh.12420 PMID: 25082025

71. Riley CH, Brimnes MK, Hansen M, Jensen MK, Hasselbalch HC, Kjær L, et al. Interferon-α induces marked alterations in circulating regulatory T cells, NK cell subsets, and dendritic cells in patients with JAK2V617F-positive essential thrombocythemia and polycythemia vera. Eur J Haematol. 2016; 97:83–92. https://doi.org/10.1111/ejh.12687 PMID: 26385526

72. Tsai JJ, Dudakov JA, Takahashi K, Shieh JH, Velardi E, Holland AM, et al. Nrf2 regulates haematopoietic stem cell function. Nature Cell Biology 2013; 15(3): 309–316. https://doi.org/10.1038/ncb2699 PMID: 23434824

**73.** Kim J, Cha YN, Surh Y-JYJ. A protective role of nuclear factor-erythroid 2- related factor-2 (Nrf2) in inflammatory disorders. Mutat Res 2010; 690: 12–23. https://doi.org/10.1016/j.mrfmmm.2009.09.007 PMID: 19799917

**74.** Skov V, Thomassen M, Riley CH, Jensen MK, Bjerrum OW, Kruse TA, et al. Gene expression profiling with principal component analysis depicts the biological continuum from essential thrombocythemia over polycythemia vera to myelofibrosis. Exp Hematol. 2012 Sep; 40(9):771–780. https://doi.org/10.1016/j.exphem.2012.05.011 PMID: 22659388

**75.** Skov V, Larsen TS, Thomassen M, Riley CH, Jensen MK, Bjerrum OW, et al. Molecular profiling of peripheral blood cells from patients with polycythemia vera and related neoplasms: identification of deregulated genes of significance for inflammation and immune surveillance. Leuk Res. 2012 Nov; 36 (11):1387–92. https://doi.org/10.1016/j.leukres.2012.07.009 PMID: 22877729

**76.** Barosi G. An immune dysregulation in MPN. Curr Hematol Malig Rep. 2014 Dec; 9(4):331–9. https://doi.org/10.1007/s11899-014-0227-0 PMID: 25139710

**77.** Yang Y, Bazhin AV, Werner J, Karakhanova S. Reactive oxygen species in the immune system. Int Rev Immunol. 2013 Jun; 32(3):249–70. https://doi.org/10.3109/08830185.2012.755176 PMID: 23617726

**78.** Holmström MO, Hasselbalch HC. Cancer immune therapy for myeloid malignancies: present and future. Semin Immunopathol. 2019 Jan; 41(1):97–109. https://doi.org/10.1007/s00281-018-0693-x PMID: 29987478

**79.** Tao L, Lemoff A, Wang G, Zarek C, Lowe A, Yan N, et al. Reactive oxygen species oxidize STING and suppress interferon production. Elife. 2020 Sep 4; 9:e57837. https://doi.org/10.7554/eLife.57837 PMID: 32886065

**80.** Pedersen KM, Çolak Y, Hasselbalch HC, Ellervik C, Nordestgaard BG, Bojesen SE. Two-fold risk of pneumonia and respiratory mortality in individuals with myeloproliferative neoplasm: A population-based cohort study. EClinicalMedicine. 2020 Apr 6; 21:100295. https://doi.org/10.1016/j.eclinm.2020.100295 PMID: 32280939

**81.** Landtblom AR, Andersson TM, Dickman PW, Smedby KE, Eloranta S, Batyrbekova N, et al. Risk of infections in patients with myeloproliferative neoplasms-a population-based cohort study of 8363 patients. Leukemia. 2021 Feb; 35(2):476–484. https://doi.org/10.1038/s41375-020-0909-7 PMID: 32546727

**82.** Moll NM, Ransohoff RM. CXCL12 and CXCR4 in bone marrow physiology. Expert Rev Hematol 2010; 3(3): 315–22. https://doi.org/10.1586/ehm.10.16 PMID: 21082982

**83.** Nie Y, Han YC, Zou YR. CXCR4 is required for the quiescence of primitive hematopoietic cells. J Exp Med 2008; 205: 777–83. https://doi.org/10.1084/jem.20072513 PMID: 18378795

**84.** Rosti V, Massa M, Vannucchi AM, Bergamaschi G, Campanelli R, Pecci A, et al. The expression of CXCR4 is down-regulated on the CD34+ cells of patients with myelofibrosis with myeloid metaplasia. Blood Cells Mol Dis 2007; 38(3): 280–6. https://doi.org/10.1016/j.bcmd.2007.01.003 PMID: 17350297

**85.** Cencioni C, Spallotta F, Martelli F, Valente S, Mai A, Zeiher AM, et al. Oxidative stress and epigenetic regulation in ageing and age-related diseases. Int J Mol Sci 2013; 14(9): 17643–63. https://doi.org/10.3390/ijms140917643 PMID: 23989608

**86.** Pe´rez C, Pascual M, Martin-Subero JI, Bellosillo B, Segura V, Delabesse E, et al. Aberrant DNA methylation profile of chronic and transformed classic Philadelphia-negative myeloproliferative neoplasms. Haematologica 2013; 98(9): 1414–20. https://doi.org/10.3324/haematol.2013.084160 PMID: 23716560

**87.** De Franceschi L, Bertoldi M, De Falco L, Santos Franco S, Ronzoni L, Turrini F, et al. Oxidative stress modulates heme synthesis and induces peroxiredoxin-2 as a novel cytoprotective response in b-thalassemic erythropoiesis. Haematologica 2011; 96, 1595–1604. https://doi.org/10.3324/haematol.2011.043612 PMID: 21750082

**88.** Ren X, Ma L, Wang N, Zhou R, Wu J, Xie X, et al. Antioxidant Gene Signature Impacts the Immune Infiltration and Predicts the Prognosis Kidney Renal Clear Cell Carcinoma. Front Genet. 2021 Aug 19; 12:721252. do https://doi.org/10.3389/fgene.2021.721252 PMID: 34490047

**89.** Čokić VP, Mossuz P, Han J, Socoro N, Beleslin-Čokić BB, Mitrović O, et al. Microarray and Proteomic Analyses of Myeloproliferative Neoplasms with a Highlight on the mTOR Signaling Pathway. PLoS One. 2015 Aug 14; 10(8):e0135463. https://doi.org/10.1371/journal.pone.0135463 PMID: 26275051

**90.** Djikic D, Markovic D, Bogdanovic A, Mitrovic-Ajtic O, Suboticki T, Diklic M, et al. Oxidative and nitrosative stress in myeloproliferative neoplasms: the impact on the AKT / mTOR signaling pathway. J BUON. 2018 Sep-Oct; 23(5):1481–1491. PMID: 30570876