博士論文

# Loss of p53 Induces Leukemic Transformation in a Murine Model of Jak2 V617F-driven Polycythemia Vera

(Jak2 V617F 変異陽性真性多血症マウスモデルにおいて p53 欠失は白血病化を引き起こす)

木住野　貴子

**Contents**


| | |
|---|---|
| Summary | 2 |
| Introduction | 4 |
| Materials and Methods | 7 |
| Results | 12 |
| Discussion | 43 |
| Acknowledgements | 50 |
| References | 51 |
| Tables | 66 |

**Summary**

Since leukemic transformation of myeloproliferative neoplasms (MPN) worsens the clinical outcome, reducing the inherent risk of the critical event in MPN cases could be beneficial. Among genetic alterations concerning the transformation, the frequent one is *TP53* mutation. Here I show that retroviral overexpression of Jak2 V617F mutant into *p53* wild-type allele murine bone marrow cells induced polycythemia vera (PV) in the recipient mice, whereas Jak2 V617F-transduced *p53* null allele mice developed lethal leukemia after the preceding PV phase. The leukemic mice had severe anemia, hepatosplenomegaly, pulmonary hemorrhage, and expansion of dysplastic erythroid progenitors. Both primitive leukemia cells (c-kit$^+$Sca1$^+$Lin$^-$ (KSL)) and erythroid progenitors (CD71$^+$) of Jak2 V617F-transduced *p53*-null leukemic cells had leukemia-initiating capacity, however, myeloid populations (Mac-1$^+$) could not recapitulate the disease. Interestingly, recipients transplanted with CD71$^+$ cells rapidly developed erythroid leukemia, which was in sharp contrast to leukemic KSL cells to cause lethal leukemia after the polycythemic state. The leukemic CD71$^+$ cells were more sensitive to INCB18424, a potent JAK inhibitor, than KSL cells. p53 restoration could ameliorate Jak2 V617F-transduced *p53*-null erythroleukemia.

Taken together, my results demonstrate that p53 loss is sufficient for inducing leukemic transformation in Jak2 V617F-postive MPN and offer a new model of post-MPN leukemia.

## Introduction

Philadelphia chromosome negative-myeloproliferative neoplasms (MPN) are clonal hematopoietic stem cell (HSC) disorders and largely induced by somatic activating mutations of Janus kinase 2 (JAK2), myeloproliferative leukemia virus oncogene (MPL), and calreticulin (CALR)[1-7]. Especially, JAK2 V617F mutation is the most prevalent gain-of-function mutation, accounting for about 50-60% of cases[8], and several groups have succeeded in modeling human MPN in mice by retroviral overexpression of Jak2 V617F or knocking-in of Jak2 V617F transgene[9-13]. Clinically, leukemic transformation is one of the frequent complications in MPN patients and associated with a dismal prognosis[14]. Recent mutational analysis in MPN patients with leukemic transformation revealed recurrent somatic mutations such as TP53, TET2, ASXL1, and RNA splicing-related factors[15,16]. Of note, TP53 has been reported to be mutated in 10-20% of post-MPN acute myeloid leukemia (AML) in sharp contrast to the quite low frequency in de novo AML[16,17]. Besides mutation of TP53 itself, high copy number of MDM4, a major negative regulator of TP53, caused by amplification of chromosome 1q has been thought to be associated with

leukemic transformation of MPN[17,18]. Considering a series of clinical evidence, TP53-related alterations seem to have some critical functions for the leukemic transforming capacity in myeloid compartment.

For AML patients, keeping remission phase is an imperative[19,20], but leukemia might relapse because of remaining leukemia cells[21]. Age of patients, molecular profiles of leukemia and therapeutic sensitivity might affect the clinical course of each patient[19,20,22]. Even the pathogenesis of leukemia has been gradually unveiled, it is obvious that about 30 % of young (< 60 years old) and 50 % of older (≥ 60 years old) AML patients have induction failure[20,22], which stresses the need for improving the poor prognosis of relapsed/ refractory AML patients[19,20,22].

TP53, a well-studied tumor suppressor gene, is somatically mutated and/or deleted in over 50% of human cancers, and the most of missense mutations identified in human malignancies including post-MPN AML samples are located in the core DNA-binding domain, which have been reported to function as a loss-of-function form[23]. In fact, genetically *p53*-deleted mice are cancer-prone, in which the most frequent is malignant lymphoma[24]. The causative role of p53 loss in leukemia progression has been elucidated in murine models of

myelodysplastic syndrome (MDS), another leukemia-prone and clonal HSC disorder. In MDS mice carrying deletion 5q, p53 loss has a positive role in restoring the hematopoietic stem/progenitor cell defect derived from 5q deletion[25]. In NUP98-HOXD13 transgenic MDS mice, genetic deletion of p53 accelerates leukemia development[26]. In spite of the accumulated evidences of p53-associated pathogenesis in hematological malignancies, the functional contribution of p53 loss to leukemic transformation from MPN remains elusive. In this study, I demonstrated that genetic deletion of *p53* leads to leukemia in Jak2 V617F-driven murine MPN model. Of interest, PV-like disease precedes erythroid leukemia in mice transplanted with Jak2 V617F-transduced *p53* knockout (*p53*$^{-/-}$) cells. Furthermore, in this model, leukemia-initiating cells were identified and the efficacy of JAK inhibitor treatment and p53 restoration was explored. My murine model of p53-deficient leukemia transformed from Jak2 V617F-positive MPN offers a useful platform of comprehensive screening for therapeutic targets in post-MPN AML patients.

## Methods

### Mice

*p53* knockout mice were obtained from RIKEN Bioresource center (Tsukuba, Japan)[27], backcrossed to C57BL/6 and genotyped by PCR under the following conditions: 30sec of denaturation at 94°C, 30 sec of annealing at 57°C and extraction at 72°C for 30 sec. PCR primers are used Forward: 5'-GTTATGCATCCATACAGTACA-3' and Reverse: 5'-CAGGATATCTTCTGGAGGAAG-3'[28]. The PCR products were analyzed by agarose gel electrophoresis. Littermate $p53^{+/+}$ mice were used as controls. Congenic C57BL/6 mice were purchased from SRL Inc. (Tokyo, Japan). All animal experiments were approved by The University of Tokyo Ethics Committee for Animal Experiments.

### Plasmids

Murine Jak2 wild-type (WT) and V617F mutant cDNAs were cloned into pBleuscript II. V617F mutant was constructed from Jak2 WT by site directed PCR-based mutagenesis. Murine p53 WT expression plasmid (pMXs-p53) was

purchased from Addgene (Addgene plasmid 22725). Human TP53 WT and G135A mutant expression plasmids were purchased from clontech. To produce protein expressing retrovirus, I used the following plasmids: pGCDNsam-IRES-EGFP, pGCDNsam-Jak2 WT-IRES-EGFP, pGCDNsam-Jak2 V617F-IRES-EGFP, pGCDNsam-Mpl W515L-IRES-GFP, pGCDNsam-IRES-Kusabira Orange (KuOr), pGCDNsam-p53-IRES-KuOr, pGCDNsam-TP53 WT-IRES-KuOr, pGCDNsam-TP53 G135A-IRES-KuOr, and MSCV-MLL ENL-IRES-GFP. All of the PCR products were verified by DNA sequencing.

Retrovirus Production and Bone Marrow Transplantation Assays

To gain retroviral supernatants, Plat-E packaging cells were transfected with retroviral vector, and the supernatants were collected 48 h after transfection and immediately utilized. c-kit$^{+}$ BM cells were purified from *p53*$^{+/+}$, *p53*$^{+/-}$, and *p53*$^{-/-}$ mice and transduced with retrovirus in the presence of RetroNectin (Takara Bio Inc.) after 24 h incubation in $\alpha$-MEM with 20% Fetal calf serum (FCS), 1% Penicillin/Streptomycin (PS), and cytokines (50 ng/mL mouse stem cell factor (SCF) and 25 ng/mL human thrombopoietin, interleukin-6 (IL-6), and FLT3) at

37°C in a 5% $CO_2$ incubator. The infected cells were harvested 48 h after retrovirus infection and injected into lethally irradiated (9.5Gy) mice. For second BMT, the indicated number of Jak2 V617F-transduced $CD71^+$, $Mac\text{-}1^+$, $CD71^-Mac\text{-}1^-$, $CD71^+Ter119^-$, $CD71^+Ter119^+$ or KSL cells was transplanted with $2\times10^5$ unfractionated competitor BM cells from congenic mice into lethally irradiated mice. For p53-restoration experiment, BM cells were obtained from moribund Jak2 V617F/*p53*$^{-/-}$ mice and were transduced with pGCDNsam-IRES-KuOr or pGCDNsam-p53-IRES-KuOr, and $3\times10^4$ GFP and KuOr double positive cells were transplanted with $5\times10^4$ unfractionated competitor BM cells from congenic mice to lethally irradiated mice. Recipients with PB engraftment of donor cells (GFP and KuOr double positive fraction) >0.1% at three weeks post-transplant were evaluated.

Analysis of BMT Mice

PB samples were collected from tail vein into EDTA-containing tubes. Complete blood cell count was determined using an automatic blood cell counter (ERMAX-18, ERMA Inc.). PB smears and cytospun BM and spleen were stained with Wright-Giemsa for morphologic analysis. For histopathology analysis,

tissues were fixed in 10% neutral-buffered-formalin, embedded in paraffin, and stained with hematoxylin-eosin. The donor engraftment in transplanted mice and the immunophenotype of leukemia cells were analyzed by flow cytometry. A list of antibodies for flow cytometry is provided in Table 1. Stained cells were sorted with a FACSAriaII/III, and analysis was performed on LSRII (both from BD). A mixture of antibodies recognizing CD3, CD4, CD8, B220, CD127, TER-119, Mac-1, or Gr-1 was used to identify $Lin^+$ cells. Analysis of flow cytometric data was performed with FlowJo software (Tree Star). For the cell cycle analysis, BM cells were stained with KSL and CD71, followed by fixation and permeabilization using BD Cytofix/Cytoperm Fixation/Permerazation Solution Kit (BD Biosciences) according to the manufacturer's recommendations. Subsequently, I stained with DAPI (molecular probes) and PE Ki-67 (BD Bioscience). For the apoptosis analysis, after KSL or CD71 staining, BM cells were stained with APC Annecin V (eBioscience) and DAPI according to the manufacturer's instruction.

JAK2 Inhibitor Treatment

$1\times10^4$ Jak2 V617F/*p53*$^{-/-}$ leukemia cells obtained from moribund mice were subjected to in vitro culture (RPMI1640 with 10% FCS, 1% PS, 25 ng/mL mouse

SCF, human erythropoietin (EPO), IL-6, and IL-3) containing DMSO, 300nM, or 900nM INCB18424 (Adooq BioScience) at 37°C in a 5% $CO_2$ incubator. Subsequently, proliferative capacity and surface marker expression were assessed on day 3.

Colony-forming Assays

$5\times10^3$ Jak2 V617F/*p53*$^{-/-}$ leukemia cells transduced with empty vector, murine p53 WT, human TP53 WT, or TP53 G135A were plated into Methocult M3434 (StemCell Technologies) media. Colonies of each dish were counted on day 7.

Statistical Analysis

Statistical significance of differences was assessed with a two-tailed unpaired t test and log-rank test. Differences were considered statistically significant at a p value of less than 0.05.

## Results

### p53 loss leads to leukemic transformation in a murine Jak2 V617F-induced PV model.

Hematopoietic progenitor cells (c-kit$^+$ cells) from bone marrow (BM) of *p53*$^{-/-}$ or littermate wild-type (*p53*$^{+/+}$) mice were retrovirally transduced with either GFP-labeled wild-type Jak2 (Jak2 WT) or Jak2 V617F mutant respectively, and these cells were transplanted into lethally irradiated recipient mice. At three weeks after BM transplantation (BMT), both recipients with Jak2 V617F-transduced *p53*$^{-/-}$ cells (Jak2 V617F/*p53*$^{-/-}$ mice) and those with Jak2 V617F-transduced *p53*$^{+/+}$ cells (Jak2 V617F/*p53*$^{+/+}$ mice) displayed elevation of white blood cell (WBC) count, hemoglobin (Hb) and mean corpuscular volume (MCV) in peripheral blood (PB), which is suggestive of MPN (Figure 1A-D). Jak2 V617F/*p53*$^{-/-}$ mice seemed cachectic and showed declined Hb and MCV in PB at five weeks post BMT, while Jak2 V617F/*p53*$^{+/+}$ mice had sustained erythrocytosis (Figure 1A-D). There showed no difference in platelet counts between Jak2 V617F/*p53*$^{-/-}$ and Jak2 V617F/*p53*$^{+/+}$ mice from three to seven weeks after BMT (Figure 1D).

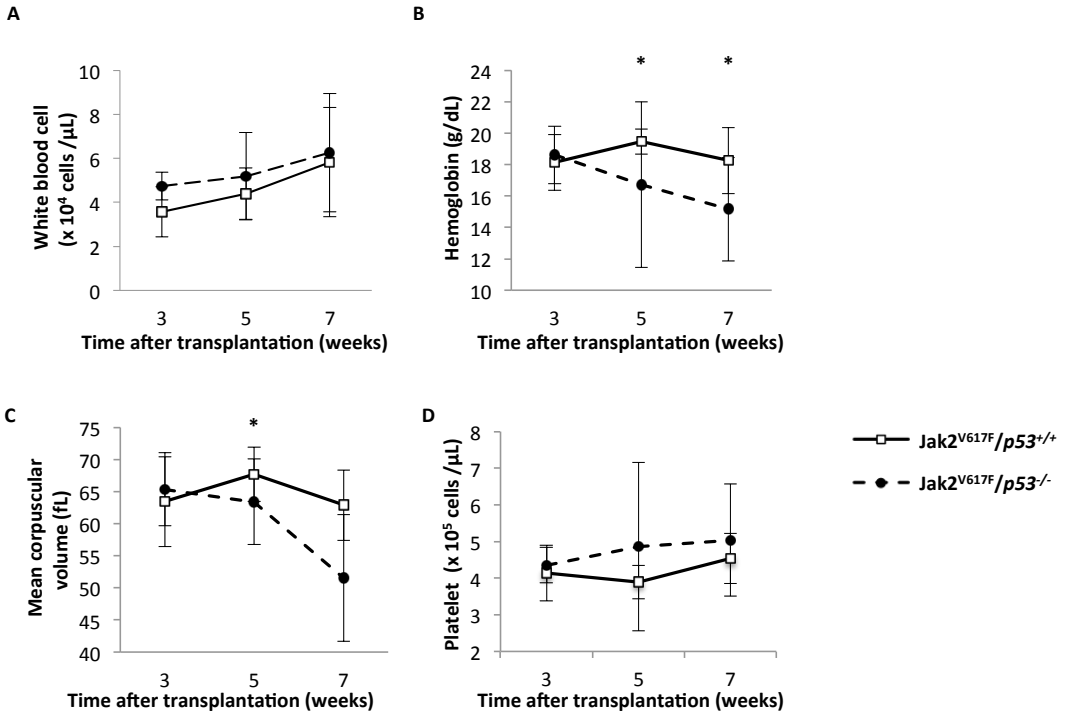


Figure1. (A-D) Data of complete blood cell count of PB from Jak2 V617F-transduced *p53*$^{-/-}$ or *p53*$^{+/+}$ mice (A: White blood cell, B: Hemoglobin, C: Mean corpuscular volume, D: Platelet). Jak2 V617F/*p53*$^{+/+}$ mice displayed sustained elevation of WBC, Hb and MCV up to 7 weeks post BMT. The level of Hb and MCV of Jak2 V617F/*p53*$^{-/-}$ mice declined from 5 weeks post BMT. Data are mean ± SD. *p < 0.05.

All of Jak2 V617F/*p53*$^{-/-}$ mice died with a median survival of 46.5 days post BMT (range, 32-56 days), in sharp contrast to Jak2 V617F/*p53*$^{+/+}$ mice, most of which seemed healthy even after 120 days after BMT (Figure 2). Additionally, *p53* heterozygosity had little impact on rendering PV mice to highly lethal phenotype (Figure 2).

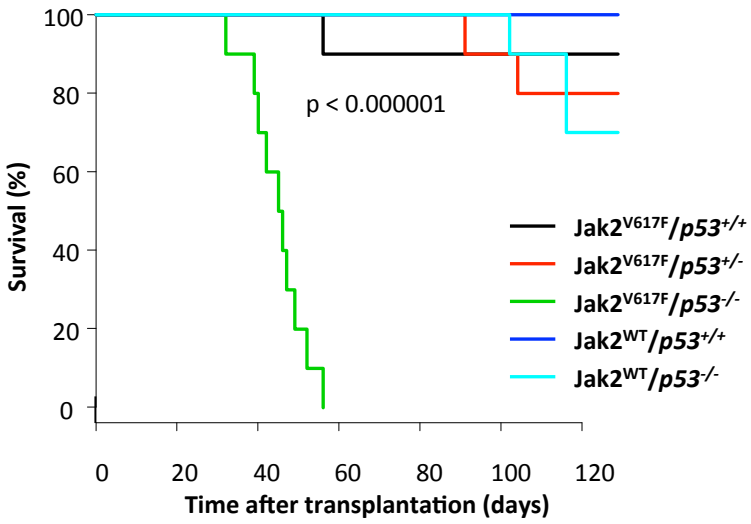


Figure2. Kaplan-Meier plot of Jak2 V617F/$p53^{-/-}$, Jak2 V617F/$p53^{+/-}$, Jak2 V617F/$p53^{+/+}$, Jak2 WT/ $p53^{-/-}$ and Jak2 WT/$p53^{+/+}$ recipients was shown (n = 10 mice per group, $p < 0.000001$; Jak2 V617F/$p53^{-/-}$ versus Jak2 V617F/$p53^{+/+}$). About 90% of Jak2 V617F/$p53^{+/+}$ mice survived 120 days after BMT, however, all of Jak2 V617F/$p53^{-/-}$ mice died by 56 days after BMT.

To assess the cause of deaths in Jak2 V617F/$p53^{-/-}$ mice, I analyzed various tissues from moribund Jak2 V617F/$p53^{-/-}$ mice. In PB smears by Wright-Giemsa staining, Jak2 V617F/$p53^{-/-}$ mice showed increase of erythroid precursors with dysplasia (Figure 3A, B). In Jak2 V617F/$p53^{-/-}$ mice, erythroblasts dominated the BM and spleen while no excess of erythroblasts was found in Jak2 V617F/$p53^{+/+}$ mice (Figure 3A, B).

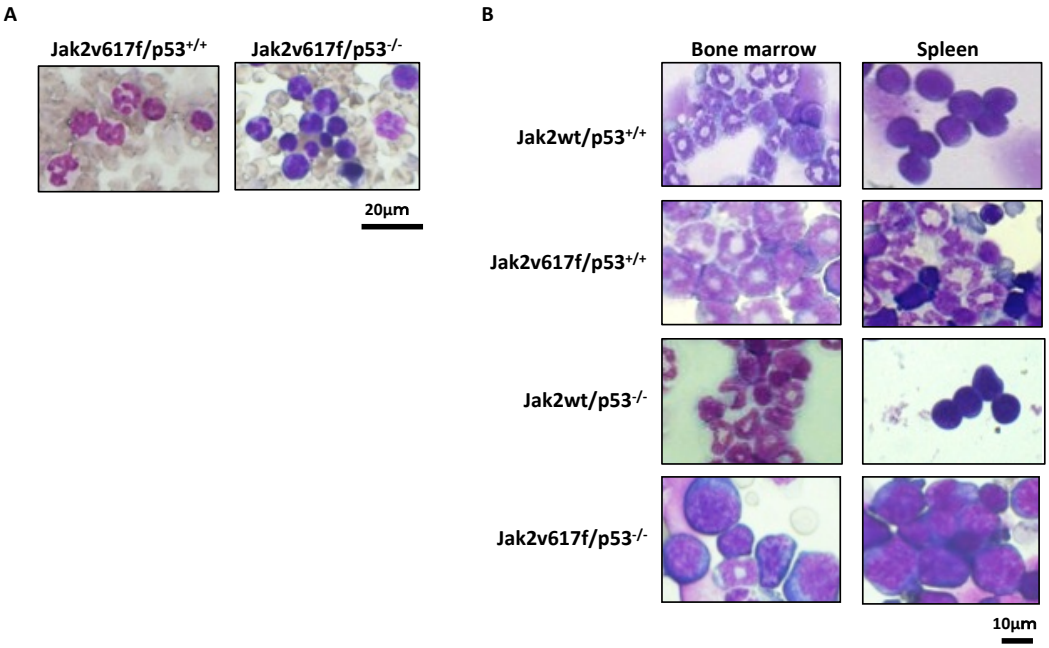


Figure 3. Photos of Wright-Giemsa-stained PB smears (A), cytospun BM (B: left) and spleen (B: right) from Jak2 V617F/*p53*$^{+/+}$ (A: left, B: the second from the top) and Jak2 V617F/*p53*$^{-/-}$ mice (A: right, B: bottom) were shown. Jak2 V617F/*p53*$^{+/+}$ mice had mature myeloid cells in PB, BM and spleen. Jak2 V617F/*p53*$^{-/-}$ mice showed erythroid precursors with dysplastic feature in PB, BM and spleen.

The percentage of Jak2 V617F (GFP)-positive cells and the cellularity of BM and spleen from Jak2 V617F/*p53*$^{-/-}$ mice was comparable to that from Jak2 V617F/*p53*$^{+/+}$ mice (Figure4 A-B).

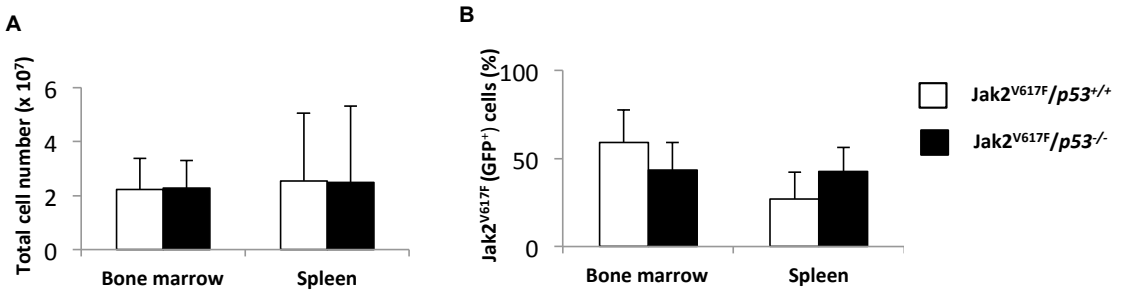


Figure 4. (A) The numbers of cells in BM and spleen from Jak2 V617F/$p53^{+/+}$ (n = 4) and Jak2 V617F/$p53^{-/-}$ mice (n = 13). (B) The percentages of Jak2 V617F (GFP) positive cells in BM and spleen from Jak2 V617F/$p53^{+/+}$ (n = 8) and Jak2 V617F/$p53^{-/-}$ mice (n = 17). Total cell number and the percentage of Jak2 V617F positive cells were similar between Jak2 V617F/$p53^{+/+}$ and Jak2 V617F/$p53^{-/-}$ mice. Data are mean ± SD.

Jak2 V617F/$p53^{-/-}$ mice also had a marked and macroscopic hepatosplenomegaly and hemorrhagic lung (Figure 5A-B and Figure 6).

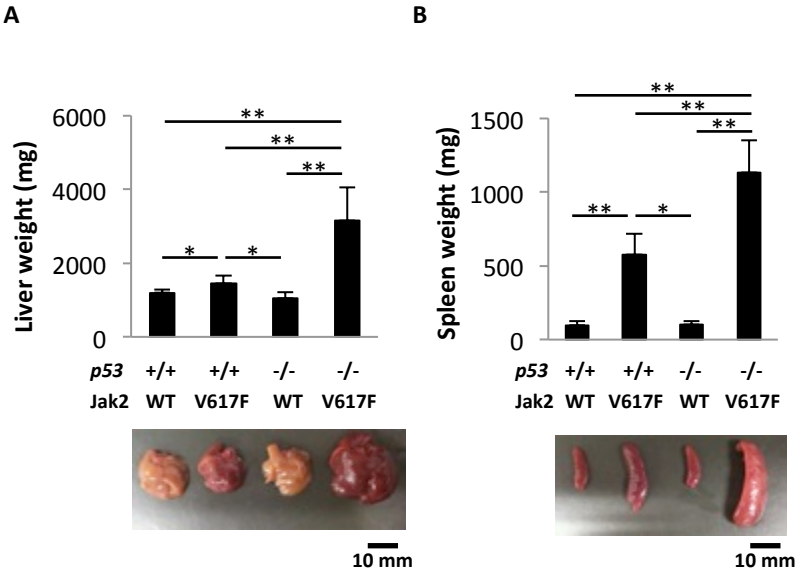


Figure 5. (A and B) Average weight of liver (A, upper) and spleen (B, upper) from Jak2 V617F/$p53^{+/+}$ (n = 9), Jak2 V617F/$p53^{-/-}$ (n = 17), Jak2 WT/$p53^{+/+}$ (n = 6)

and Jak2 WT/*p53*$^{-/-}$ mice (n = 5) at seven weeks after BMT. Representative photos of livers (A, lower) and spleens (B, lower) were also shown. Jak2 V617F/*p53*$^{+/+}$ mice showed mild hepatosplenomegaly and Jak2 V617F/*p53*$^{-/-}$ mice displayed remarkable hepatosplenomegaly compared with Jak2 WT/*p53*$^{+/+}$ and Jak2 WT/*p53*$^{-/-}$ mice. Data are mean ± SD. *p < 0.05, **p < 0.0005.

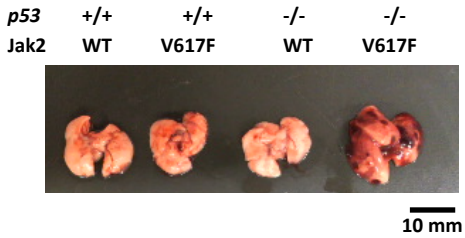


Figure 6. Representative photos of lungs. Lungs from Jak2 V617F/*p53*$^{-/-}$ mice showed macroscopic hemorrhages.

When livers, spleens and lungs from these two types of recipients were histologically assessed, Jak2 V617F/*p53*$^{-/-}$ mice had infiltration of blastic cells at each tissue, and especially the spleen showed effacement of splenic follicular structure. Additionally, lungs from Jak2 V617F/*p53*$^{-/-}$ mice had diffuse hemorrhage originated from collapse on pulmonary capillaries by infiltrated blastic cells, which might reflect the severity of the disease (Figure 7). By contrast, Jak2 V617F/*p53*$^{+/+}$ mice only had mild to moderate hepatic and splenic infiltration of erythroid progenitors and neutrophils with no effacement of sinusoidal or splenic architectures, which was compatible with the histology of PV mice (Figure 7)[9].

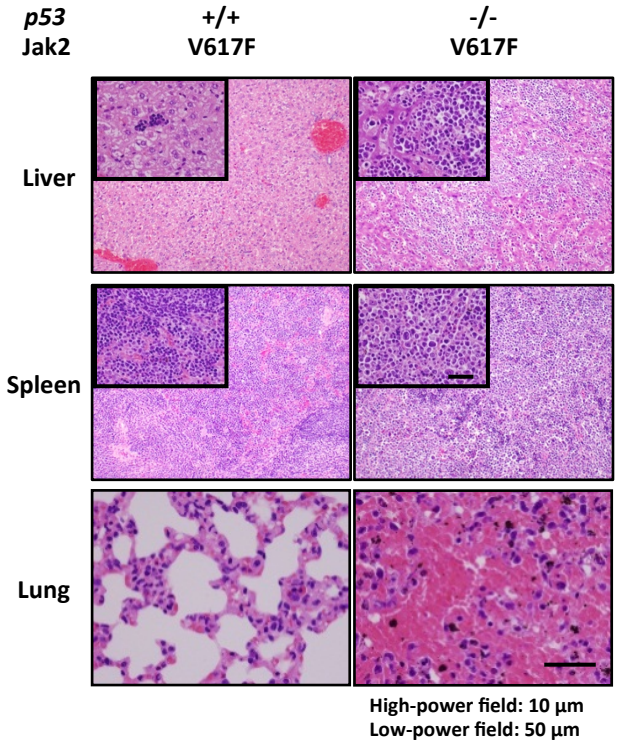


Figure 7. HE-stained livers, spleens and lungs from Jak2 V617F/*p53*$^{+/+}$ (left) and Jak2 V617F/*p53*$^{-/-}$ mice (right) were shown. All of these organs from Jak2 V617F/*p53*$^{-/-}$ mice had infiltration of blastic cells.

From flow cytometric analysis of BM and spleen cells from Jak2 V617F/*p53*$^{-/-}$ mice in comparison to Jak2 V617F/*p53*$^{+/+}$ mice, it was revealed that BM contained an increasing CD71$^{+}$ erythroid population and a decreasing Mac-1$^{+}$ myeloid population and that CD71$^{+}$ cells dominated the spleen (Figure 8A-C).

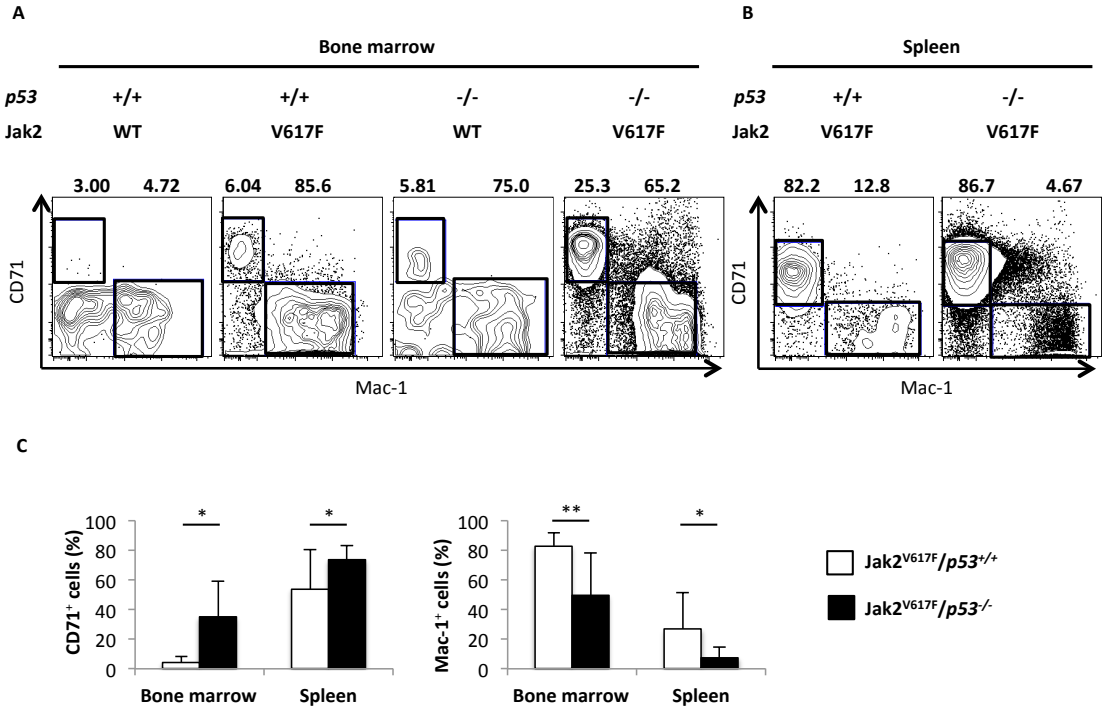


Figure 8. (A) Representative flow cytmetric plots of BM cells from Jak2 WT/*p53*$^{+/+}$, Jak2 V617F/*p53*$^{+/+}$, Jak2 WT/*p53*$^{-/-}$, and Jak2 V617F/*p53*$^{-/-}$ mice. (B) Representative flow cytmetric plots of spleen cells from Jak2 V617F/*p53*$^{+/+}$ and Jak2 V617F/*p53*$^{-/-}$ mice. (C) The percentages of CD71$^{+}$ cells (left) and Mac-1$^{+}$ cells (right) in the BM and spleen from Jak2 V617F/*p53*$^{+/+}$ and Jak2 V617F/*p53*$^{-/-}$ mice. Jak2 V617F/*p53*$^{+/+}$ mice showed increased Mac-1$^{+}$ myeloid population in BM and spleen. Jak2 V617F/*p53*$^{-/-}$ mice had expansion of CD71$^{+}$ erythroid precursors in BM and spleen. Data are mean ± SD. *p < 0.05, **p < 0.005

In the expanding CD71$^{+}$ population from Jak2 V617F/*p53*$^{-/-}$ mice, there were more immature erythroid progenitors (CD71$^{+}$Ter119$^{-}$) than mature erythroid cells (CD71$^{+}$Ter119$^{+}$) in the BM (Figure 9A-B). In contrast to Jak2 V617F/*p53*$^{+/+}$ mice, CD71$^{+}$Ter119$^{-}$ population turned to be dominant in the spleen of Jak2 V617F/*p53*$^{-/-}$ mice. From these analyses, it was revealed that Jak2 V617F/*p53*$^{-/-}$

recipients developed the infiltrative leukemia with accumulation of erythroblasts, fulfilling the Bethesda Criteria of erythroleukemia in mice[29].

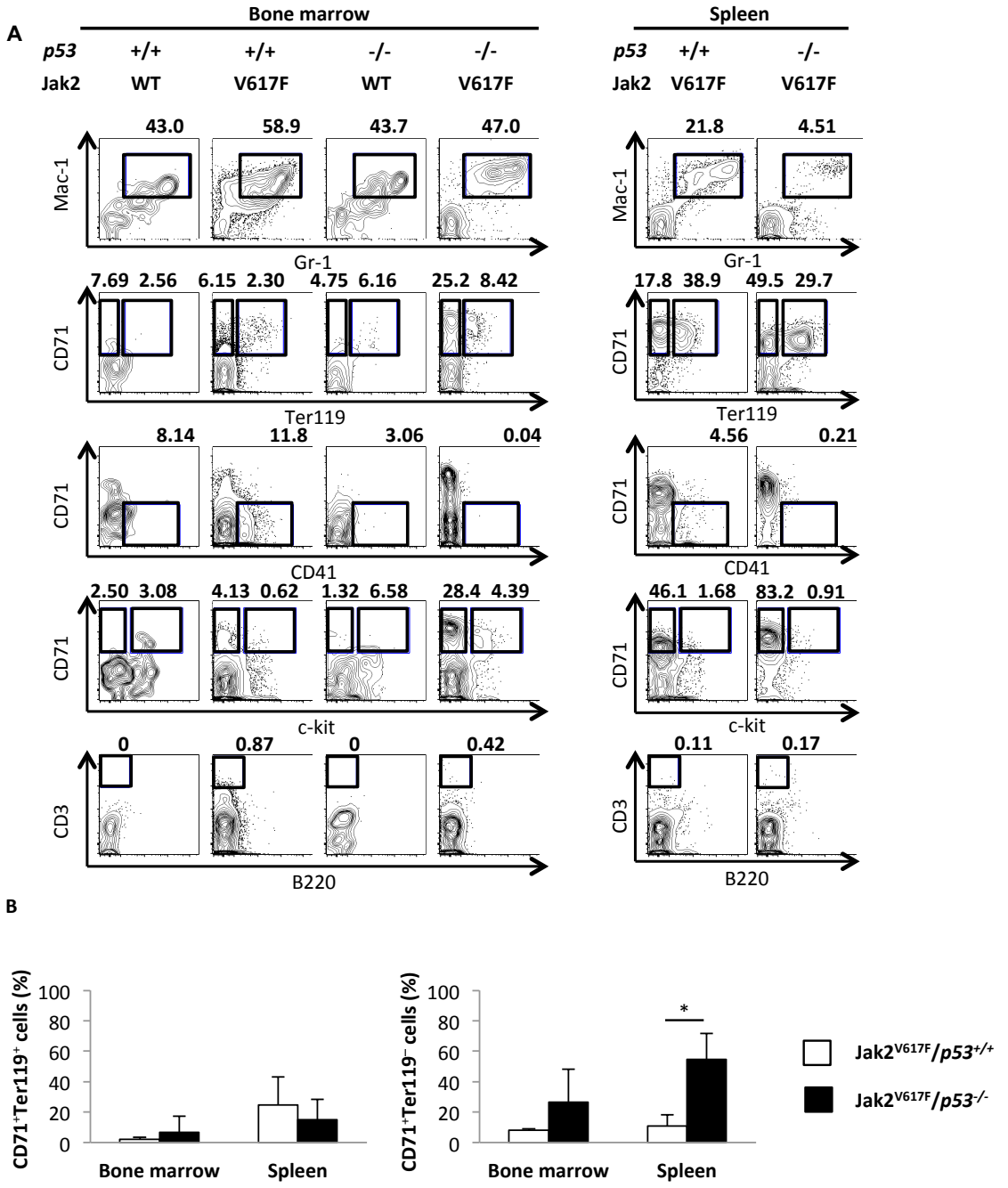


Figure 9. (A) Representative flow cytometric plots of BM and spleen cells from Jak2 WT/*p53*$^{+/+}$, Jak2 V617F/*p53*$^{+/+}$, Jak2 WT/*p53*$^{-/-}$ and Jak2 V617F/*p53*$^{-/-}$ mice. (B) The percentages of CD71$^{+}$Ter119$^{+}$ (left) and CD71$^{+}$Ter119$^{-}$ cells (right) in BM and spleen from Jak2 V617F/*p53*$^{+/+}$ (n = 8) and Jak2 V617F/*p53*$^{-/-}$ mice (n = 17). CD71$^{+}$Ter119$^{-}$ population was increased in BM and spleen from Jak2 V617F/*p53*$^{-/-}$ mice. Data are mean ± SD. *p < 0.05.

To explore the functional role of p53 loss in the leukemic transformation in Jak2 V617F-driven MPN, I analyzed cell cycle and apoptosis status of these cells. Cell cycle analysis revealed that the percentage of cells in S/G2/M phase was increased in bulk Jak2 V617F/*p53*$^{-/-}$ leukemia cells compared to bulk Jak2 V617F/*p53*$^{+/+}$ PV cells. In contrast, more detailed subfraction analysis showed that the percentage of quiescent cells (G0 status) was higher in Jak2 V617F/*p53*$^{-/-}$ KSL cells (Figure 10A-C).

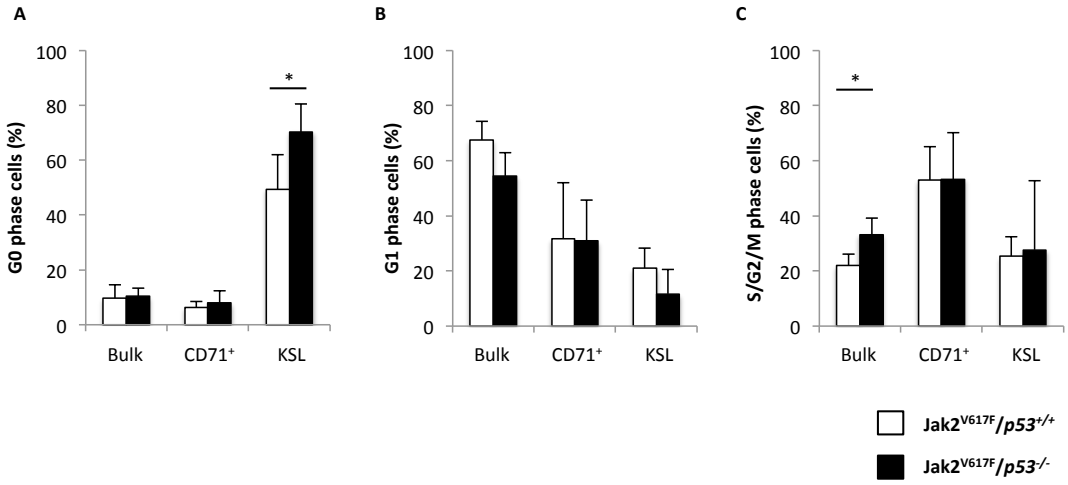


Figure 10. Percentages of cells in G0 phase (A), G1 phase (B) and S/G2/M phase (C) in bulk, CD71$^{+}$ and KSL cells from Jak2 V617F/*p53*$^{+/+}$ and Jak2 V617F/*p53*$^{-/-}$ mice (n = 4 mice per group). Jak2 V617F/*p53*$^{-/-}$ KSL cells were more in G0 and bulk Jak2 V617F/*p53*$^{-/-}$ cells were more in S/G2/M phase. Data are mean ± SD. *p < 0.05.

In apoptosis analysis, apoptotic cells were decreased in Jak2 V617F/*p53*$^{-/-}$ KSL cells compared to Jak2 V617F/*p53*$^{+/+}$ KSL cells (Figure 11), while there was no significant alteration bulk and CD71$^{+}$ Jak2 V617F/*p53*$^{-/-}$ leukemia cells. These results indicated that retaining KSL Jak2 V617F/p53$^{-/-}$ leukemia cells depend on resting state of cell cycle and inhibition of apoptosis.

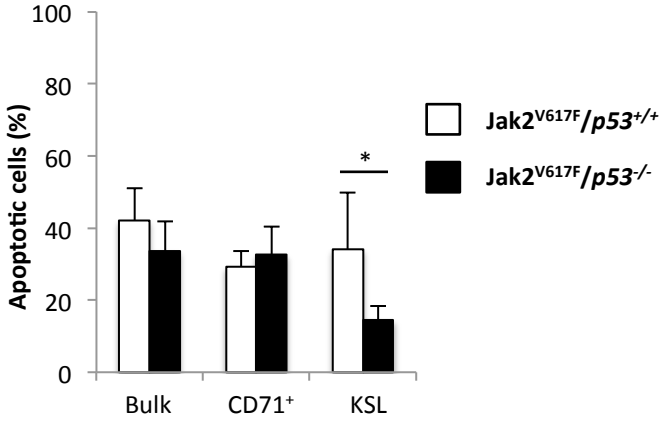


Figure 11. Percentages of apoptotic cells in bulk, CD71$^{+}$ and KSL cells from Jak2 V617F/*p53*$^{+/+}$ and Jak2 V617F/*p53*$^{-/-}$ mice (n = 4 mice per group). Jak2 V617F/*p53*$^{-/-}$ KSL cells had lower percentage of apoptotic cells than the Jak2 V617F/*p53*$^{+/+}$ counterpart. Data are mean ± SD. *p < 0.05.

To clarify whether p53 deficiency collaborates with some oncogenes other than Jak2 V617F to cause hematological malignancies, I performed transplantation assay to generate MLL-ENL-driven AML[30-33] and Mpl W515L mutant-driven MPN mouse models on p53$^{+/+}$ or p53$^{-/-}$ background as previously done by Jak2 V617F[1] (MLL-ENL/*p53*$^{+/+}$, MLL-ENL/*p53*$^{-/-}$, Mpl W515L/*p53*$^{+/+}$ and

Mpl W515L/*p53*$^{-/-}$ mice). While p53 loss had no impact on disease progression in MLL-ENL-induced AML (Figure 12A), the latency of Mpl W515L-induced MPN was clearly shortened by p53 nullizygousity (Figure 12B), Morphology of BM and spleen from Mpl W515L-induced MPN was not affected by p53 nullizygousity (Figure 13).

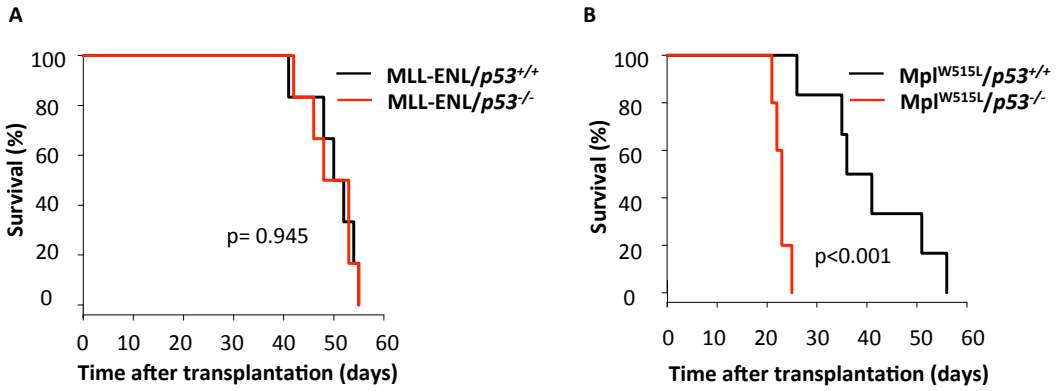


Figure 12. (A) Kaplan-Meier plot of MLL-ENL/*p53*$^{-/-}$ (n = 6) and MLL-ENL/*p53*$^{-/-}$ (n = 6). (B) Kaplan-Meier plot of Mpl W515L/*p53*$^{-/-}$ (n = 6) and Mpl W515L/*p53*$^{-/-}$ (n = 5). Both MLL-ENL/*p53*$^{+/+}$ and MLL-ENL/*p53*$^{-/-}$ mice showed similar survival, however, Mpl W515L/*p53*$^{-/-}$ animals had shorter survival compared with Mpl W515L/*p53*$^{+/+}$ mice.

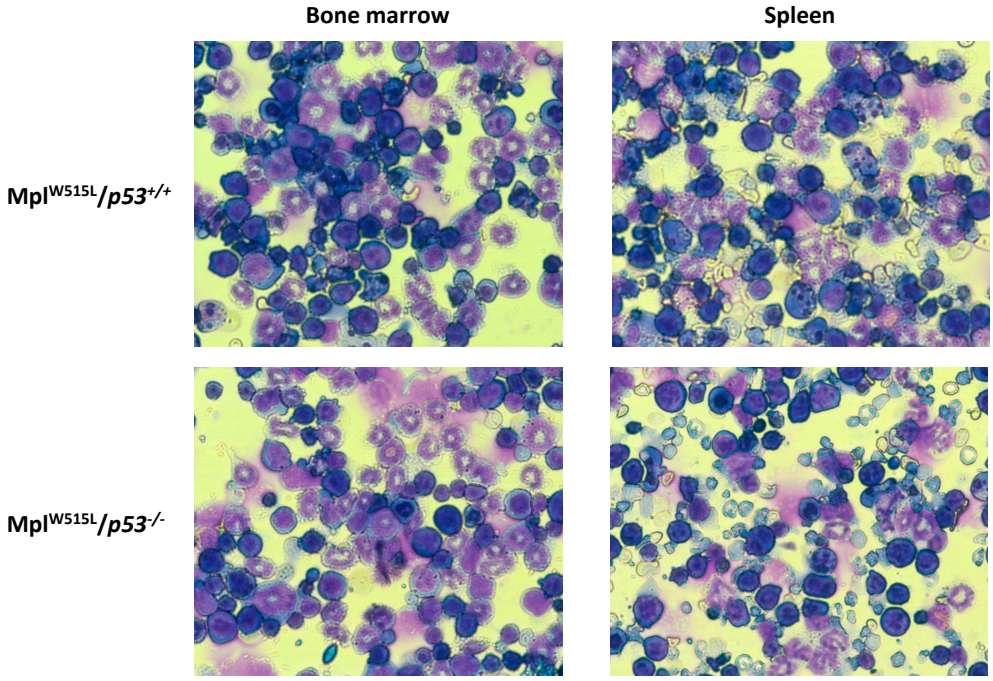


Figure 13. Photos of Wright-Giemsa-stained cytospin BM (left) and spleen (right) from Mpl W515L/*p53*$^{+/+}$ (top) and Mpl W515L/*p53*$^{-/-}$ (bottom). Mpl W515L/*p53*$^{-/-}$ BM and spleen cells showed similar morphology to those from Mpl W515L/*p53*$^{+/+}$ mice.

From these data, I revealed that p53 loss derived leukemic transformation is specific to Jak2 V617F positive MPN.

**Jak2 V617F/*p53*$^{-/-}$ leukemia is serially transplantable in recipient mice.**

To test the transplantability of Jak2 V617F/*p53*$^{-/-}$ leukemia cells, I injected unfractionated BM cells from Jak2 V617F/*p53*$^{-/-}$ mice into lethally irradiated mice. All secondary recipients with Jak2 V617F/*p53*$^{-/-}$ leukemic cells developed lethal

leukemia earlier than primary recipients (median survival of secondary recipients; 22 days)(Figure 14).

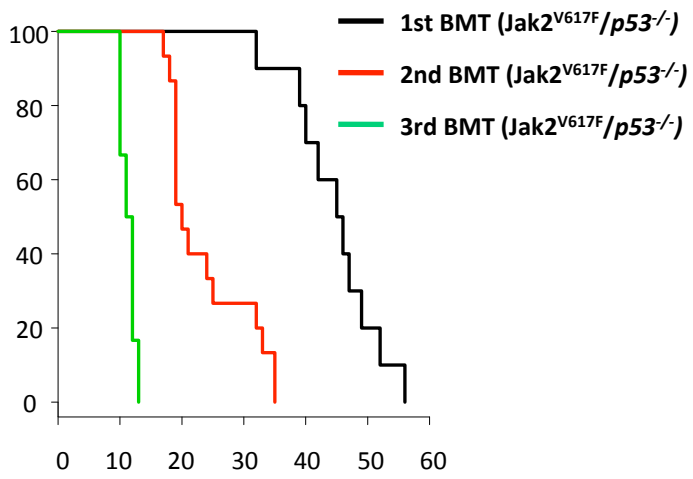


Figure 14. Kaplan-Meier plot of primary (1st BMT, n = 10), secondary (2nd BMT, n = 10) and tertiary (3rd BMT, n= 6) recipients with Jak2 V617F/*p53*$^{-/-}$ cells was shown. ($p < 0.0001$; 1st BMT versus 2nd or 3rd BMT, $p < 0.001$; 2nd BMT versus 3rd BMT).

These secondary transplants also showed hepatosplenomegaly and pulmonary hemorrhage (Table 2), and the increase of $CD71^+$ population in the BM and spleen (Figure 15A-B).

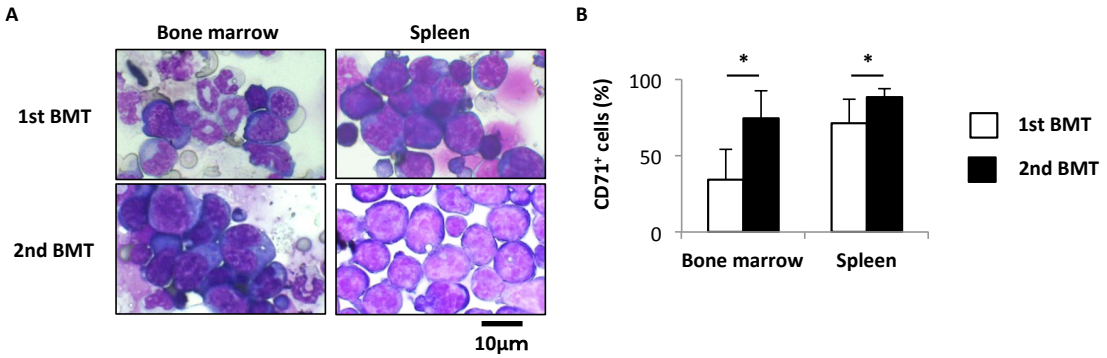


Figure 15. (A) Photos of cytospun and Wright-Giemsa-stained BM (left) and spleen (right) from primary (upper) and secondary recipients (lower) with Jak2 V617F/*p53*$^{-/-}$ leukemic cells were shown. (B) The percentages of CD71$^{+}$ cells in BM and spleen from primary (n = 17) and secondary recipients (n = 13) with Jak2 V617F/*p53*$^{-/-}$ leukemic cells. Secondary recipients displayed expansion of CD71$^{+}$ population compared with primary recipients. Data are mean ± SD. *p < 0.05.

In addition, tertiary transplantation hastened the latency to develop leukemia. (median survival of thirdly recipients; 11.5 days) (Figure 14). These data demonstrated that Jak2 V617F/*p53*$^{-/-}$ leukemia is serially transplantable, and the erythroid population is the predominant cells involved in Jak2 V617F/*p53*$^{-/-}$ leukemia model.

**Two distinct population of Jak2 V617F/*p53*$^{-/-}$ leukemia possess leukemia-initiating capacity.**

Based on the above finding, I next examined the leukemia-initiating capacity of Jak2 V617F/*p53*$^{-/-}$ leukemia cells. From surface marker profiles of Jak2

V617F/*p53*$^{-/-}$ leukemia cells, three populations were sorted and transplanted into secondary recipients as follows; CD71$^{+}$ erythroid precursors, mature myeloid cells (Mac-1$^{+}$), and lineage negative (CD71$^{-}$Mac-1$^{-}$) primitive leukemia cells (Figure 16).

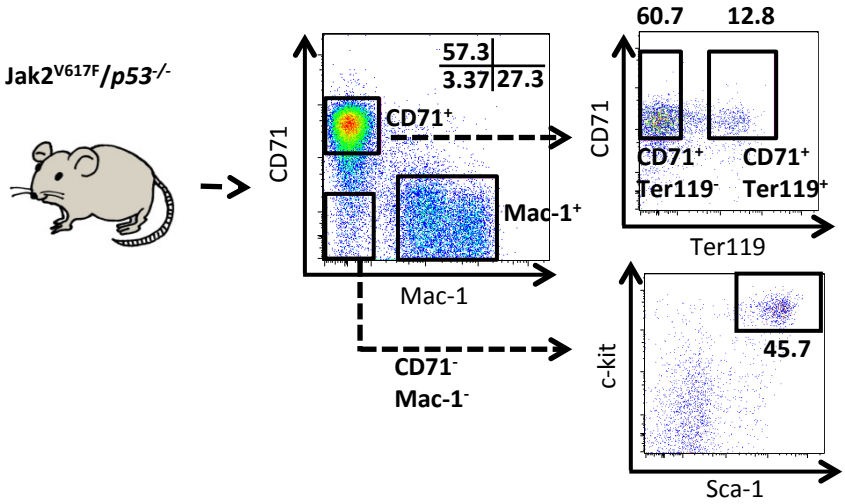


Figure 16. Flow cytometric gating of BM cells from Jak2 V617F/*p53*$^{-/-}$ erythroleukemia mice. Jak2 V617F/*p53*$^{-/-}$ leukemia cells could be divided into CD71$^{+}$, Mac-1$^{+}$ and CD71$^{-}$Mac-1$^{-}$ populations. CD71$^{+}$ population was further divided into Ter119$^{+}$ and Ter119$^{-}$ populations. About 45% of CD71$^{-}$Mac-1$^{-}$ cells showed lineage$^{-}$c-kit$^{+}$Sca-1$^{+}$ immunophenotype.

Morphologically, sorted CD71$^{-}$Mac-1$^{-}$ cells were mainly immature hematopoietic cells, and CD71$^{+}$ cells were erythroblasts, although almost all of Mac-1$^{+}$ cells were mature myeloid cells (Figure 17).

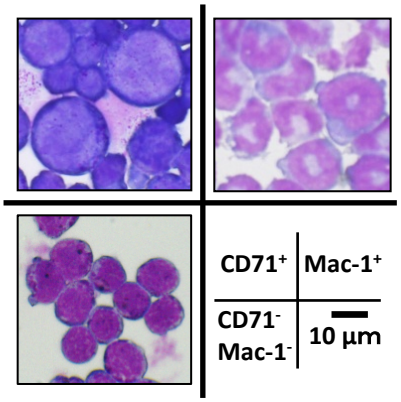


Figure 17. Representative photos of Wright-Giemsa-stained $CD71^+$, $Mac\text{-}1^+$ and $CD71^-Mac\text{-}1^-$ cells from Jak2 V617F/*p53*$^{-/-}$ mice.

Among three types of recipients with Jak2 V617F/*p53*$^{-/-}$ leukemia cells, all of transplants with $CD71^-Mac\text{-}1^-$ cells and those with $CD71^+$ cells died of leukemia while around 80% of recipients with $Mac\text{-}1^+$ cells from Jak2 V617F/*p53*$^{-/-}$ leukemic mice survived 60 days or more after BMT (Figure 18).

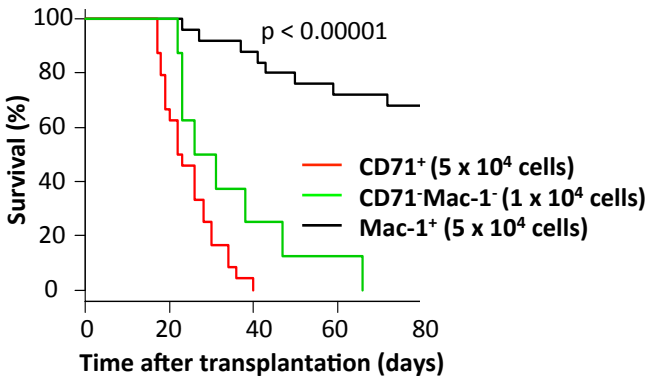


Figure 18. Kaplan-Meier plot of recipients with $CD71^+$ (n = 20), $CD71^-Mac\text{-}1^-$ (n = 6) and $Mac\text{-}1^+$ cells (n = 19). $p < 0.00001$; $CD71^+$ versus $Mac\text{-}1^+$ recipients. All

recipients with $CD71^+$ or $CD71^-Mac\text{-}1^-$ cells died of leukemia while about 70% of recipients with $Mac\text{-}1^+$ cells were free from leukemia.

It is of note that recipients with $CD71^+$ cells had earlier incidence of erythroleukemia with leukocytosis and anemia (Figure 18 and Figure 19).

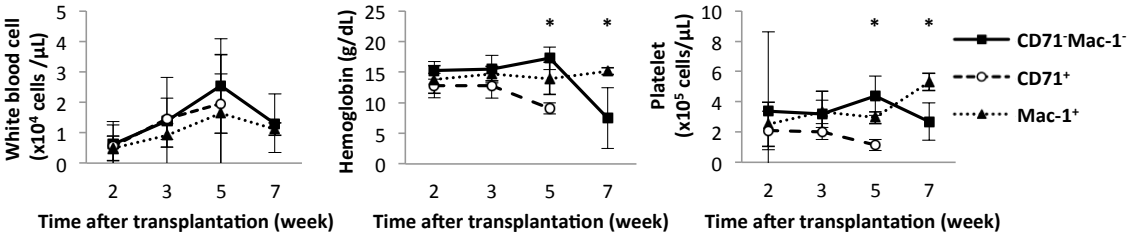


Figure 19. Data of complete blood cell count of PB from secondary recipients with $CD71^-Mac\text{-}1^-$ (n = 6), $CD71^+$ (n = 20) and $Mac\text{-}1^+$ (n = 19) Jak2 V617F/*p53*$^{-/-}$ leukemic cells (left: White blood cell, center: Hemoglobin, right: Platelet). The difference was significant between $CD71^+$ recipients and $CD71^-Mac\text{-}1^-$ at five weeks, and between $Mac\text{-}1^+$ recipients and $CD71^-Mac\text{-}1^-$ recipients at seven weeks post BMT. Data are mean ± SD. *$p < 0.05$.

These $CD71^+$ cell-transplanted mice had predominance of $CD71^+$ cells in the BM and spleen and showed hepatosplenomegaly, suggesting the leukemia-initiating capacity of $CD71^+$ cells in Jak2 V617F/*p53*$^{-/-}$ erythroleukemia (Figure 18).

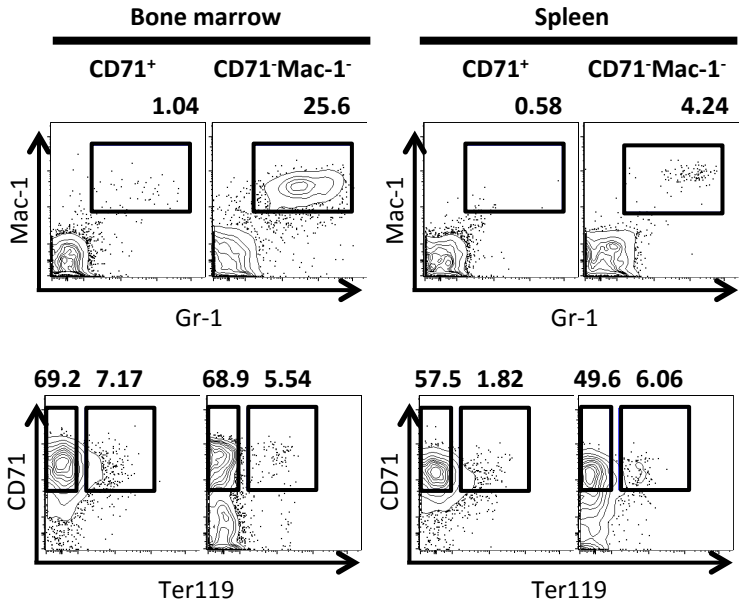


Figure 20. Representative flow cytmetric plots of BM and spleen from recipients with $CD71^+$ and $CD71^-Mac\text{-}1^-$ leukemic cells. BM and spleen from $CD71^+$ cell-transplanted mice were mostly consisted of $CD71^+$ cells. $CD71^-Mac\text{-}1^-$ cell-transplanted animals had both $CD71^+$ and myeloid populations in BM and spleen.

By contrast, recipients with $CD71^-Mac\text{-}1^-$ cells gave a temporal increase of WBC and Hb at three weeks after BMT, and succumbed to death after five weeks post BMT with progressive anemia, which implied the precedent polycythemic state in these recipients followed by highly-lethal leukemia (Figure 19). Indeed, the moribund mice transplanted with $CD71^-Mac\text{-}1^-$ cells had $CD71^+$ cell predominance in the BM and spleen with hepatosplenomegaly (Figure 20 and Table 3). Of interest, the BM from transplants with $CD71^-Mac\text{-}1^-$ cells had engraftment of both $CD71^+$ and $Gr\text{-}1^+Mac\text{-}1^+$ populations, suggesting the

erythroid and myeloid reconstituting capacity of Jak2 V617F/*p53*$^{-/-}$ CD71$^{-}$Mac-1$^{-}$ cells (Figure 20).

Since CD71$^{+}$ population from Jak2 V617F/*p53*$^{-/-}$ mice could be further subfractionated into CD71$^{+}$Ter119$^{-}$ immature erythroid precursors and CD71$^{+}$Ter119$^{+}$ mature erythroid progenitors, these two subpopulations were subjected to serial BMT for further characterization of leukemia-initiating cells (Figure 16). All of recipients with CD71$^{+}$Ter119$^{-}$ cells died of erythroleukemia (median; 33 days) with accumulation of CD71$^{+}$ cells in BM and spleen and with hepatosplenomegaly (Figure 21, Figure 22, Figure 23 and Table 4).

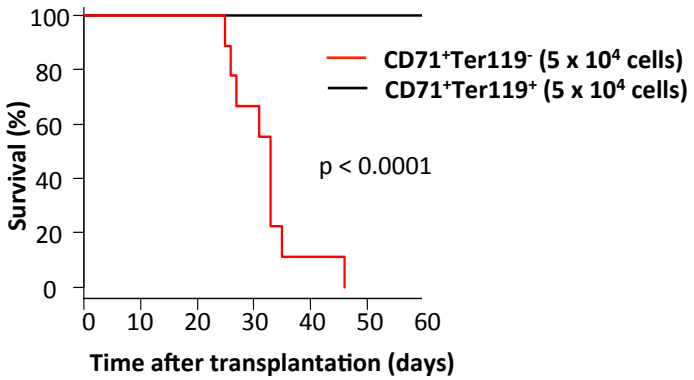


Figure 21. Kaplan-Meier plot of recipients with CD71$^{+}$Ter119$^{-}$ (n = 9) and CD71$^{+}$Ter119$^{+}$ cells (n = 8). All of CD71$^{+}$Ter119$^{-}$ cell-transplanted mice died by 50 days post BMT.

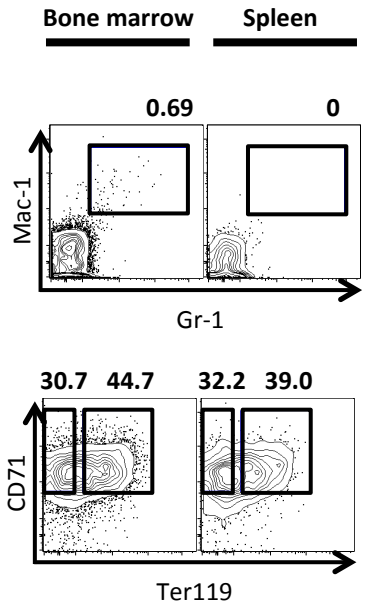


Figure 22. Representative flow cytmetric plots of BM and spleen from recipients with $CD71^{+}Ter119^{-}$ leukemic cells. Most of BM and spleen cells from these recipients showed $CD71^{+}$ immunophenotype.

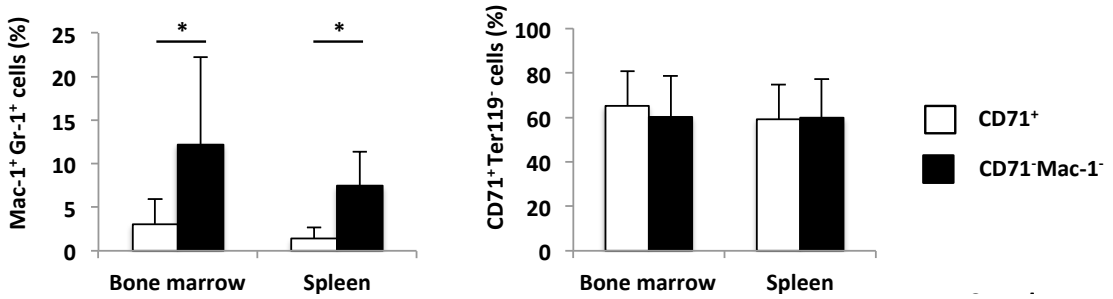


Figure 23. The percentages of $Mac\text{-}1^{+}Gr\text{-}1^{+}$ cells (left) and $CD71^{+}Ter119^{-}$ cells (right) in BM and spleen from secondary recipients with $CD71^{-}Mac\text{-}1^{-}$ (n = 6) and $CD71^{+}$ (n = 20) Jak2 V617F/*p53*$^{-/-}$ leukemic cells. Data are mean ± SD. *p < 0.05.

The lethality of $CD71^{+}Ter119^{-}$ cells was in sharp contrast to the negative potential of $CD71^{+}Ter119^{+}$ cells in Jak2 V617F/*p53*$^{-/-}$ erythroleukemia incidence (Figure 21). From these data, it was revealed that $CD71^{+}Ter119^{-}$ immature

erythroid compartment possesses leukemia-initiating capacity in Jak2 V617F/*p53*$^{-/-}$ mice.

When CD71$^{-}$Mac-1$^{-}$ cells in Jak2 V617F/*p53*$^{-/-}$ erythroleukemia, another population with leukemogenic potential, were assessed in detail for surface marker expression, most of these cells showed c-kit$^{+}$Sca-1$^{+}$ immunophenotype (Figure 16). Because of this finding, c-kit$^{+}$Sca-1$^{+}$lineage- (KSL) cells instead of CD71$^{-}$Mac-1$^{-}$ cells were subjected to the following experiments. To further assess the leukemogenic potential of CD71$^{+}$ and KSL cells from Jak2 V617F/*p53*$^{-/-}$ mice were transplanted into lethally irradiated mice. All of recipients with $5\times10^{4}$ CD71$^{+}$ cells died of erythroleukemia, and 20% of transplants with $1\times10^{4}$ CD71$^{+}$ cells could survive. By contrast, $1\times10^{4}$ KSL cells were enough to cause lethal leukemia in all of recipients (Figure 24)

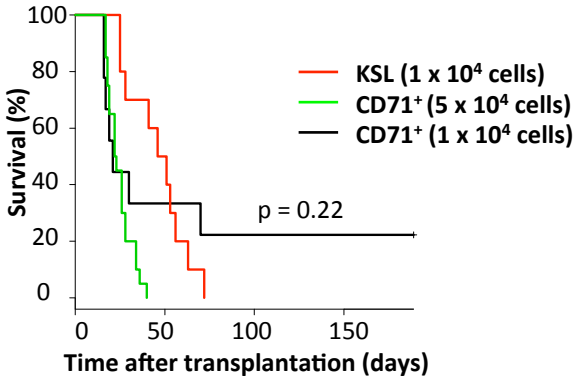


Figure 24. Kaplan-Meier plot of recipients with 1 x $10^4$ KSL (n = 10), 5 × $10^4$ $CD71^+$ (n = 20) and 1 × $10^4$ $CD71^+$ cells (n = 9). p = 0.22; 1 × $10^4$ KSL versus 1 × $10^4$ $CD71^+$ injected mice.

Recipients with KSL cells showed engraftment of mature myeloid cells in addition to dominating $CD71^+$ cells in the BM and spleen (Figure 25).

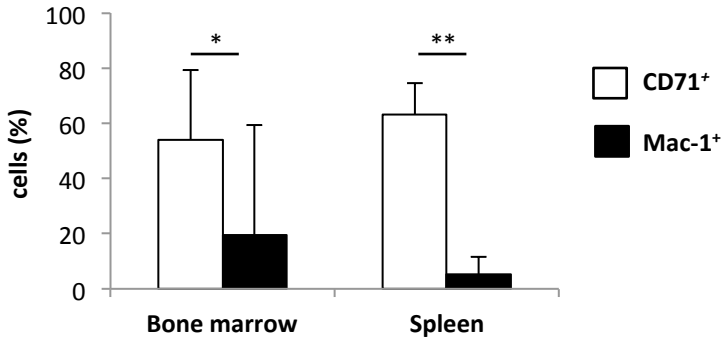


Figure 25. The percentages of $CD71^+$ cells (white bar) and Mac-$1^+$ cells (black bar) in BM and spleen from secondary recipients with KSL (n = 10) Jak2 V617F/*p53*$^{-/-}$ leukemic cells. Data are mean ± SD. *p < 0.05, **p < 0.005

Taken together, in my Jak2 V617F/*p53*$^{-/-}$ erythroleukemia model, it was revealed that both CD71$^{+}$ and KSL cells possess leukemia-initiating capacity and that the latter is more potent.

**Restoration of p53 limits in vitro proliferation of *p53*-null erythroleukemic cells and ameliorates in vivo leukemogenesis.**

From my above-mentioned data, p53 loss could initiate erythroid leukemia in Jak2 mutant-driven PV model. To examine the role of p53 in the maintenance of Jak2 V617F/*p53*$^{-/-}$ erythroleukemia, Jak2 V617F/*p53*$^{-/-}$ leukemia cells were retrovirally transduced with Kusabira Orange-labeled murine p53 WT, human TP53 WT, TP53 G135A mutant, or control vector (mock) and these cells were assessed in vitro and in vivo (Figure 26A-B).

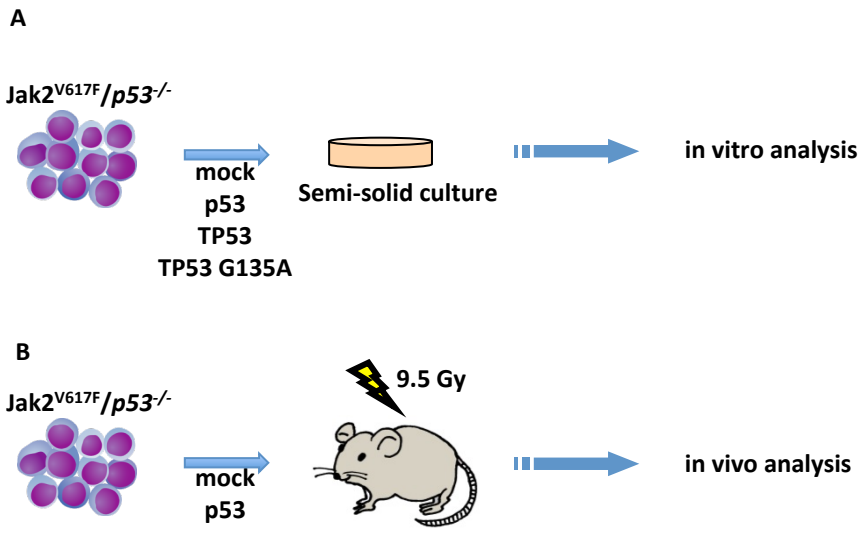


Figure 26. Schematic outlines of in vitro (A) and in vivo (B) experiments.

In in vitro colony forming assay, both murine p53 WT- and human TP53 WT-transduced Jak2 V617F/*p53*$^{-/-}$ cells showed a reduced colony-forming capacity (Figure 27 and Figure 28).

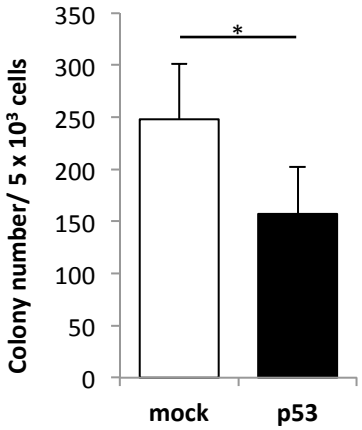


Figure 27. Colony assay using mock or p53-transduced Jak2 V617F/*p53*$^{-/-}$ cells (n = 5). Data are mean ± SD. *p < 0.05. The colony number of p53 WT-induced Jak2 V617F/*p53*$^{-/-}$ leukemia cells was lower that that of control cells.

On the other hand, colony forming capacity was not altered by TP53 G135A mutant, suggesting the functional role of TP53 WT in the maintenance of Jak2 V617F/*p53*$^{-/-}$-driven leukemogenesis (Figure 28).

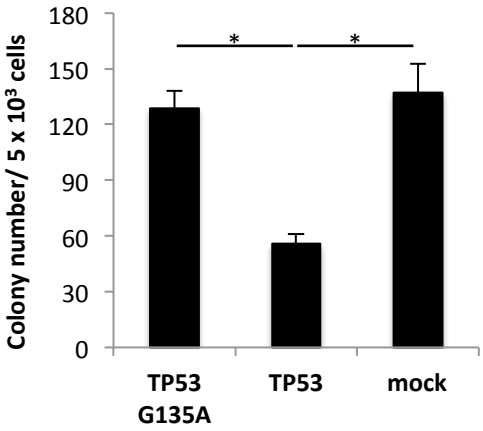


Figure 28. Colony assay using mock (n = 3), TP53 (n = 5) or TP53 G135A (n = 5)-transduced Jak2 V617F/*p53*$^{-/-}$ cells. TP53 WT-transduced leukemia cells had reduced colony forming capacity, however, TP53 mutant-treated leukemia cells showed comparable colony forming capacity to mock-treated leukemia cells. Data are mean ± SD. *$p < 0.05$.

The initial assessment of donor cell engraftment at three weeks after BMT in PB showed no difference between recipients with mock-transduced and p53-transduced erythroleukemia cells, suggesting the important role of p53 in Jak2 V617F/*p53*$^{-/-}$ erythroleukemia (Figure 29).

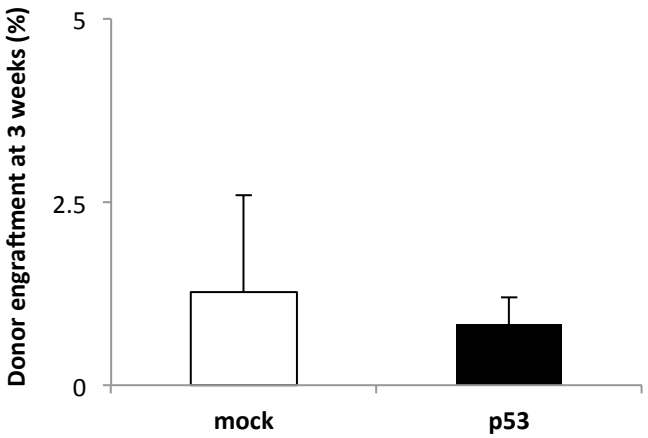


Figure 29. The donor cell engraftment rates (percentages of Jak2 V617F$^{+}$GFP(mock)$^{+}$ or Jak2 V617F$^{+}$p53 WT cells)in PB from recipients with mock (n = 3) or p53-transduced (n = 3) Jak2 V617F/*p53*$^{-/-}$ leukemic cells at three weeks post BMT. Data are mean ± SD.

Strikingly, transplants with p53-transduced Jak2 V617F/*p53*$^{-/-}$ cells appeared healthy even after seven weeks post BMT whereas half of recipient mice with mock-transduced Jak2 V617F/*p53*$^{-/-}$ cells died of leukemia with predominance of CD71$^{+}$ cells in the BM and spleen by day 50 post BMT (Figure 30).

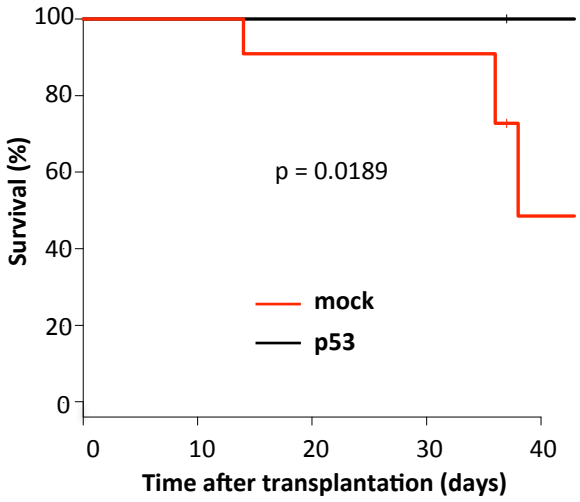


Figure 30. Kaplan-Meier plot of recipients with mock or p53-transduced Jak2 V617F/*p53*$^{-/-}$ cells (n = 6). About 50% of mice transplanted with mock-transduced Jak2 V617F/*p53*$^{-/-}$ cells died by 40 days after BMT. All recipients with p53-transduced Jak2 V617F/*p53*$^{-/-}$ cells survived 40 days post BMT.

**Jak2 V617F/*p53*$^{-/-}$ KSL cells are JAK inhibitor resistant.**

JAK2 is a promising druggable target and indeed JAK2 inhibitors show beneficial effect on JAK2-mutated MPN[34,35]. To examine the sensitivity of leukemia-initiating cells from Jak2 V617F/*p53*$^{-/-}$ mice to INCB18424, a potent JAK1/2 inhibitor, BM cells from Jak2 V617F/*p53*$^{-/-}$ or Jak2 V617F/*p53*$^{+/+}$ mice were treated with INCB18424 or DMSO and subjected to in vitro liquid culture (Figure 31).

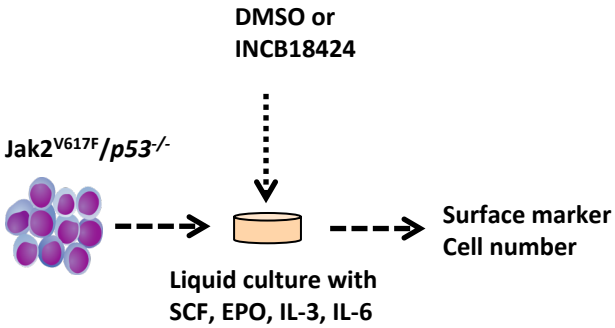


Figure 31. Schematic design of the experiment.

When $CD71^+$ and KSL cells from Jak2 V617F/*p53*$^{-/-}$ mice were assessed, INCB18424 could reduce $CD71^+$ leukemia cells in number while KSL cells showed insensitive to the drug (Figure. 32).

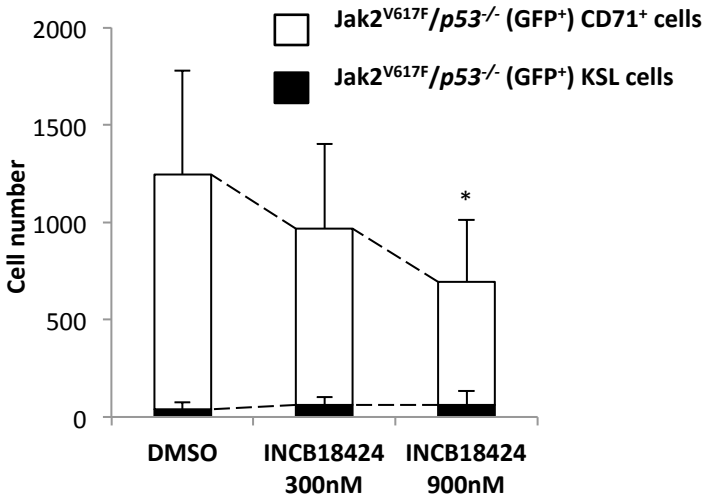


Figure 32. The numbers of Jak2 V617F/*p53*$^{-/-}$ $CD71^+$ and KSL cells were counted after cultivation with DMSO or INCB18424 (300 nM or 900 nM) (n = 3). INCB18424 treatment reduced the number of Jak2 V617F/*p53*$^{-/-}$ $CD71^+$ cells while KSL cells remained unchanged in number. Data are mean ± SD. *$p < 0.05$.

Consequently, these data indicate that these two types of leukemia-initiating cells have different responsiveness to pharmacologic Jak2 inhibition and that KSL cells might be the major cause of refractoriness of this erythroleukemia model against JAK inhibitor.

## Discussion

Despite its benign phenotype at initial stage, MPN is leukemia-prone, and once transformed, MPN patients have dramatic increase in mortality[4-7,14]. To cause leukemic transformation of MPN, some additional genetic mutations would be required, and p53 mutation is the leading one[8]. It is well known that p53 mutation is mainly a loss-of-function type and that p53 knockout mice are vulnerable to cancers[23,24]. In line with clinical relevance between JAK2 V617F mutant and p53 mutation, there exist erythroid and megakaryocytic leukemia cell lines coexpressing these two mutations[36], and molecularly, JAK2 V617F can inhibit DNA damage-induced p53 expression[37].

Here, in this study, I show that p53 loss could collaborate with Jak2 V617F mutant to cause erythroleukemia in mice after the precedent PV phase. The aggressive disease by p53 loss and Jak2 V617F is highlighted as extramedullary infiltration not only in the liver and the spleen but also in the lung, in which infiltrated leukemia cells collapsed pulmonary capillaries to induce severe hemorrhage. From my finding that p53 restoration could alleviate the disease (Figure 29), p53 loss in Jak2 V617F-induced PV mice is important for

both the initiation and the maintenance of leukemia. The reestablishment of p53 function has been shown effective in murine chronic myeloid leukemia[38]. My leukemia model is unique in that two distinct immunophenotypic populations have leukemia-initiating potential. $CD71^+$ Jak2 V617F/*p53*$^{-/-}$ cells compose a large part of the leukemic mice, and when serially transplanted, all recipients succumb to more rapid erythroleukemia than primary recipients (Figure 24). By contrast, KSL Jak2 V617F/*p53*$^{-/-}$ cells, a minority of leukemic cells, develop PV-like disease followed by full-blown $CD71^+$ erythroleukemia and these KSL cells still possess the differential potential to myeloid lineage (Figure 24). Notably, these KSL cells were quiescent and showed inhibition of apoptosis in comparison to KSL Jak2 V617F/$p53^{+/+}$ cells (Figure 10 and 11). Leukemia-initiating cells (LICs) were mainly in G0 phase and resistant to chemotherapy which depends on cell cycle[39,40]. In addition, LICs have similar self-renewal systems in BM as normal hematopoietic stem cells[21]. These data suggest that KSL Jak2 V617F/p53-/- has leukemia-initiating potential and reduction of apoptosis led by p53 defect might contribute to leukemic transformation from Jak2 V617F derived MPN. Since Mac-$1^+$ Jak2 V617F/*p53*$^{-/-}$ cells are less potent in leukemia development (Figure 18), LICs in this leukemia

model comprise a distinct lineage hierarchy. However, the existence of two immunophenotypically different LICs suggests an atypical hierarchy in this model (Figure 33).

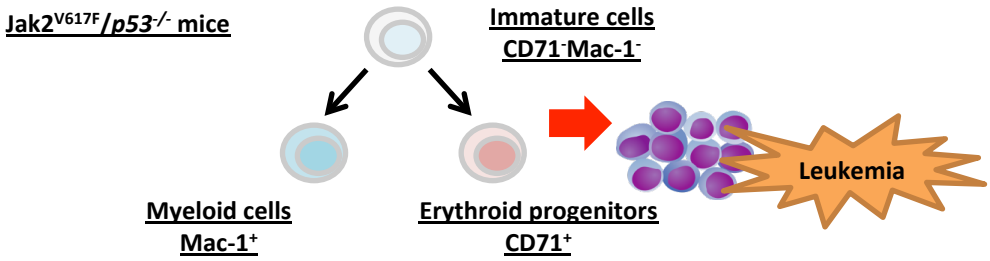


Figure 33. Summary of Jak2 V617F/*p53*$^{-/-}$ erythroleukemia.

Several groups have reported the immunophenotypic heterogeneity of LICs. In a murine leukemia model by HoxA9 and Meis1, both Gr-1$^{+}$c-kit$^{+}$ cells and c-kit$^{+}$ cells coexpressing lymphoid markers can initiate leukemia[41]. In human AML, both multipotent progenitors and granulocyte-macrophage progenitors have leukemia-initiating potential in immunodeficient mice[42,43].

Of interest, the combination of p53 loss and Jak2 V617F caused not myeloid leukemia but erythroleukemia in mice. It has been reported that retroviral overexpression or transgenic expression of Jak2V617F is sufficient to cause MPN in mice and the level of Jak2 mutant defines MPN phenotype[9,12,13].

In my study, all of Jak2 V617F/*p53*$^{+/+}$ mice had PV, not essential thrombocythemia (ET)-like phenotype, suggesting the high level of Jak2 expression. This highly-expressed Jak2 mutant would have led erythroleukemia in combination with cancer-prone *p53*$^{-/-}$ cells. It is also possible that expanded HSCs by p53 deletion would be relevant to the maintenance of KSL Jak2 V617F/*p53*$^{-/-}$ leukemic cells[44-47]. It is to be elucidated whether low Jak2 V617F in *p53*$^{-/-}$ cells could induce megakaryocytic leukemia in mice or not. Considering no leukemia incidence in Jak2 V617F/*p53*$^{+/-}$ mice by 120 days post BMT and the suppressive role of p53 in Jak2 V617F/*p53*$^{-/-}$ leukemia (Figure 2 and 29), it is possible that p53 nullizygousity would hold the key for leukemic transformation of Jak2 V617F MPN. In this study, 30% of Jak2 V617F/*p53*$^{+/-}$ and 10% of Jak2 V617F/*p53*$^{+/+}$ mice died by 120 days post BMT, but these mice showed no leukemia development. Because Jacks et al have reported that almost 30% of *p53*$^{+/-}$ mice tend to develop various neoplasms by 300 days of age[24], it is still of note that Jak2 V617F/*p53*$^{+/-}$ mice would develop leukemia with long latency. Most of TP53-related alterations in JAK2 V617F positive MPN patients with leukemic transformation are bi-allelic[17]. In addition to the loss-of-function role of p53, carcinogenensis has been reported to be associated with gain-of-function

role of p53[48]. It remains a matter of research which type of AML in FAB subclassification is more prevalent in mutant TP53-related JAK2 V617F positive leukemia. For the further clarification of the mechanisms in leukemic transformation of MPN, the functional role of mutation-derived truncated p53 should be elaborated.

In this study, MLL-ENL did not collaborate with p53 loss on leukemogenesis (Figure 12). Indeed, some groups have shown that p53 mutant and MLL fusion rarely coexist in human AML[49,50]. Beer et al have reported that MPL W515L induces MPN, TET2 mutation and TP53 mutation (loss of function) collaborate to develop leukemia[51,52]. I revealed that Mpl W515L/*p53*$^{-/-}$ MPN mice demonstrated shorter survival than those of Mpl W515L/*p53*$^{+/+}$ MPN mice, but no sign of leukemia development. When I consider the difference of phenotype between Jak2 V617F/*p53*$^{-/-}$ leukemia mice and Mpl W515L/*p53*$^{-/-}$ MPN mice, it is still possible that Mpl W515L would collaborate with p53 loss to cause leukemia with long duration. To prove that possibility, some animal models other than retroviral Mpl W515L overexpression are warranted because in this study Mpl W515L/*p53*$^{+/+}$ MPN mice died of MPN exacerbation by 56 days post BMT in clear contrast to viable Jak2 V617F/ *p53*$^{+/+}$mice. Genetic instability caused by

p53 defect could also be causative for shorter survival of Mpl W515L/*p53*$^{-/-}$ mice[24,53,54]. These results suggest that leukemic transformation of absence of p53 function might be specific to Jak2 V617F induced MPN.

The importance of my new erythroleukemia model lies in the rarity of murine erythroleukemia model so far. In my understanding, overexpression of BCR-ABL into *Cebpa* knockout fetal liver cells can induce erythroleukemia in mice, in which CD71$^{+}$Ter119$^{+}$ differentiated erythroid cells dominate[55]. The disease would be caused by the differentiation block of myeloid lineage and the skewed erythroid differentiation in *Cebpa* knockout cells. The novelty of my model also lies in that Jak2 V617F/*p53*$^{-/-}$ leukemia is immature erythroid leukemia with a dominated CD71$^{+}$Ter119$^{-}$ population.

Furthermore, KSL Jak2 V617F/*p53*$^{-/-}$ leukemic cells showed resistance to JAK inhibitor, while CD71$^{+}$ erythroid cells were sensitive to JAK inhibition. It has been reported that JAK2 inhibitor can not eradicate the disease burden in most MPN cases[56], indicating the further exploration of new drugs. The resistance to selective tyrosine kinases in leukemic stem cells has been reported to be derived from several mechanisms including quiescence and less addiction to oncogene[57-59]. Seen in that light, my leukemia model would also be a valuable

tool for drug screening. Drugs which can eliminate not only CD71+ cells but also KSL Jak2 V617F/*p53*$^{-/-}$ leukemic stem cells would promise the cure of leukemias transformed from Jak2 V617F-positive MPN.

In conclusion, p53 loss induces leukemic transformation of Jak2 V617F-driven PV with two distinct populations as leukemia-initiating cells. This leukemia model is CD71$^{+}$Ter119$^{-}$ immature erythroid leukemia, providing a useful platform for the screening of new drugs against Jak2 V617F-positive leukemias. Utilizing this model, it is highly expected that an outstanding drug to compensate mutated p53-derived alteration in p53-mutated MPN patients would appear on the scene.

**Acknowledgement**

I express my hearty thanks to Professor Mineo Kurokawa for the leadership and advices. I also thank K. Kataoka, J. Koya, H. Kobayashi, K. Narukawa, Y. Sumitomo and T. Sato for their continued support. I thank T. Kitamura for Plat-E packaging cells; H. Nakauchi and M. Onodera for pGCDNsam-IRES-EGFP and Kusabira Orange retroviral vector; S. Aizawa for *p53* knockout mice (RIKEN BRC, Acc. No. CDB 0001K); F. Ueki, R. Takizawa-Toyama and Y. Oikawa for expert assistance. This study was supported by the Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

## References

1 Pikman, Y., Lee, B. H., Mercher, T., McDowell, E., Ebert, B. L., Gozo, M., Cuker, A., Wernig, G., Moore, S., Galinsky, I., DeAngelo, D. J., Clark, J. J., Lee, S. J., Golub, T. R., Wadleigh, M., Gilliland, D. G. & Levine, R. L. MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. *PLoS Med* **3**, e270, (2006).

2 Klampfl, T., Gisslinger, H., Harutyunyan, A. S., Nivarthi, H., Rumi, E., Milosevic, J. D., Them, N. C., Berg, T., Gisslinger, B., Pietra, D., Chen, D., Vladimer, G. I., Bagienski, K., Milanesi, C., Casetti, I. C., Sant'Antonio, E., Ferretti, V., Elena, C., Schischlik, F., Cleary, C., Six, M., Schalling, M., Schonegger, A., Bock, C., Malcovati, L., Pascutto, C., Superti-Furga, G., Cazzola, M. & Kralovics, R. Somatic mutations of calreticulin in myeloproliferative neoplasms. *N Engl J Med* **369**, 2379-2390, (2013).

3 Nangalia, J., Massie, C. E., Baxter, E. J., Nice, F. L., Gundem, G., Wedge, D. C., Avezov, E., Li, J., Kollmann, K., Kent, D. G., Aziz, A., Godfrey, A. L., Hinton, J., Martincorena, I., Van Loo, P., Jones, A. V., Guglielmelli, P., Tarpey, P., Harding, H. P., Fitzpatrick, J. D., Goudie, C. T., Ortmann, C. A.,

Loughran, S. J., Raine, K., Jones, D. R., Butler, A. P., Teague, J. W., O'Meara, S., McLaren, S., Bianchi, M., Silber, Y., Dimitropoulou, D., Bloxham, D., Mudie, L., Maddison, M., Robinson, B., Keohane, C., Maclean, C., Hill, K., Orchard, K., Tauro, S., Du, M. Q., Greaves, M., Bowen, D., Huntly, B. J., Harrison, C. N., Cross, N. C., Ron, D., Vannucchi, A. M., Papaemmanuil, E., Campbell, P. J. & Green, A. R. Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. *N Engl J Med* **369**, 2391-2405, (2013).

4 James, C., Ugo, V., Le Couedic, J. P., Staerk, J., Delhommeau, F., Lacout, C., Garcon, L., Raslova, H., Berger, R., Bennaceur-Griscelli, A., Villeval, J. L., Constantinescu, S. N., Casadevall, N. & Vainchenker, W. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. *Nature* **434**, 1144-1148, (2005).

5 Levine, R. L., Wadleigh, M., Cools, J., Ebert, B. L., Wernig, G., Huntly, B. J., Boggon, T. J., Wlodarska, I., Clark, J. J., Moore, S., Adelsperger, J., Koo, S., Lee, J. C., Gabriel, S., Mercher, T., D'Andrea, A., Frohling, S., Dohner, K., Marynen, P., Vandenberghe, P., Mesa, R. A., Tefferi, A., Griffin, J. D., Eck, M. J., Sellers, W. R., Meyerson, M., Golub, T. R., Lee, S. J. & Gilliland, D. G. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera,

essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer Cell* **7**, 387-397, (2005).

6 Baxter, E. J., Scott, L. M., Campbell, P. J., East, C., Fourouclas, N., Swanton, S., Vassiliou, G. S., Bench, A. J., Boyd, E. M., Curtin, N., Scott, M. A., Erber, W. N. & Green, A. R. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *Lancet* **365**, 1054-1061, (2005).

7 Kralovics, R., Passamonti, F., Buser, A. S., Teo, S. S., Tiedt, R., Passweg, J. R., Tichelli, A., Cazzola, M. & Skoda, R. C. A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med* **352**, 1779-1790, (2005).

8 Campbell, P. J. & Green, A. R. The myeloproliferative disorders. *N Engl J Med* **355**, 2452-2466, (2006).

9 Lacout, C., Pisani, D. F., Tulliez, M., Gachelin, F. M., Vainchenker, W. & Villeval, J. L. JAK2V617F expression in murine hematopoietic cells leads to MPD mimicking human PV with secondary myelofibrosis. *Blood* **108**, 1652-1660, (2006).

10 Mullally, A., Lane, S. W., Ball, B., Megerdichian, C., Okabe, R., Al-Shahrour, F., Paktinat, M., Haydu, J. E., Housman, E., Lord, A. M., Wernig,

G., Kharas, M. G., Mercher, T., Kutok, J. L., Gilliland, D. G. & Ebert, B. L. Physiological Jak2V617F expression causes a lethal myeloproliferative neoplasm with differential effects on hematopoietic stem and progenitor cells. *Cancer Cell* **17**, 584-596, (2010).

11 Mullally, A., Poveromo, L., Schneider, R. K., Al-Shahrour, F., Lane, S. W. & Ebert, B. L. Distinct roles for long-term hematopoietic stem cells and erythroid precursor cells in a murine model of Jak2V617F-mediated polycythemia vera. *Blood* **120**, 166-172, (2012).

12 Wernig, G., Mercher, T., Okabe, R., Levine, R. L., Lee, B. H. & Gilliland, D. G. Expression of Jak2V617F causes a polycythemia vera-like disease with associated myelofibrosis in a murine bone marrow transplant model. *Blood* **107**, 4274-4281, (2006).

13 Marty, C., Lacout, C., Martin, A., Hasan, S., Jacquot, S., Birling, M. C., Vainchenker, W. & Villeval, J. L. Myeloproliferative neoplasm induced by constitutive expression of JAK2V617F in knock-in mice. *Blood* **116**, 783-787, (2010).

14 Kennedy, J. A., Atenafu, E. G., Messner, H. A., Craddock, K. J., Brandwein, J. M., Lipton, J. H., Minden, M. D., Schimmer, A. D., Schuh, A. C.,

Yee, K. W. & Gupta, V. Treatment outcomes following leukemic transformation in Philadelphia-negative myeloproliferative neoplasms. *Blood* **121**, 2725-2733, (2013).

15 Abdel-Wahab, O., Manshouri, T., Patel, J., Harris, K., Yao, J., Hedvat, C., Heguy, A., Bueso-Ramos, C., Kantarjian, H., Levine, R. L. & Verstovsek, S. Genetic analysis of transforming events that convert chronic myeloproliferative neoplasms to leukemias. *Cancer Res* **70**, 447-452, (2010).

16 Zhang, S. J., Rampal, R., Manshouri, T., Patel, J., Mensah, N., Kayserian, A., Hricik, T., Heguy, A., Hedvat, C., Gonen, M., Kantarjian, H., Levine, R. L., Abdel-Wahab, O. & Verstovsek, S. Genetic analysis of patients with leukemic transformation of myeloproliferative neoplasms shows recurrent SRSF2 mutations that are associated with adverse outcome. *Blood* **119**, 4480-4485, (2012).

17 Harutyunyan, A., Klampfl, T., Cazzola, M. & Kralovics, R. p53 lesions in leukemic transformation. *N Engl J Med* **364**, 488-490, (2011).

18 Pant, V., Quintas-Cardama, A. & Lozano, G. The p53 pathway in hematopoiesis: lessons from mouse models, implications for humans. *Blood* **120**, 5118-5127, (2012).

19 Breems, D. A., Van Putten, W. L., Huijgens, P. C., Ossenkoppele, G. J., Verhoef, G. E., Verdonck, L. F., Vellenga, E., De Greef, G. E., Jacky, E., Van der Lelie, J., Boogaerts, M. A. & Lowenberg, B. Prognostic index for adult patients with acute myeloid leukemia in first relapse. *J Clin Oncol* **23**, 1969-1978, (2005).

20 Ofran, Y. & Rowe, J. M. Treatment for relapsed acute myeloid leukemia: what is new? *Curr Opin Hematol* **19**, 89-94, (2012).

21 Kikushige, Y., Shima, T., Takayanagi, S., Urata, S., Miyamoto, T., Iwasaki, H., Takenaka, K., Teshima, T., Tanaka, T., Inagaki, Y. & Akashi, K. TIM-3 is a promising target to selectively kill acute myeloid leukemia stem cells. *Cell stem cell* **7**, 708-717, (2010).

22 Kurosawa, S., Yamaguchi, T., Miyawaki, S., Uchida, N., Sakura, T., Kanamori, H., Usuki, K., Yamashita, T., Okoshi, Y., Shibayama, H., Nakamae, H., Mawatari, M., Hatanaka, K., Sunami, K., Shimoyama, M., Fujishima, N., Maeda, Y., Miura, I., Takaue, Y. & Fukuda, T. Prognostic factors and outcomes of adult patients with acute myeloid leukemia after first relapse. *Haematologica* **95**, 1857-1864, (2010).

23 Olivier, M., Hollstein, M. & Hainaut, P. TP53 mutations in human cancers: origins, consequences, and clinical use. *Cold Spring Harb Perspect Biol* **2**, a001008, (2010).

24 Donehower, L. A., Harvey, M., Slagle, B. L., McArthur, M. J., Montgomery, C. A., Jr., Butel, J. S. & Bradley, A. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature* **356**, 215-221, (1992).

25 Barlow, J. L., Drynan, L. F., Hewett, D. R., Holmes, L. R., Lorenzo-Abalde, S., Lane, A. L., Jolin, H. E., Pannell, R., Middleton, A. J., Wong, S. H., Warren, A. J., Wainscoat, J. S., Boultwood, J. & McKenzie, A. N. A p53-dependent mechanism underlies macrocytic anemia in a mouse model of human 5q- syndrome. *Nat Med* **16**, 59-66, (2010).

26 Xu, H., Menendez, S., Schlegelberger, B., Bae, N., Aplan, P. D., Gohring, G., Deblasio, T. R. & Nimer, S. D. Loss of p53 accelerates the complications of myelodysplastic syndrome in a NUP98-HOXD13-driven mouse model. *Blood* **120**, 3089-3097, (2012).

27 Tsukada, T., Tomooka, Y., Takai, S., Ueda, Y., Nishikawa, S., Yagi, T., Tokunaga, T., Takeda, N., Suda, Y., Abe, S. & et al. Enhanced proliferative

potential in culture of cells from p53-deficient mice. *Oncogene* **8**, 3313-3322, (1993).

28 Nam, C., Doi, K. & Nakayama, H. Etoposide induces G2/M arrest and apoptosis in neural progenitor cells via DNA damage and an ATM/p53-related pathway. *Histol Histopathol* **25**, 485-493, (2010).

29 Kogan, S. C., Ward, J. M., Anver, M. R., Berman, J. J., Brayton, C., Cardiff, R. D., Carter, J. S., de Coronado, S., Downing, J. R., Fredrickson, T. N., Haines, D. C., Harris, A. W., Harris, N. L., Hiai, H., Jaffe, E. S., MacLennan, I. C., Pandolfi, P. P., Pattengale, P. K., Perkins, A. S., Simpson, R. M., Tuttle, M. S., Wong, J. F. & Morse, H. C., 3rd. Bethesda proposals for classification of nonlymphoid hematopoietic neoplasms in mice. *Blood* **100**, 238-245, (2002).

30 Look, A. T. Oncogenic transcription factors in the human acute leukemias. *Science* **278**, 1059-1064, (1997).

31 Rowley, J. D. The critical role of chromosome translocations in human leukemias. *Annu Rev Genet* **32**, 495-519, (1998).

32 Hess, J. L. MLL: a histone methyltransferase disrupted in leukemia. *Trends Mol Med* **10**, 500-507, (2004).

33 Cozzio, A., Passegue, E., Ayton, P. M., Karsunky, H., Cleary, M. L. & Weissman, I. L. Similar MLL-associated leukemias arising from self-renewing stem cells and short-lived myeloid progenitors. *Genes & development* **17**, 3029-3035, (2003).

34 Harrison, C., Kiladjian, J. J., Al-Ali, H. K., Gisslinger, H., Waltzman, R., Stalbovskaya, V., McQuitty, M., Hunter, D. S., Levy, R., Knoops, L., Cervantes, F., Vannucchi, A. M., Barbui, T. & Barosi, G. JAK inhibition with ruxolitinib versus best available therapy for myelofibrosis. *N Engl J Med* **366**, 787-798, (2012).

35 Verstovsek, S., Mesa, R. A., Gotlib, J., Levy, R. S., Gupta, V., DiPersio, J. F., Catalano, J. V., Deininger, M., Miller, C., Silver, R. T., Talpaz, M., Winton, E. F., Harvey, J. H., Jr., Arcasoy, M. O., Hexner, E., Lyons, R. M., Paquette, R., Raza, A., Vaddi, K., Erickson-Viitanen, S., Koumenis, I. L., Sun, W., Sandor, V. & Kantarjian, H. M. A double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis. *N Engl J Med* **366**, 799-807, (2012).

36 Zhao, W., Du, Y., Ho, W. T., Fu, X. & Zhao, Z. J. JAK2V617F and p53 mutations coexist in erythroleukemia and megakaryoblastic leukemic cell lines. *Exp Hematol Oncol* **1**, 15, (2012).

37 Nakatake, M., Monte-Mor, B., Debili, N., Casadevall, N., Ribrag, V., Solary, E., Vainchenker, W. & Plo, I. JAK2(V617F) negatively regulates p53 stabilization by enhancing MDM2 via La expression in myeloproliferative neoplasms. *Oncogene* **31**, 1323-1333, (2012).

38 Velasco-Hernandez, T., Vicente-Duenas, C., Sanchez-Garcia, I. & Martin-Zanca, D. p53 restoration kills primitive leukemia cells in vivo and increases survival of leukemic mice. *Cell Cycle* **12**, 122-132, (2013).

39 Guan, Y., Gerhard, B. & Hogge, D. E. Detection, isolation, and stimulation of quiescent primitive leukemic progenitor cells from patients with acute myeloid leukemia (AML). *Blood* **101**, 3142-3149, (2003).

40 Ishikawa, F., Yoshida, S., Saito, Y., Hijikata, A., Kitamura, H., Tanaka, S., Nakamura, R., Tanaka, T., Tomiyama, H., Saito, N., Fukata, M., Miyamoto, T., Lyons, B., Ohshima, K., Uchida, N., Taniguchi, S., Ohara, O., Akashi, K., Harada, M. & Shultz, L. D. Chemotherapy-resistant human AML stem cells home to and engraft within the bone-marrow endosteal region. *Nature biotechnology* **25**, 1315-1321, (2007).

41 Gibbs, K. D., Jr., Jager, A., Crespo, O., Goltsev, Y., Trejo, A., Richard, C. E. & Nolan, G. P. Decoupling of tumor-initiating activity from stable

immunophenotype in HoxA9-Meis1-driven AML. *Cell stem cell* **10**, 210-217, (2012).

42 Goardon, N., Marchi, E., Atzberger, A., Quek, L., Schuh, A., Soneji, S., Woll, P., Mead, A., Alford, K. A., Rout, R., Chaudhury, S., Gilkes, A., Knapper, S., Beldjord, K., Begum, S., Rose, S., Geddes, N., Griffiths, M., Standen, G., Sternberg, A., Cavenagh, J., Hunter, H., Bowen, D., Killick, S., Robinson, L., Price, A., Macintyre, E., Virgo, P., Burnett, A., Craddock, C., Enver, T., Jacobsen, S. E., Porcher, C. & Vyas, P. Coexistence of LMPP-like and GMP-like leukemia stem cells in acute myeloid leukemia. *Cancer Cell* **19**, 138-152, (2011).

43 Sarry, J. E., Murphy, K., Perry, R., Sanchez, P. V., Secreto, A., Keefer, C., Swider, C. R., Strzelecki, A. C., Cavelier, C., Recher, C., Mansat-De Mas, V., Delabesse, E., Danet-Desnoyers, G. & Carroll, M. Human acute myelogenous leukemia stem cells are rare and heterogeneous when assayed in NOD/SCID/IL2Rgammac-deficient mice. *J Clin Invest* **121**, 384-395, (2011).

44 Liu, Y., Elf, S. E., Miyata, Y., Sashida, G., Huang, G., Di Giandomenico, S., Lee, J. M., Deblasio, A., Menendez, S., Antipin, J., Reva, B.,

Koff, A. & Nimer, S. D. p53 regulates hematopoietic stem cell quiescence. *Cell stem cell* **4**, 37-48, (2009).

45 TeKippe, M., Harrison, D. E. & Chen, J. Expansion of hematopoietic stem cell phenotype and activity in Trp53-null mice. *Exp Hematol* **31**, 521-527, (2003).

46 Akala, O. O., Park, I. K., Qian, D., Pihalja, M., Becker, M. W. & Clarke, M. F. Long-term haematopoietic reconstitution by Trp53-/-p16Ink4a-/-p19Arf-/- multipotent progenitors. *Nature* **453**, 228-232, (2008).

47 Chen, J., Ellison, F. M., Keyvanfar, K., Omokaro, S. O., Desierto, M. J., Eckhaus, M. A. & Young, N. S. Enrichment of hematopoietic stem cells with SLAM and LSK markers for the detection of hematopoietic stem cell function in normal and Trp53 null mice. *Exp Hematol* **36**, 1236-1243, (2008).

48 Oren, M. & Rotter, V. Mutant p53 gain-of-function in cancer. *Cold Spring Harb Perspect Biol* **2**, a001107, (2010).

49 Megonigal, M. D., Rappaport, E. F., Nowell, P. C., Lange, B. J. & Felix, C. A. Potential role for wild-type p53 in leukemias with MLL gene translocations. *Oncogene* **16**, 1351-1356, (1998).

50 Haferlach, C., Dicker, F., Herholz, H., Schnittger, S., Kern, W. & Haferlach, T. Mutations of the TP53 gene in acute myeloid leukemia are strongly associated with a complex aberrant karyotype. *Leukemia* **22**, 1539-1541, (2008).

51 Beer, P. A., Ortmann, C. A., Stegelmann, F., Guglielmelli, P., Reilly, J. T., Larsen, T. S., Hasselbalch, H. C., Vannucchi, A. M., Moller, P., Dohner, K. & Green, A. R. Molecular mechanisms associated with leukemic transformation of MPL-mutant myeloproliferative neoplasms. *Haematologica* **95**, 2153-2156, (2010).

52 He, X., Chen, Z., Jiang, Y., Qiu, X. & Zhao, X. Different mutations of the human c-mpl gene indicate distinct haematopoietic diseases. *Journal of hematology & oncology* **6**, 11, (2013).

53 Kagoya, Y., Yoshimi, A., Tsuruta-Kishino, T., Arai, S., Satoh, T., Akira, S. & Kurokawa, M. JAK2V617F+ myeloproliferative neoplasm clones evoke paracrine DNA damage to adjacent normal cells through secretion of lipocalin-2. *Blood* **124**, 2996-3006, (2014).

54 Donehower, L. A., Godley, L. A., Aldaz, C. M., Pyle, R., Shi, Y. P., Pinkel, D., Gray, J., Bradley, A., Medina, D. & Varmus, H. E. Deficiency of p53

accelerates mammary tumorigenesis in Wnt-1 transgenic mice and promotes chromosomal instability. *Genes & development* **9**, 882-895, (1995).

55 Wagner, K., Zhang, P., Rosenbauer, F., Drescher, B., Kobayashi, S., Radomska, H. S., Kutok, J. L., Gilliland, D. G., Krauter, J. & Tenen, D. G. Absence of the transcription factor CCAAT enhancer binding protein alpha results in loss of myeloid identity in bcr/abl-induced malignancy. *Proc Natl Acad Sci U S A* **103**, 6338-6343, (2006).

56 Tefferi, A. JAK inhibitors for myeloproliferative neoplasms: clarifying facts from myths. *Blood* **119**, 2721-2730, (2012).

57 Pei, S. & Jordan, C. T. How close are we to targeting the leukemia stem cell? *Best Pract Res Clin Haematol* **25**, 415-418, (2012).

58 Saito, Y., Kitamura, H., Hijikata, A., Tomizawa-Murasawa, M., Tanaka, S., Takagi, S., Uchida, N., Suzuki, N., Sone, A., Najima, Y., Ozawa, H., Wake, A., Taniguchi, S., Shultz, L. D., Ohara, O. & Ishikawa, F. Identification of therapeutic targets for quiescent, chemotherapy-resistant human leukemia stem cells. *Sci Transl Med* **2**, 17ra19, (2010).

59 Pellicano, F., Mukherjee, L. & Holyoake, T. L. Cancer cells escape from oncogene addiction: Understanding the mechanisms behind treatment failure for more effective targeting. *Stem Cells*, (2014).

## Tables

Table 1. A list of antibodies for flowcytometry

| Epitope | Clone | Fluorophore | Dilution | Supplier |
|---|---|---|---|---|
| Gr-1 | RB6-8C5 | Biotin | 1:200 | Biolegend |
| | RB6-8C5 | APC | 1:200 | Biolegend |
| Mac-1 | M1/70 | Biotin | 1:200 | Biolegend |
| | M1/70 | APC | 1:200 | Biolegend |
| B220 | RA3-6B2 | Biotin | 1:200 | Biolegend |
| | RA3-6B2 | APC | 1:200 | Biolegend |
| Ter119 | TER-119 | Biotin | 1:200 | Biolegend |
| Ter119 | TER-119 | APC | 1:200 | Biolegend |
| CD3ε | 145-2C11 | Biotin | 1:200 | Biolegend |
| CD4 | GK1.5 | Biotin | 1:200 | Biolegend |
| CD8a | 53-6.7 | Biotin | 1:200 | Biolegend |
| CD127 | A7R34 | Biotin | 1:200 | Biolegend |
| Sca-1 | D7 | PE-Cy7 | 1:200 | Biolegend |
| c-kit | 2B8 | APC | 1:200 | Biolegend |
| CD71 | C2 | PE | 1:200 | BD |
| CD41 | eBioMWReg30 | APC | 1:200 | eBio |

Table 2. Average weight of liver and spleen and a number of mice which have pulmonary hemorrhage in secondary recipients (n = 10), Jak2 WT/*p53*$^{+/+}$ (control) (n = 10) and Jak2 WT/*p53*$^{-/-}$ (control) (n = 10)).

| | 2nd BMT (Jak2 V617F/*p53*$^{-/-}$) | Jak2 WT/*p53*$^{+/+}$ (control) | Jak2 WT/*p53*$^{-/-}$ (control) |
|---|---|---|---|
| Liver (mg) | 2582.9±338.1 | 1199.1±87.9 | 1035.6±165.4 |
| Spleen (mg) | 727.4±239.8 | 98.2±30.0 | 102.9±22.3 |
| Pneumonorrhagia | 10/10 mice | 0/10 mice | 0/10 mice |

Table 3. Average weight of liver and spleen in $CD71^+$(n = 20), $CD71^-Mac\text{-}1^-$ (n = 6), Jak2 WT/$p53^{+/+}$ (control) (n = 10) and Jak2 WT/$p53^{-/-}$ (control) (n = 10)).

| | $CD71^+$ | $CD71^-Mac\text{-}1^-$ | Jak2 WT/$p53^{+/+}$ (control) | Jak2 WT/$p53^{-/-}$ (control) |
|---|---|---|---|---|
| Liver (mg) | 2792.9±211.9 | 2550.0±754.9 | 1199.1±87.9 | 1035.6±165.4 |
| Spleen (mg) | 1057.6±62.9 | 895.1±615.5 | 98.15±30.0 | 102.94±22.34 |

Table 4. Average weight of liver and spleen in $CD71^{+}Ter119^{-}$ (n = 9), Jak2 WT/$p53^{+/+}$ (control) (n = 10) and Jak2 WT/$p53^{-/-}$ (control) (n = 10)).

| | $CD71^{+}Ter119^{-}$ | Jak2 WT/$p53^{+/+}$ (control) | Jak2 WT/$p53^{-/-}$ (control) |
|---|---|---|---|
| Liver (mg) | 2489.0±1014.5 | 1199.1±87.9 | 1035.6±165.4 |
| Spleen (mg) | 784.8±160.8 | 98.15±30.0 | 102.94±22.34 |