Review

Murine Models of Myelofibrosis

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Abstract: Myelofibrosis (MF) is subtype of myeloproliferative neoplasm (MPN) characterized by a relatively poor prognosis in patients. Understanding the factors that drive MF pathogenesis is crucial to identifying novel therapeutic approaches with the potential to improve patient care. Driver mutations in three main genes (janus kinase 2 (JAK2), calreticulin (CALR), and myeloproliferative leukemia virus oncogene (MPL)) are recurrently mutated in MPN and are sufficient to engender MPN using animal models. Interestingly, animal studies have shown that the underlying molecular mutation and the acquisition of additional genetic lesions is associated with MF outcome and transition from early stage MPN such as essential thrombocythemia (ET) and polycythemia vera (PV) to secondary MF. In this issue, we review murine models that have contributed to a better characterization of MF pathobiology and identification of new therapeutic opportunities in MPN.

Keywords: myeloproliferative neoplasm; MPN; mouse model; myelofibrosis; cancer; hematopoietic stem cells; oncogene; mutation

1. Introduction

Myeloproliferative neoplasms (MPN) are a group of myeloid-derived malignancies that lead to excessive production of blood cells (erythrocytes, leukocytes, or platelets). Genetic gain of function mutations drive abnormal clonal proliferation of myeloid progenitors, leading to accumulation of fully differentiated hematopoietic elements. Unlike in acute myeloid leukemia (AML), MPN hematopoietic progenitors retain a relatively normal differentiation program. However, they are characterized by hypersensitivity to cytokines, in particular, to erythropoietin (EPO) and thrombopoietin (TPO) [1,2]. Three MPN subtypes have been identified: essential thrombocythemia (ET), polycythemia vera (PV), and primary myelofibrosis (PMF). PMF was previously known as chronic idiopathic myelofibrosis (CIMF), agnogenic myeloid metaplasia (AMM), and myelofibrosis with myeloid metaplasia (MMM). In 2007, the International Working Group for Myelofibrosis Research and Treatment (IWG-MRT) standardized the nomenclature referring to PMF [3]. Myelofibrosis arising secondary to PV or ET have since been named post-PV MF and post-ET MF, respectively. PMF is the least frequent of the three classic MPN but is the most aggressive and is associated with a significantly shortened survival [4,5]. The abbreviation MF will be used in this review to denote PMF and post ET/PV MF. MF is defined as proliferation, atypia of megakaryocytes (MK), and bone marrow (reticulin and/or collagen) fibrosis (major diagnostic criteria) with additional features of extramedullary hematopoiesis, splenomegaly, and abnormal cytokine expression.

The vast majority of patients carry mutations that activate the JAK-STAT signaling pathway, namely janus kinase 2 (JAK2), calreticulin (CALR), and myeloproliferative leukemia virus oncogene...
(MPL). In PMF, JAK2V617F mutations are the most common (58%), followed by CALR (25%) and MPL (7%) [5–7]. Constitutive or aberrant activation of JAK-STAT signaling plays a pivotal role in the pathogenesis of MF. Following ligand receptor binding to cytokine receptors such as EPOR or MPL, homodimeric JAK2 signaling molecules activate downstream signaling pathways, leading to the phosphorylation of transcription factor Stat5. This in turn triggers nuclear translocation of these proteins and activation of multiple targets including inflammatory and survival-related genes [8–10].

Aberrant inflammatory cytokines and signaling molecules affect both hematopoietic stem cells (HSC) and stromal cells within the hematopoietic niche. Little is known about the cellular targets of bone marrow fibrosis; however, mesenchymal stromal cells (MSC) from pre-fibrotic MPN patients can increase extracellular matrix remodeling, can decrease hematopoiesis supporting capacity, and can thus play a role in MF pathology [11]. In this review, we will review murine models of MF (Figure 1), discuss new findings that shed light on the underlying molecular mechanisms, and identify therapeutic opportunities that might improve outcomes in this challenging disease.

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![Timeline of published myelofibrosis mouse models.](image-url)

**Figure 1.** Timeline of published myelofibrosis mouse models.

## 2. JAK2V617F

JAK2 is a non-receptor tyrosine kinase that mediates cytokine receptor signaling pathways downstream of MPL and the EPO receptor, where it homodimerizes to initiate STAT1,3,5-dependent signaling in response to EPO-EPOR or TPO-MPL binding. JAK2 also signals downstream of numerous other cytokines including interleukin (IL) 3, granulocyte-macrophage colony-stimulating factor (GM-CSF), and interferon (IFN)γ [12]. An activating mutation in JAK2 which involves a valine to phenylalanine substitution JAK2V617F was first identified in 2005 and is the most prevalent mutation in MPN, found in 95% of PV, and in more than 50% of PMF and ET [1,13–16].

Following the discovery of the JAK2V617F mutation (JAK2V617F), retroviral models were generated to assess the phenotypic effects of this mutation in vivo [1,17–20] (Table 1). Mice transplanted with JAK2V617F expressing donor bone marrow developed a phenotype resembling PV, but MF transformation was described in two studies [18,19]. Common MPN features such as high hemoglobin, leukocytosis, and megakaryocyte hyperplasia were observed at early stages in these two studies and splenomegaly was identified at endpoints, consistent with early PV progressing to MF. However, Lacout and colleagues described increased fibrosis at later time points (>4 months) after transplantation that were associated with marrow hypocellularity, anemia, thrombocytopenia, and granulocytosis, more reminiscent of PMF features. Wernig et al. observed MF in Balb/c but not in C57/Bl6 recipients after retroviral expression of JAK2V617F, suggesting that additional host factors may regulate the development of fibrosis [19].
Table 1. Models of myelofibrosis generated by retroviral overexpression and bone marrow transplantation in mice. ET: essential thrombocythemia, MF: myelofibrosis, TPO: thrombopoietin, PV: polycythemia vera.

| Model   | Strain       | Vector | Phenotype                                           | References |
|---------|--------------|--------|----------------------------------------------------|------------|
| Jak2VF  | C57Bl/6      | MEGIX  | PV with progression to MF                          | [18]       |
| Jak2VF  | C57Bl/6 and  | MSCV   | PV, progression to MF was only observed in Balb/c   | [19]       |
| MplW515L| Balb/C       | MSCV   | Features of ET with progression to MF              | [21]       |
| Calrdel52| C57Bl/6     | MSCV   | ET with progression to MF                          | [22]       |
| TPO     | BDF1         | MSCV   | ET with progression to MF                          | [23]       |
| TPO     | C57Bl/6      | MPZen2 | ET with progression to MF                          | [24]       |

MF transformation potentiation could be explained by different plasmid backbone (pMEGIX vs. MSCV-IRES-EGFP), transfection efficiencies, or possibly expression level of the Jak2VF transgene. Of note, in the study of Lacout et al., phenotypic MF was transplantable into secondary recipients while, in the model generated by Wernig et al., it was not [18,19].

Although informative, retroviral models engender marked overexpression of JAK2V617F, and to more closely recapitulate physiological level expression, genetically engineered mouse models (GEMM) of Jak2VF were developed (previously reviewed in detail [25,26]). The development of GEMM models allowed correlation of disease phenotype with different gene expression levels, resulting in the conclusion that JAK2VF expression levels influence the MPN phenotype. In one transgenic mouse model, Shide et al. were able to demonstrate that Jak2VF expression could lead to MF transformation when Jak2VF expression was higher than wild-type (WT) Jak2 expression [27]. In a second transgenic model, Xing et al. expressed Jak2VF under the control of the vav promoter [28], resulting in higher levels of Jak2VF expression and age-related MF in 38% of Jak2VF mice.

Five Jak2VF knock-in models were published [29–32] in which murine Jak2VF from the endogenous murine jak2 promoter was expressed in different manners (Table 2). Akada et al. and Mullally et al. generated conditional Jak2VF knock-in models [29,32] while Jak2VF expression was constitutive in the model generated by Marty et al. [31]. Hasan and colleagues generated conditional Jak2VF mice, where mutant Jak2 was expressed from the endogenous locus using the VavCre system [33]; the same model was later used by Mansier et al. using recombination by PF4Cre [34]. Li et al. developed a model within which human JAK2VF was conditionally expressed from the endogenous murine jak2 promoter. In all these models, erythrocytosis, leukocytosis, extramedullary hematopoiesis, and MF development (in a minority of mice after 26 weeks) were observed. Interestingly, in the conditional Jak2VF knock-in model we developed, we have not observed MF in primary mice (likely due to thrombosis-related decreased survival); however, late progression to MF is infrequently observed in secondary recipients [35]. Once again, heterogeneity in the fibrosis phenotype between the Jak2VF knock-in models may relate to the level of Jak2VF expression.
Table 2. Transgenic and knock-in mouse models of myelofibrosis. BMT: bone marrow transplant, ET: essential thrombocythemia, MF: myelofibrosis, PMF: primary myelofibrosis, TPO: thrombopoietin, PV: polycythemia vera.

| Model Type | Strain | Locus | Activation | Phenotype | References |
|------------|--------|-------|------------|-----------|------------|
| Driver mutations | | | | | |
| Jak2VF Transgenic | BDF1 | Line 1: Dcc intron 12 on chr18<br>Line 2: between Mef2a and Lrrc28 on chr7, expression under H-2Kb promoter | Constitutive | Line 3: about 50% PV or ET, 50% no clear phenotype<br>Line 2: PMF | [27] |
| Jak2VF Transgenic | C57BL/6 XDBA | Locus n/a, expression under Vav promoter | Conditional | ET, PV or PMF | [28] |
| JAK2VF Transgenic | C57Bl/6 | Human JAK2VF, expression under human JAK2 promoter | Conditional, inducible (Mx1Cre) | VavCre: ET with progression to MF<br>Mx1Cre: PV with progression to MF | [36] |
| Jak2VF Knock-in | 129Sv C57Bl/6 | Endogenous Jak2 locus on chr19 | Constitutive | PV with progression to MF in heterozygous, stronger phenotype in homozygous mice | [29] |
| Jak2VF Knock-in | 129Sv C57Bl/6 | Endogenous Jak2 locus on chr19 | Constitutive | PV with progression to MF in heterozygous mice<br>PMF in homozygous mice | [31] |
| Jak2VF Knock-in | C57Bl/6 | Endogenous Jak2 locus on chr19 | Conditional, inducible (E2ACre) | PV, homozygosity embryonically lethal, MF only in secondary recipients | [32] |
| JAK2VF Knock-in | C57Bl/6 | Human JAK2VF in endogenous Jak2 locus on chr19 | Conditional, inducible (Mx1Cre) | ET, PV and one mouse with MF in BMT | [30] |
| JAK2VF Knock-in | C57Bl/6 | Endogenous Jak2 locus on chr19 | Conditional (VavCre) | PV with progression to MF | [33] |
| Calr<sup>Δ52</sup> Knock-in | C57Bl/6 | Endogenous Calr locus on chr8 | Conditional, inducible (Mx1Cre) | ET in heterozygous, ET with progression to MF in homozygous mice | [37] |
| Other models | | | | | |
| TPO Transgenic Knock-out (upstream promoter region of Gata1) | BDF1 | Locus n/a, driven by the IgH promoter | Constitutive | Features of ET with progression to MF | [38] |
| Gata-1<sup>low</sup> Chromosomal translocation | C57Bl/6CD1 | Endogenous promotor region on chrX | Constitutive | >90% mortality in C57Bl/6, normal life span with development of PMF in CD1 | [39] |
| Trisomy 21 (Ts65Dn) | C57Bl/6 | Chr16 (harboring 2/3 of human chr21 genes) translocation to chr17 | Constitutive | Down syndrome, features of ET with progression to MF | [40] |
3. MPL Mutation (W515L and W515K)

In 2006, point mutations in the TPO receptor MPL were discovered among JAK2\(^{VF}\)-negative MPN [21]. Somatic mutations in MPL\(^{W515L/K}\) have been found in 5% and 1% respectively of MF and ET patients [41]. Pikman et al. used MPL\(^{W515L}\) expressed retrovirally in hematopoietic stem and progenitor cells (HSPC) to induce MPN characterized by leukocytosis, thrombocytosis, splenomegaly, and reticulin fibrosis [21] (Table 1). MPL\(^{W515L}\) expression recapitulated phenotypic features of human MF such as megakaryocytic hyperplasia, splenomegaly, extra medullary hematopoiesis, and thrombocytosis. Using this model, Kleppe et al. showed that JAK-STAT activation (STAT3) and cytokine production is found in malignant and nonmalignant hematopoietic cells in MF [9]. MPL\(^{W515L}\) -mutant HSPC were the largest source of IL6; however, mutant and WT cells secreted tumor necrosis factor (TNF), IL10, and C-C motif chemokine 2 (CCL2). Global cytokine production and the proportion of cells secreting multiple cytokines were increased in differentiated cells (both mutant and nonmutant) and in MPL\(^{W515L}\) -mutant-expressing HSPC from MF mice. These data suggest a potential cross talk between malignant and nonmalignant cells and show that cytokine production by both populations is an important feature of MF. The authors identified six cytokines (IL6, IL10, IL12, TNF\(_{\alpha}\), CCL2, and CCL5) aberrantly produced in their murine models, which were also found to be increased in granulocytes from patients with MF (as compared to healthy individuals). This work provided preclinical evidence in support of the use of JAK2 inhibitors in MPN patients positive for MPL\(^{W515L}\) mutation [42,43].

4. CALR

CALR is a molecular chaperone protein localizing in the endoplasmic reticulum. In 2013, recurrent mutations in CALR were described as a key MPN driver mutations, which occur in a mutually exclusive manner with JAK2 and MPL mutations [6,7]. Two main mutations account for approximately 80% of all identified CALR mutations: a 52-base deletion (del52) (type 1: c.1092_1143del) and 5-base insertion (ins5) (type 2: c.1154_1155insTTGTC). However, more than 50 types of CALR mutation have been reported. All of them are base-pair insertions (+2) or deletions (−1) leading to a +1 base-pair frameshift that generates a mutant-specific 36 amino acid sequence in the CALR C-terminus. The mutant-specific C-terminus is found in all CALR mutant-expressed proteins and central to the pathogenesis of disease [6,7]. Subsequent studies by different groups demonstrated that the mutant CALR protein causes constitutive activation of MPL and downstream JAK-STAT signaling [44–49]. CALR alongside JAK2 and MPL mutations are included as major diagnostic criteria for PMF by the World Health Organization (WHO) classification of myeloproliferative neoplasms. CALR mutations are associated with better prognosis and lower risk of thrombosis in patients with PMF compared to JAK2 mutations [6,50,51]. However, the favorable prognostic effect of CALR mutations in PMF might be restricted to the type 1 mutant [52].

In 2016, Marty et al. showed that both CALR\(^{del52}\) and CALR\(^{ins5}\) mutants can be expressed in mouse lineage-negative bone marrow (BM) cells using retroviral vectors (pMSCV-IRES-GFP) to induce MPN, with MF developing in CALR\(^{del52}\)-expressing mice [22] (Table 1). CALR\(^{delex9}\) (lacking the entire exon 9) did not induce an MPN phenotype, suggesting that gain of function of the CALR mutant C terminus rather than loss of the WT C-terminal sequence drives the MPN phenotype. CALR\(^{del52}\) conferred a competitive advantage over WT HSC in vivo and in vitro, while this phenotype was not reported for CALR\(^{ins5}\). However, CALR\(^{del52}\) and CALR\(^{ins5}\) both amplify the megakaryocyte (MK) lineage. All CALR mutant-expressing mice rapidly developed thrombocytosis due to MK hyperplasia. After 6 months, CALR\(^{del52}\)-transduced mice developed MF associated with splenomegaly and a marked osteosclerosis, however, this was rarely observed in CALR\(^{ins5}\). MK progenitors expressing CALR\(^{del52}\) displayed a hypersensitivity to TPO, exhibited an increased growth in the absence of TPO, and induced MPL activation and JAK/STAT signaling. Interestingly, growth of CALR\(^{del52}\) MK progenitors and thrombocytosis was dependent on Mpl expression. CALR\(^{del52}\)-Tpo\(^{−/−}\) recipient mice developed ET, suggesting that TPO is not required for in vivo induction of MPN by CALR\(^{del52}\) [22].
Transgenic [53] or knock-in mice [37,54] expressing human CALRdel52 mutant protein [37] have also been generated; however, MF was only observed in one model, potentially due to the difference in transgene loci insertion and expression (Table 2). Shide et al. introduced mutant CALR complementary DNA in intron 5 of Tmigd3 on mouse chromosome 3 [53], driven by the H-2KB promoter and Moloney murine leukemia virus long terminal repeat. These CALR mutant mice develop ET, which is sensitive to ruxolitinib treatment. In contrast, Li et al. generated a conditional mouse knock-in model of the human mutant C-terminus (CALRdel52) knocked into the mouse Calr gene locus, resulting in mutant CALR expression under the control of the endogenous mouse Calr locus [37]. These heterozygous CALR mutants developed a transplantable ET-like disease with marked thrombocytosis associated with increased and morphologically abnormal MK and increased HSC numbers. Homozygous CALRdel52 mice developed extreme thrombocytosis accompanied by features of MF, including leukocytosis, reduced hematocrit, splenomegaly, and increased bone marrow reticulin fibrosis. Interestingly, CALRdel52 HSC did not display a competitive advantage upon transplantation in primary or secondary recipient mice. These data suggest that the expression level of the CALRdel52 mutant contributes to MF development. More recently, Balligand et al. generated germline Calrdel52 knock-in mice using Clustered Regularly Interspaced Short Palindromic Repeats-Cas9 (CRISPR/Cas9) [54]. These mice had a transplantable ET phenotype but did not develop MF. Finally, CALR sequencing data from patients has shown that CALRdel52 is more enriched in MF (70%) compared to CALRins5 (13%) [55,56] and that there is evidence of increased susceptibility to MF for CALRdel52 mutations as compared to CALRins5 mutations [57].

5. Other Models of MF

5.1. TPO Overexpression

Although overexpression of TPO is not found in patients with MF, two groups initially reported induction of MF by retroviral expression of murine TPO within bone marrow cells [23,24] (Table 1). Very similar phenotypes were observed in these studies, that is, enforced expression of murine TPO in bone marrow transplanted cells leads to fatal MPN with associated MF and osteosclerosis. Interestingly, biphasic disease reminiscent of human MF was observed. During the first 10 weeks, posttransplant mice presented with megakaryocytosis and granulocytosis in both the spleen and bone marrow with erythroblastic hypoplasia in the bone marrow. In the second phase, pancytopenia, a drop in hematopoietic progenitors, osteosclerosis, together with spleen and bone marrow fibrosis were observed.

Using the retroviral TPO overexpression model and genetic fate mapping, Decker et al. investigated the role of Leptin receptor (Lepr) expressing MSC in MF development [58]. Cells that were positive for Lepr and platelet-derived growth factor receptor (PDGFR) α were the source of fibrosis-driving myofibroblasts and expanded extensively in the bone marrow fibrosis induced by TPO overexpression. Depletion of PDGFRα in myofibroblasts or treatment with imatinib, which also targets the PDGFR kinase domain, improved the MF phenotype in these mice, resulting in higher BM cellularity and amelioration of BM fibrosis. Although megakaryocyte dysplasia and fibrosis in the spleen was not changed upon PDGFRα depletion, the favorable effects on BM fibrosis suggest that targeting PDGFRα might be a potential strategy in treating MF.

Kakumitsu et al. generated murine TPO transgenic (TPO Tg) mice [38] driven by the IgH promoter. Similar to the retroviral system, the authors showed elevated platelets and neutrophils in peripheral blood and increased numbers of megakaryocytes and granulo-myelomonocytic progenitor cells in the bone marrow. Anemia was also observed in TPO Tg mice, while erythrocyte progenitors were increased. This suggests a block or a shift in erythroid differentiation toward megakaryocyte/platelet differentiation. TPO Tg mice developed MF and osteosclerosis starting at 9 months with progression at 12 months, associated with splenic extramedullary hematopoiesis. It is thought that the local
production of TPO in the bone marrow and spleen drives the development of fibrosis and osteosclerosis in that model.

5.2. Gata-1 low Mouse

Gata-1 is an essential hematopoietic transcription factor regulated by an 8-kb upstream region containing a DNase hypersensitive site or enhancer region. Gata-1 low transgenic mice were generated by replacing this cis-acting enhancer element by a neomycin-resistance cassette using homologous recombination in mouse embryonic stem cells [39]. In the CD1 background, most of mice survived until adulthood but were anemic at birth. These mice present with impaired megakaryocyte maturation and consequent thrombocytopenia. Gata-1 low mice develop slow progressive MF, resembling the development of PMF in patients (Table 2) [59]. They become anemic from 5 months of age and anemia is associated with tear-drop poikilocytes, circulating progenitor cells, and fibrosis in the marrow and in the spleen. Bone marrow from Gata-1 low mutant mice is enriched for osteocalcin, TGFβ-1, platelet-derived growth factors (PDGF), and vascular endothelial growth factor transcript compare to control mice [60]. Subsequent mechanistic investigation performed in that model identified alteration of TGFβ-1, hedgehog, and p53 signaling pathways [61]. Inhibition of TGFβ-1 with SB431542 normalized TGFβ-1 signaling and expression of p53-related genes. This was associated with restoration of hematopoiesis and normal megakaryocyte development, while the authors observed reduced fibrosis, neovascularization, and osteogenesis in the bone marrow. These data further support a pivotal role of TGFβ-1 in the pathogenesis of MF.

In recent years, Zingariello et al. characterized the Tpo/Mpl axis and downstream Jak/Stat activation in the Gata-1 low mouse model in more detail, finding good agreement with key pathological features that are characteristic for PMF patients [62]. Tpo expression was elevated in liver and plasma of Gata-1 low mice, and higher expression levels of Mpl were detected in lineage –, Sca1+, cKit+ (LSK) cells. Jak2 and Stat5 protein expression was increased in BM and spleen of Gata-1 low mice. Treating Gata-1 low mice with ruxolitinib reduced spleen size and Jak2 expression in the spleen but did not have an effect on Jak2/Stat5 levels in the bone marrow or bone marrow cellularity and did not ameliorate BM fibrosis. Interestingly, Zingariello et al. observed poorly developed endoplasmic reticulum with rare polysomes in megakaryocytes of Gata-1 low mice using electron microscopy, supporting their finding of ribosomal deficiencies.

More recently, the Gata-1 low model was used to investigate the PDGF system and regulation of PDGFRβ in bone marrow fibrosis. Using multispectral imaging, Kramer et al. showed increased PDGFRβ expression in bone marrow stromal cells in MF [59]. PDGF-B expression was likewise increased but found to be mainly derived from megakaryocytes, supporting the hypothesis that growth factors produced by malignant cells drive bone marrow fibrosis. Furthermore, T-cell protein tyrosine phosphatase (TC-PTP) was identified as a negative regulator of PDGFRβ in bone marrow fibrosis.

5.3. Trisomy 21 Mouse

Trisomy 21 is frequently found as a somatic aberration in myeloid malignancies. Kirsammer et al. observed highly penetrant MPN with modest MF in Ts65Dn mice [40] (Table 2). These mice are trisomic for 104 orthologs of the mouse chromosome 21 (Hsa21). Cellular compartment analysis showed accumulation of stem cells (LSK) and megakaryocytes, together with thrombocytosis, granulocytosis, and anemia. Interestingly, as opposed to many models, splenomegaly was not observed in Ts65Dn mice, despite the bone marrow fibrosis.

5.4. Abi-1 Knockout Mouse

Chorzalska et al. recently demonstrated that Abelson interactor 1 (ABI-1), a negative regulator of ABL, is downregulated in CD34+ cells from PMF patients. Reduced ABI-1 gene expression was observed in granulocytes from PMF patients and patients with MF secondary to PV but not in ET, PV, or MF secondary to ET. Hence, Chorzalska et al. induced conditional deletion of Abi-1 in mice.
using the Mx1Cre system [63] (Table 2). Loss of Abi-1 in the bone marrow of mice resulted in a PMF phenotype featuring leukocytosis, thrombocytosis, anemia, splenomegaly, megakaryocytosis, and fibrosis in the bone marrow. HSC self-renewal was impaired in competitive bone marrow transplant experiments, and Src family kinases, Stat3, and NF-κB signaling were found to be more activated in Abi-1-deficient mice. Interestingly, there was no increase in Jak2/Stat5 signaling in the BM of Abi-1 knockout mice. Transducing heterozygous Abi-1 knockout mice with MplW515L accelerated development of the MPN phenotype, showing that loss of Abi-1 cooperates with mutant Mpl to induced MPN. Thus, Abi-1-deficient mice are an interesting new model to interrogate Src, Stat3, and NF-κB signaling in MF.

6. Disease Evolution Through Additional Genetic Mutations

JAK2VF-LNK

Upon TPO stimulation of MPL, the adaptor protein LNK binds to JAK2 and negatively regulates JAK-STAT signaling [64]. However, JAK2VF and LNK mutations are not necessarily mutually exclusive, as they have been found co-mutated in patients with PMF [65,66]. Interestingly, in a mouse model, LNK loss exacerbates JAK2VF-driven MPN and accelerates the development of MF through the potentiation of JAK2VF signaling [67]. LNK-deficient mice exhibit a mild, chronic myeloid leukemia (CML)-like phenotype [68], demonstrating how acquisition of additional mutations can modulate disease progression (Table 3).
Table 3. Mouse models of myelofibrosis targeting multiple genes. BMT: bone marrow transplant, ET: essential thrombocythemia, MF: myelofibrosis, PMF: primary myelofibrosis, TPO: thrombopoietin, PV: polycythemia vera.

| Model | Type | Strain   | Jak2<sup>VF</sup> model (locus) | Activation | Phenotype                                      | References |
|-------|------|----------|----------------------------------|------------|-----------------------------------------------|------------|
| Jak2<sup>VF</sup>-LNK | Retroviral overexpression (MIG)/BMT (Jak2<sup>VF</sup>) Knockout (LNK) | C57BL6/J | / | Constitutive (LNK knockout) | PV with progression to MF is accelerated in double mutant | [67] |
| JAK2<sup>VF</sup>-Ezh2 | Transgenic (Jak2<sup>VF</sup>) Knockout (Ezh2) | C57BL6/J | Tiedt et al. JAK2<sup>VF</sup> Jak2<sup>VF</sup> mice (human JAK2<sup>VF</sup>, expression under human JAK2 promoter) | Conditional, inducible (Mx1Cre, SclCre<sup>ER</sup>) | ET with progression to MF is accelerated in double mutant | [69] |
| Jak2<sup>VF</sup>-Ezh2 | Knock-in (Jak2<sup>VF</sup>) Knockout (Ezh2) | C57Bl/6 | Akada et al. 2010 Jak2<sup>VF</sup> mice (endogenous Jak2 locus on Chr19) | Conditional, inducible (Mx1Cre) | PV (JAK2<sup>VF</sup>) shifts towards ET in combined model with rapid progression to MF | [70] |
| Jak2<sup>VF</sup>-Ezh2 | Transgenic (Jak2<sup>VF</sup>) Knockout (Ezh2) BMT | C57Bl/6 | Shide et al. 2008 Jak2<sup>VF</sup> mice (line 1 chr18, line 2 chr7, expression under H-2Kb promoter) | Conditional (Cre-ERT2) | MPN features Ezh2/- with rapid progression to MF | [71] |
| Jak2<sup>VF</sup>-Asxl1 | Transgenic (Jak2<sup>VF</sup>) Knockout (Asxl1) | C57BL6/6 XDBA | Xing et al. 2008 Jak2<sup>VF</sup> mice (locus n/a, expression under Vav promoter) | Conditional | ET, PV, or PMF with accelerated MF in combined model | [72] |
| Jak2<sup>VF</sup>-Dnmt3a | Knock-in (Jak2<sup>VF</sup>) Knockout (Dnmt3a) | C57Bl/6 | Mullally et al. 2010 Jak2<sup>VF</sup> mice (endogenous Jak2 locus on Chr19) | Conditional | PV with progression to MF in combined model (MF was absent in JAK2<sup>VF</sup> model) | [73] |
7. Genetic Lesion in Epigenetic Regulators

Genes encoding epigenetic regulators such as enhancer of zeste homologue 2 (EZH2), DNA methyltransferase 3A (DNMT3A), and additional sex combs-like 1 (ASXL1) are among the most frequently mutated genes in patients with MPN [74–76]. These mutations usually coexist with one of the three main driver mutations of MPN (i.e., JAK2, CALR, or MPL) [77], and the presence of certain additional mutations (e.g., ASXL1) has a powerful, adverse effect on clinical outcomes [78]. Recent publications have now provided evidence of cooperation between driver mutation-associated constitutive activation of JAK-STAT signaling and mutations in epigenetic regulators, as discussed below.

7.1. JAK2VF-EZH2

EZH2, a histone methyltransferase member of the polycomb repressive complex 2 (PRC2), is a master regulator of chromatin topology that mediates silencing through di- and trimethylation of lysine H3 (H3K27me2/3) [79,80]. Overexpression of Ezh2 induces MPN in mice [81]. Activating mutations in EZH2 have been observed in malignant B cell lymphomas [82,83]; however, loss of function mutations in EZH2 are frequently identified in patients with myelodysplastic syndrome (MDS) and MF [84] and loss of function is associated with drug resistance and adverse clinical outcome in hematologic malignancies [85–87].

In vivo cooperativity between jak2VF and Ezh2 loss of function was reported by three independent groups [69–71], using intercrossed genetically engineered mouse models of jak2VF [27,29,36] and Ezh2 [88–90] (Table 3). In two studies, expression of jak2VF and the deletion of Ezh2 were simultaneously induced in hematopoietic stem cells by polyinosinic–polycytidylic acid (pIpC) injection and induction of Mx1=Cre [69,70]. Conversely, Sashida et al. deleted Ezh2 by tamoxifen injection within secondary hosts transplanted with bone marrow cell from jak2VF-Ezh2^{lox/lox}.CreER{T2} [71]. These complementary studies demonstrated that coincident induction of jak2VF and deletion of Ezh2 leads to impairment of erythropoiesis and alterations of megakaryopoiesis, highlighted by expansion of megakaryocytic precursors and increase in platelet counts compared to jak2VF mice. Moreover, reticulin fibrosis develops in the bone marrow and spleen of these mice. Loss of Ezh2 enhanced the repopulation capacity of jak2VF expressing HSC, and transplantation of bone marrow from Ezh2-deleted jak2VF resulted in accelerated progression to MF. Mechanistically, Ezh2 deletion altered PRC2 function and led to marked decrease H3K27 tri-methylation (H3K27me3) repressive marks in HSPC with associated increase in H3K27 acetylation activation marks (H3K27ac) in jak2VF mice. PRC2 targets, such as the oncogenes Mlf1 and Pbx3, and inflammatory regulators such as S100a8, S100a9, Ifi27l2a, Lin28b Hmga2, and TGFβ1 were upregulated. HMGA2 has been reported to be activated in CD34+ cells from patients with PMF [91,92].

Sashida et al. demonstrated that targeting this epigenetic deregulation using bromodomain inhibition leads to abrogation of MF-initiating cells and is associated with a significant H3K27ac attenuation at the promoter regions of PRC2 target gene in jak2VF-Ezh2^−/−-recipients mice [71]. Also, as previously reported in MF, TNF/NF-κB inflammation and TGFβ-1 signaling were enriched in these mouse models, confirming the important role of these pathways in the development of MF [70].

7.2. JAK2VF-ASXL1

Mutations in the Additional Sex Combs-Like 1 (ASXL1) epigenetic regulator have been found to occur at high frequency in patients with myeloid malignancies (48% of CML, 20% of MDS, 10% of MPN, and 20% of AML) [85,93–102]. ASXL1 mutations are associated with poor prognosis and leukemic transformation [103]. Interestingly, these mutations are more common with advanced age and are also found in patients with clonal hematopoiesis of indeterminate potential (CHIP) [104–106]. ASXL1 contains an N-terminal ASX homology (ASXH) domain and a C-terminal plant homeodomain (PHD). The majority of patient-derived ASXL1 mutations are nonsense or frameshift downstream of the ASXH domain, leading to truncation and loss of the PHD domain [98,100,102,107]. It remains...
unclear if ASXL1 confers a gain of function due to expression of a truncated protein or whether a somatic mutation leads to loss of function. Additional studies are required to more accurately understand ASXL1 mutations in the context of MPN. In mouse models, both loss and Truncated Asxl1 expression leads to altered erythropoiesis [108,109]. Mechanistically, ASXL1 physically interacts with PRC2 and ASXL1 silencing is associated with loss of PRC2-mediated gene repression and global loss of H3K27me3 [107]. Recently, Yang et al. described a gain of function mediated by expression of ASXL1 truncating protein in the pathogenesis of myeloid malignancy [110]. Here they demonstrated that expression of ASXL1<sup>aa1-587</sup> truncating protein in the hematopoietic system was sufficient to lead to diverse myeloid malignancies such as MDS, MPN, and AML. ASXL1<sup>aa1-587</sup> expression increases HSC/HSPC functions and increases chromatin accessibility in cKit+ hematopoietic precursors cells. Importantly, they demonstrate that ASXL1<sup>aa1-587</sup> interacts with BRD4 in bone marrow cells, providing a rationale to treat these malignancies with BET bromodomain inhibitors.

ASXL1 mutations are found in 34.5% of PMF patients [111]. In line with other epigenetic-related mutations, coincidence of JAK2<sup>VF</sup> and ASXL1 mutations are more frequent in secondary acquired MF (post-PV; 26%) than in PV patients without MF (4%) [72]. Guo et al. crossed Jak2<sup>VF</sup> mice [28] with Asxl1<sup>+/−</sup> [112] to generated a Jak2<sup>VF</sup>-Asxl1<sup>+/−</sup> transgenic mice (Table 3). They demonstrated that cooperation of Jak2<sup>VF</sup> and Asxl1 heterozygous loss accelerates secondary MF when compared to Jak2<sup>VF</sup> and/or Asxl1<sup>+/−</sup> mice alone. Notably, 12% of Jak2<sup>VF</sup>-Asxl1<sup>+/−</sup> mice, but not the littermate controls, progressed to secondary AML at 6–8 months of age. Even at early time points (2–3 months), Jak2<sup>VF</sup>-Asxl1<sup>+/−</sup> mice showed increased white blood cell and neutrophil counts in the peripheral blood when compared with the WT but not with Jak2<sup>VF</sup> mice, with increased MEPs and CD41+CD61+ megakaryocytic precursors and erythroid precursors in BM and spleen. Jak2<sup>VF</sup>-Asxl1<sup>+/−</sup> mice exhibited splenomegaly with disrupted splenic architecture and prominent megakaryocytes and myeloid precursors. Finally, reticulin staining revealed extensive fibrosis in the BM at the age of 3 months. However, after 8–10 months, Jak2<sup>VF</sup>-Asxl1<sup>+/−</sup> mice display BM failure with anemia and reduced BM cellularity, consistent with progression to advanced MF. More investigation may provide additional information about chromatin landscape and epigenetic changes in this model.

7.3. JAK2<sup>VF</sup>–DNMT3A

DNMT3A is a de novo DNA methyltransferase that catalyzes the addition of methyl groups into active chromatin in CpG-rich regions, leading to gene inactivation [113,114]. DNMT3A mutations are found in up to 22% of AML patients [115]. Interestingly, DNMT3A mutations are found at low rates below 5% in primary MPN (PV and ET) but appear to be more frequent in advanced MPN (10–15% of PMF, 15% of secondary MF, and 17% of AML arising from MPN) [115–117]. Mutations in DNMT3A cluster in the methyltransferase domain, leading to dominant negative function with loss of DNA binding and reduced catalytic activity [118,119]. Dnmt3a<sup>−/−</sup> HSC have enhanced self-renewal capacities and a block in differentiation upon serial transplantation in vivo [120]. This may relate to the observation that DNMT3A mutations are frequently found in CHIP [121,122]. In AML, DNMT3A mutations mediate resistance to chemotherapy drugs through altering chromatin conformation [123].

In 2018, we reported that Dnmt3a loss and Jak2<sup>VF</sup> could cooperate to induce MF, using in vivo CRISPR-Cas9 targeting to disrupt Dnmt3a function within Jak2<sup>VF</sup> LSK [73]. Jak2<sup>VF</sup>–Dnmt3a-Cas9 progenitors were transplanted into irradiated recipients and showed a biphasic disease reminiscent of secondary transformation of PV to MF (Table 3). At early time points (8 weeks), Jak2<sup>VF</sup>–Dnmt3a-Cas9 recipients showed high hematocrit, platelet, and white blood cells counts; however, by 32 weeks, mice became pancytopenic with progressive BM failure. Furthermore, a dense fibrocellular infiltrate and BM osteosclerosis developed in Jak2<sup>VF</sup>–Dnmt3a-Cas9 recipients, together with disorganized and effaced splenic architecture, dense reticulin fibrosis, and massive splenomegaly. Blood smear from Jak2<sup>VF</sup>–Dnmt3a-Cas9 recipients showed severe anemia with left shifted myelopoiesis, anisocytosis, poikilocytosis, and tear-drop erythrocytes, reminiscent of human MF. In this model, myelofibrotic transformation was associated with HSC depletion and the accumulation of multipotent
progenitors (MPP). Transcriptional analysis revealed gene expression changes associated with not only HSC identity and function but also proinflammation (TNFα via NF-κB), and these changes were also seen when comparing PMF patients vs. ET, PV, or healthy individuals. Mechanistically, loss of Dnmt3a caused PRC2 alteration and increased chromatin accessibility at active enhancers of jak2V617F-Dnmt3a-Cas9 upregulated genes, including the aforementioned stemness and inflammatory-related pathways such as TNFα/NF-κB signaling. These data demonstrate that Dnmt3a loss of function was sufficient to drive MF through activated TNFα signaling and pro-inflammatory gene expression.

8. Signaling Pathway Activation

Heterogeneity in the fibrosis phenotype between driver mutants in JAK2, MPL, or CALR and the type of models (knock-in/retroviral) appears to be related to allele burden/express level. However, recent studies point out that acquisition of additional mutations (Asxl1, Ezh2, or Dnmt3a) can also alter transcriptional program and can accelerate disease transformation from early stage MPN (PV/ET) toward MF in a scenario where low allele expression of the mutant driver might not be sufficient. It would be interesting to test in CALR mutant models if additional mutation such as Ezh2, Dnmt3a, or Asxl1 could accelerate MF transformation (in CALRdel52) or MF incidence (in CALRins5).

9. Using Mouse Models to Validate Preclinical Therapeutic Strategies for MF

Murine models of MF provide excellent opportunities to develop and validate preclinical therapeutic strategies for patients with MF. Inflammation has been a key and recurrent feature of MF development, and strategies targeting cytokine inflammation hold great promise clinically. Kleppe et al. further demonstrated that ruxolitinib treatment normalized cytokine expression and that JAK2 inhibition reduced cytokine production from both normal and mutant cells in vivo [9]. Among the aberrantly activated networks, they identified TNF/NF-κB inflammatory signaling as a key pathway activated in both malignant and nonmalignant cells and as a common pathway in MF progression, thus providing insights into the molecular basis of MPN-associated inflammation and a therapeutic approach to target aberrant inflammatory signaling in MPN.

In an alternative approach to JAK2 inhibition, Yue et al. used the MPLW515L model to target transforming growth factor β-1 (TGFβ-1 signaling) [124]. Administration of galunisertib, an inhibitor of TGF-β receptor I kinase (ALK5), decreased collagen deposition by MSC in the bone marrow and improved MF in the MPLW515L model. However, ALK5 inhibition did not have an effect on blood cell counts or splenomegaly. Yue and colleagues successfully reproduced the beneficial effects of ALK5 inhibition on BM fibrosis in the transgenic JAK2V617F mice generated by Xing et al., indicating that targeting the TGFβ-1 axis might be a promising treatment strategy [28]. These findings are also supported from work using TPO overexpression, as TGFβ-1 is required for fibrosis development in that model. TPO expression in Tgfb1 null mice donor cells could not recapitulate MF physiopathology [125]. Complementary works demonstrated later that thrombospondin (Tsp) is not the major activator of TGF-β1 [126] and that osteoprotegerin (Opq) is required for osteosclerotic transformation in this model [127].

Interestingly, Verstovsek et al. have reported that monocyte-derived fibrocytes are abundant in primary myelofibrosis and produce collagen [128]. In this study, immunodeficient mice transplanted with bone marrow cells from patients with myelofibrosis developed a lethal myelofibrosis-like phenotype and, importantly, that treatment with the fibrocyte inhibitor, serum amyloid P, slowed the development of fibrosis.

More recently, Schneider and colleagues demonstrated that TPO transduced cells promote the outgrowth of Gli1+ MSC that expand into fibrosis in the bone marrow of recipient mice [129]. Mechanistically, the authors elegantly demonstrated that hematopoietic cells overexpressing TPO induced expression of profibrotic factors such as Cxcl4, Endothelin 1, and MMP9 by stromal cells. Release of chemokine Cxcl4 by hematopoietic and stromal cells is necessary and sufficient to attract Gli1+ stromal cells and to induce their differentiation into myofibroblastic cells. Gli1 antagonist 61
(GANT61), a small-molecule inhibitor of fibrosis [131,132] with the potential to normalize production of fibrotic factors, significantly reduced the number of Gli1+ myofibroblasts and malignant hematopoietic cells and abolished the development of reticulin fibrosis. This mechanism is relevant to patients, emphasized by the finding that Gli1+ MSC also expand in human MF and are sensitive to GANT61 inhibition [130]. Thus, this model was able to provide a rationale for targeting the GLI1 pathway in MF.

10. Discussion

The mechanisms leading to MF converge on the amplification and modification of signaling pathways, i.e., JAK2 signaling via JAK/STAT, IFNγ response, INFα response, TGFβ-1, p53, and hedgehog and TNFα signaling via NF-κB [8,69,70,73,129,133]. There are many similarities across the MF models including cytokine dysregulation, inflammatory pathway activation, megakaryocytic morphologic abnormalities, and enhancer reprogramming, leading to HSC dysfunction. In the constitutively active MPL mutant (MPLW515L), JAK-STAT activation (STAT3) was required in malignant and nonmalignant hematopoietic cells, and this identified six cytokines (Il6, IL10, IL12, TNFα, CCL2, and CCL5) aberrantly produced that were also found to be increased in granulocyte from patients with MF. Importantly, ruxolitinib treatment normalized cytokine expression and reduced cytokine production from both normal and mutant cells in vivo [9]. Interestingly, highly sensitive protein studies suggest that cytokine levels remain markedly abnormal in MF, even in the presence of JAK2 inhibition [134].

Kleppe et al. showed that, both in the retroviral MPLW515L model and Jak2VF knock-in mice, MF-related inflammation is associated with alterations of chromatin regulatory element such as enhancer and promoter regions in the MF clone, also seen in other studies [72,73]. These findings suggest that different genetic lesions may alter similar pathways within hematopoietic progenitors in MF.

TNFα via the NF-κB inflammatory signaling pathway was also identified as central to pathophysiology in two additional MF models: Jak2VF-Ezh2+/− [70] and Jak2VF-Dnmt3a [73]. However, in those two MF models, TGFβ-1 signaling was only enriched in Jak2VF-Ezh2+/− recipients, perhaps explaining the absence of megakaryocytosis in Jak2VF-Dnmt3a-Cas9 mice [61]. It cannot be excluded that this difference is due to disease kinetics as bone marrow observation and transcriptional analysis were not performed under strictly comparable condition across studies. Interestingly, elevated TGFβ-1 was found in plasma from Jak2VF homozygous but not Jak2VF heterozygous patients compared to healthy controls [61].

MF is a chemo-refractory disease associated with a poor clinical prognosis. So far, the only curative treatment is allogeneic stem cell transplantation which is only available to a minority of patients. Conventional treatment is mainly aimed at controlling symptoms and complications. For many years, the cytoreductive agent hydroxyurea was the therapy of choice for MF splenomegaly. Here, the overall response is 40% and the median duration is 13.2 months [135]. Oral or leg ulcers and progressive cytopenias are related toxicities of hydroxyurea and may be dose limiting. More recently, JAK2 inhibitors have become the standard of care in patients with high or intermediate risk MF [136–138]. JAK inhibitors such as ruxolitinib and fedratinib can control the symptoms of MF, in particular splenomegaly, but MPN cells are not cleared by these drugs, perhaps due to the inherent resistance of stem cell populations [139] or by persistent cross activation of JAK-STAT signaling [140]. In the latter example, JAK inhibitor persistence is reversible and is associated with site-specific changes in the chromatin state, consistent with an epigenetic mechanism by which MPN cells evade JAK kinase inhibition.

The findings from murine models of MF give some direction to new possibilities in the treatment of MF. Kleppe et al. have shown that BET Inhibition with (JQ1) attenuates NF-κB transactivation in vivo and that combined ruxolitinib/JQ1 delays JAK inhibitor persistence and shows efficacy against MPN cells [8]. These findings are supported by the work of Sashida et al., who demonstrated that bromodomain inhibition leads to abrogation of MF-initiating cells and is associated with H3K27ac
attenuation at the promoter regions of PRC2 target genes in a Jak2VF-Ezh2-/- model [71]. Together, these studies provide strong evidence and rationale for targeting the bromodomain protein in MF. Despite their strong efficiency in MF, the use of BET inhibitor molecules has initially been limited due to dose-limiting toxicities including thrombocytopenia, fatigue, nausea, vomiting, and diarrhea [141]. However, use of CPI-0610 alone or in combination with ruxolitinib showed encouraging results with good tolerability [142,143].

Altered chromatin landscape has been described in a number of mouse models [8,69–71,73]. Loss of H3K27me3 repressive marks at chromatin regulatory element (promoter or enhancer) controlling inflammation-related genes appears to be a common mechanism driving MF pathology, also implicating PRC2 as a key element in MF pathology. PRC2 is essential for controlling hematopoietic cell differentiation, shapes chromatin regulatory components by turning off stemness genes, and facilitates a normal differentiation program. Chromatin landscape is thoroughly regulated during hematopoiesis [144]. Thus, alteration of PRC2 function leads to topological changes in chromatin, failure to repress developmental enhancers, abnormal stem cell differentiation, and inflammatory programs in the context of MF.

11. Conclusions

In MPN, progression to MF represents a high-risk clinical scenario and murine models of disease demonstrate the important role that MPN phenotypic driver gene dosage and the acquisition of additional genetic lesions play in this process. Mechanistically, pathways to MF appear to converge on epigenetic deregulation and pro-inflammatory signaling pathway activation, identifying these pathways as tractable therapeutic opportunities. Moving forward, translational clinical trials will be needed to address the optimal ways to target these pathways in patients with MF to improve long-term outcomes, survival, and potentially even cure.

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References
1. James, C.; Ugo, V.; Le Couedic, J.P.; Staerk, J.; Delhommeau, F.; Lacout, C.; Garcon, L.; Raslova, H.; Berger, R.; Bennaceur-Griscelli, A.; et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. Nature 2005, 434, 1144–1148. [CrossRef] [PubMed]
2. Lu, X.; Huang, L.J.; Lodish, H.F. Dimerization by a cytokine receptor is necessary for constitutive activation of JAK2V617F. J. Biol. Chem. 2008, 283, 5258–5266. [CrossRef] [PubMed]
3. Mesa, R.A.; Verstovsek, S.; Cervantes, F.; Barosi, G.; Reilly, J.T.; Dupriez, B.; Levine, R.; Le Bousse-Kerdiles, M.C.; Wadleigh, M.; Campbell, P.J.; et al. Primary myelofibrosis (PMF), post polycythemia vera myelofibrosis (post-PV MF), post essential thrombocythemia myelofibrosis (post-ET MF), blast phase PMF (PMF-BP): Consensus on terminology by the international working group for myelofibrosis research and treatment (IWG-MRT). Leuk. Res. 2007, 31, 737–740. [CrossRef] [PubMed]
4. Mehta, J.; Wang, H.; Igbal, S.U.; Mesa, R. Epidemiology of myeloproliferative neoplasms in the United States. Leuk. Lymphoma 2014, 55, 595–600. [CrossRef]
5. Tefferi, A. Primary myelofibrosis: 2012 update on diagnosis, risk stratification, and management. Am. J. Hematol. 2011, 86, 1017–1026. [CrossRef]
6. Klampfl, T.; Gisslinger, H.; Harutyunyan, A.S.; Nivarthi, H.; Rumi, E.; Milosevic, J.D.; Them, N.C.; Berg, T.; Gisslinger, B.; Pietra, D.; et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. *N. Engl. J. Med.* 2013, 369, 2379–2390. [CrossRef]
7. 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.; et al. Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. *N. Engl. J. Med.* 2013, 369, 2391–2405. [CrossRef]
8. Kleppe, M.; Koche, R.; Zou, L.; Van Galen, P.; Hill, C.E.; Dong, L.; De Groote, S.; Papalexi, E.; Hanasoge Somasundara, A.V.; Cordner, K.; et al. Dual Targeting of Oncogenic Activation and Inflammatory Signaling Increases Therapeutic Efficacy in Myeloproliferative Neoplasms. *Cancer Cell* 2018, 33, 785–787. [CrossRef]
9. Kleppe, M.; Kwak, M.; Koppikar, P.; Riester, M.; Keller, M.; Bastian, L.; Hricik, T.; Bhagwat, N.; McKenney, A.S.; Papalexi, E.; et al. JAK-STAT pathway activation in malignant and nonmalignant cells contributes to MPN pathogenesis and therapeutic response. *Cancer Discov.* 2015, 5, 316–331. [CrossRef]
10. Yan, D.; Hutchison, R.E.; Mohi, G. Critical requirement for Stat5 in a mouse model of polycythemia vera. *Blood* 2012, 119, 3539–3549. [CrossRef]
11. Schneider, R.K.; Ziegler, S.; Leisten, I.; Ferreira, M.S.; Schumacher, A.; Rath, B.; Fahrenkamp, D.; Muller-Newen, G.; Crysandt, M.; Wilop, S.; et al. Activated fibronectin-secretory phenotype of mesenchymal stromal cells in pre-fibrotic myeloproliferative neoplasms. *J. Hematol. Oncol.* 2014, 7, 92. [CrossRef] [PubMed]
12. Rawlings, J.S.; Rosler, K.M.; Harrison, D.A. The JAK/STAT signaling pathway. *J. Cell Sci.* 2004, 117, 1281–1283. [CrossRef] [PubMed]
13. Baxter, E.J.; Scott, L.M.; Campbell, P.J.; East, C.; Fournouclas, N.; Swanton, S.; Vassiliou, G.S.; Bench, A.J.; Boyd, E.M.; Curtin, N.; et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *Lancet* 2005, 365, 1054–1061. [CrossRef]
14. 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.; et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer Cell* 2005, 7, 387–397. [CrossRef] [PubMed]
15. Jones, A.V.; Kreil, S.; Zoi, K.; Wasilewski, K.; Curtis, C.; Zhang, L.; Score, J.; See, R.; Chase, A.J.; Grand, F.H.; et al. Widespread occurrence of the JAK2 V617F mutation in chronic myeloproliferative disorders. *Blood* 2005, 106, 2162–2168. [CrossRef]
16. 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.* 2005, 352, 1779–1790. [CrossRef]
17. Bumm, T.G.; Elsea, C.; Corbin, A.S.; Loriaux, M.; Sherbenou, D.; Wood, L.; Deininger, J.; Silver, R.T.; Druker, B.J.; Deininger, M.W. Characterization of murine JAK2V617F-positive myeloproliferative disease. *Cancer Res.* 2006, 66, 11156–11165. [CrossRef]
18. 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* 2006, 108, 1652–1660. [CrossRef]
19. Wernig, G.; Merchier, 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* 2006, 107, 4274–4281. [CrossRef]
20. Zaleskas, V.M.; Krause, D.S.; Lazarides, K.; Patel, N.; Hu, Y.; Li, S.; Van Etten, R.A. Molecular pathogenesis and therapy of polycythemia induced in mice by Jak2 V617F. *PLoS ONE* 2006, 1, e18. [CrossRef]
21. Pikman, Y.; Lee, B.H.; Merchier, T.; McDowell, E.; Ebert, B.L.; Gozo, M.; Cuker, A.; Wernig, G.; Moore, S.; Galinsky, I.; et al. MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. *PLoS Med* 2006, 3, e270. [CrossRef] [PubMed]
22. Marty, C.; Pecquet, C.; Nivarthi, H.; El-Khoury, M.; Chachoua, I.; Tulliez, M.; Villeval, J.L.; Raslova, H.; Kralovics, R.; Constantinescu, S.N.; et al. Calreticulin mutants in mice induce an MPL-dependent thrombocytosis with frequent progression to myelofibrosis. *Blood* 2016, 127, 1317–1324. [CrossRef] [PubMed]
23. Yan, X.Q.; Lacey, D.; Fletcher, F.; Hartley, C.; McElroy, P.; Sun, Y.; Xia, M.; Mu, S.; Saris, C.; Hill, D.; et al. Chronic exposure to retroviral vector encoded MGDF (mpl-ligand) induces lineage-specific growth and differentiation of megakaryocytes in mice. *Blood* 1995, 86, 4025–4033. [CrossRef]
24. Villeval, J.L.; Cohen-Solal, K.; Tulliez, M.; Giraudier, S.; Guichard, J.; Burstein, S.A.; Cramer, E.M.; Vainchenker, W.; Wendling, F. High thrombopoietin production by hematopoietic cells induces a fatal myeloproliferative syndrome in mice. *Blood* **1997**, *90*, 4369–4383. [CrossRef] [PubMed]

25. Li, J.; Kent, D.G.; Chen, E.; Green, A.R. Mouse models of myeloproliferative neoplasms: JAK of all grades. *Dis. Model Mech.* **2011**, *4*, 311–317. [CrossRef] [PubMed]

26. Mullally, A.; Lane, S.W.; Brumme, K.; Ebert, B.L. Myeloproliferative neoplasm animal models. *Hematology/oncology clinics of North America* **2012**, *26*, 1065–1081. [CrossRef]

27. Shide, K.; Shimoda, H.K.; Kumano, T.; Karube, K.; Kameda, T.; Takenaka, K.; Oku, S.; Abe, H.; Katayose, K.S.; Kubuki, Y.; et al. Development of ET, primary myelofibrosis and PV in mice expressing JAK2V617F. *Leukemia* **2008**, *22*, 87–95. [CrossRef]

28. Xing, S.; Wanting, T.H.; Zhao, W.; Ma, J.; Wang, S.; Xu, X.; Li, Q.; Fu, X.; Xu, M.; Zhao, Z.J. Transgenic expression of JAK2V617F causes myeloproliferative disorders in mice. *Blood* **2008**, *111*, 5109–5117. [CrossRef]

29. Akada, H.; Yan, D.; Zou, H.; Fiering, S.; Hutchison, R.E.; Mohi, M.G. Conditional expression of heterozygous or homozygous Jak2V617F from its endogenous promoter induces a polycythemia vera-like disease. *Blood* **2010**, *115*, 3589–3597. [CrossRef]

30. Li, J.; Spensberger, D.; Ahn, J.S.; Anand, S.; Beer, P.A.; Ghevaert, C.; Chen, E.; Forrai, A.; Scott, L.M.; Ferreira, R.; et al. JAK2 V617F impairs hematopoietic stem cell function in a conditional knock-in mouse model of JAK2 V617F-positive essential thrombocythemia. *Blood* **2010**, *116*, 1528–1538. [CrossRef]

31. 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* **2010**, *116*, 783–787. [CrossRef] [PubMed]

32. Mullally, A.; Lane, S.W.; Ball, B.; Megerdichian, C.; Okabe, R.; Al-Shahrour, F.; Paktinat, M.; Haydu, J.E.; Housman, E.; Lord, A.M.; et al. Physiological Jak2V617F expression causes a lethal myeloproliferative neoplasm with differential effects on hematopoietic stem and progenitor cells. *Cancer Cell* **2010**, *17*, 584–596. [CrossRef]

33. Hasan, S.; Lacout, C.; Marty, C.; Cuingnet, M.; Solary, E.; Vainchenker, W.; Villeval, J.L. JAK2V617F expression in mice amplifies early hematopoietic cells and gives them a competitive advantage that is hampered by IFNalpha. *Blood* **2013**, *122*, 1464–1477. [CrossRef] [PubMed]

34. Mansier, O.; Kilani, B.; Guitart, A.V.; Guy, A.; Gourdou-Latyszenok, V.; Marty, C.; Parrens, M.; Plo, I.; Vainchenker, W.; James, C. Description of a knock-in mouse model of JAK2V617F MPN emerging from a minority of mutated hematopoietic stem cells. *Blood* **2019**, *134*, 2383–2387. [CrossRef] [PubMed]

35. 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 polycythaemia vera. *Blood* **2012**, *120*, 166–172. [CrossRef]

36. Tiedt, R.; Hao-Shen, H.; Sobas, M.A.; Looser, R.; Dirnhofer, S.; Schwaller, J.; Skoda, R.C. Ratio of mutant JAK2V617F to wild-type Jak2 determines the MPD phenotypes in transgenic mice. *Blood* **2008**, *111*, 3931–3940. [CrossRef]

37. Li, J.; Prins, D.; Park, H.J.; Grinfeld, J.; Gonzalez-Arias, C.; Loughran, S.; Dovey, O.M.; Klampfl, T.; Bennett, C.; Hamilton, T.L.; et al. Mutant calreticulin knockin mice develop thrombocytosis and myelofibrosis without a stem cell self-renewal advantage. *Blood* **2018**, *131*, 649–661. [CrossRef]

38. Kakumitsu, H.; Kamezaki, K.; Shimoda, K.; Karube, K.; Haro, T.; Numata, A.; Shide, K.; Matsuda, T.; Oshima, K.; Harada, M. Transgenic mice overexpressing murine thrombopoietin develop myelofibrosis and osteosclerosis. *Leuk. Res.* **2005**, *29*, 761–769. [CrossRef]

39. McDevitt, M.A.; Shivdasani, R.A.; Fujiwara, Y.; Yang, H.; Orkin, S.H. A “knockdown” mutation created by cis-element gene targeting reveals the dependence of erythroid cell maturation on the level of transcription factor GATA-1. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 6781–6785. [CrossRef]

40. Kirsammer, G.; Jilani, S.; Liu, H.; Davis, E.; Gurbuxani, S.; Le Beau, M.M.; Crispino, J.D. Highly penetrant myeloproliferative disease in the Ts65Dn mouse model of Down syndrome. *Blood* **2008**, *111*, 767–775. [CrossRef]

41. Pardanani, A.D.; Levine, R.L.; Lasho, T.; Pikman, Y.; Mesa, R.A.; Wadleigh, M.; Steensma, D.P.; Elliott, M.A.; Wolanskyj, A.P.; Hogan, W.J.; et al. MPLS15 mutations in myeloproliferative and other myeloid disorders: A study of 1182 patients. *Blood* **2006**, *108*, 3472–3476. [CrossRef] [PubMed]
42. Koppikar, P.; Abdel-Wahab, O.; Hedvat, C.; Marubayashi, S.; Patel, J.; Goel, A.; Kucine, N.; Gardner, J.R.; Combs, A.P.; Vaddi, K.; et al. Efficacy of the JAK2 inhibitor INCB16562 in a murine model of MPLW515L-induced thrombocytosis and myelofibrosis. *Blood* 2010, 115, 2919–2927. [CrossRef] [PubMed]

43. Wernig, G.; Kharas, M.G.; Mullanly, A.; Leeman, D.S.; Okabe, R.; George, T.; Clary, D.O.; Gilliland, D.G.; EXEL-8232, a small-molecule JAK2 inhibitor, effectively treats thrombocytosis and extramedullary hematopoiesis in a murine model of myeloproliferative neoplasm induced by MPLW515L. *Leukemia* 2012, 26, 720–727. [CrossRef] [PubMed]

44. Araki, M.; Yang, Y.; Masubuchi, N.; Hironaka, Y.; Takei, H.; Morishita, S.; Mizukami, Y.; Kan, S.; Shirane, S.; Edahiro, Y.; et al. Activation of the thrombopoietin receptor by mutant calreticulin in CALR-mutant myeloproliferative neoplasms. *Blood* 2016, 127, 1307–1316. [CrossRef] [PubMed]

45. Chachoua, I.; Pecquet, C.; El-Khoury, M.; Nivarthi, H.; Albu, R.I.; Marty, C.; Gryshkova, V.; Defour, J.P.; Vertenoeil, G.; Ngo, A.; et al. Thrombopoietin receptor activation by myeloproliferative neoplasm associated calreticulin mutants. *Blood* 2016, 127, 1325–1335. [CrossRef] [PubMed]

46. Elf, S.; Abdelfattah, N.S.; Chen, E.; Perales-Paton, J.; Rosen, E.A.; Ko, A.; Peisker, F.; Florescu, N.; Giannini, S.; Wolach, O.; et al. Mutant Calreticulin Requires Both Its Mutant C-terminus and the Thrombopoietin Receptor for Oncogenic Transformation. *Cancer Discov.* 2016, 6, 368–381. [CrossRef]

47. Imai, M.; Araki, M.; Komatsu, N. Somatic mutations of calreticulin in myeloproliferative neoplasms. *Int. J. Hematol.* 2017, 105, 743–747. [CrossRef]

48. Pecquet, C.; Chachoua, I.; Roy, A.; Balligand, T.; Vertenoeil, G.; Leroy, E.; Albu, R.I.; Defour, J.P.; Nivarthi, H.; Hug, E.; et al. Calreticulin mutants as oncogenic rogue chaperones for TpoR and traffic-defective pathogenic TpoR mutants. *Blood* 2019, 133, 2669–2681. [CrossRef]

49. Nivarthi, H.; Chen, D.; Cleary, C.; Nivarthi, H.; Defour, J.P.; Pecquet, C.; Vainchenker, W.; Constantinescu, S.N.; et al. Thrombopoietin receptor is required for the oncogenic function of CALR mutants. *Leukemia* 2016, 30, 1759–1763. [CrossRef]

50. Finazzi, M.C.; Carobbio, A.; Cervantes, F.; Isola, I.M.; Vannucchi, A.M.; Guglielmelli, P.; Rambaldi, A.; Finazzi, G.; Barosi, G.; Barbui, T. CALR mutation, MPL mutation and triple negativity identify patients with the lowest vascular risk in primary myelofibrosis. *Leukemia* 2015, 29, 1209–1210. [CrossRef]

51. Tefferi, A.; Guglielmelli, P.; Laslo, T.L.; Rotunno, G.; Finke, C.; Mannarelli, C.; Belachew, A.A.; Pancrazzi, A.; Wassie, E.A.; Ketterling, R.P.; et al. CALR and ASXL1 mutations-based molecular prognostication in primary myelofibrosis: An international study of 570 patients. *Leukemia* 2014, 28, 1494–1500. [CrossRef] [PubMed]

52. Tefferi, A.; Laslo, T.L.; Tischer, A.; Wassie, E.A.; Finke, C.M.; Belachew, A.A.; Ketterling, R.P.; Hanson, C.A.; Pardanani, A.D. The prognostic advantage of calreticulin mutations in myelofibrosis might be confined to type 1 or type 1-like CALR variants. *Blood* 2014, 124, 2465–2466. [CrossRef] [PubMed]

53. Shide, K.; Kameda, T.; Yamaji, T.; Sekine, M.; Inada, N.; Kumiunten, A.; Akizuki, K.; Nakamura, K.; Hidaka, T.; Kubuki, Y.; et al. Calreticulin mutant mice develop essential thrombocythemia that is ameliorated by the JAK inhibitor ruxolitinib. *Leukemia* 2017, 31, 1136–1144. [CrossRef] [PubMed]

54. Balligand, T.; Aouhri, Y.; Pecquet, C.; Gaudray, G.; Colau, D.; Hug, E.; Rahmani, Y.; Stroobant, V.; Plo, I.; Vainchenker, W.; et al. Knock-in of murine Calr del52 induces essential thrombocythemia with slow-rising dominance in mice and reveals key role of Calr exon 9 in cardiac development. *Leukemia* 2020, 34, 510–521. [CrossRef]

55. Cabagnols, X.; Defour, J.P.; Ugo, V.; Janotto, J.C.; Mossuz, P.; Mondet, J.; Girodon, F.; Alexandre, J.H.; Mansier, O.; Viallard, J.F.; et al. Differential association of calreticulin type 1 and type 2 mutations with myelofibrosis and essential thrombocytemia: Relevance for disease evolution. *Leukemia* 2015, 29, 249–252. [CrossRef]

56. Tefferi, A.; Wassie, E.A.; Guglielmelli, P.; Gangat, N.; Belachew, A.A.; Laslo, T.L.; Finke, C.; Ketterling, R.P.; Hanson, C.A.; Pardanani, A.; et al. Type 1 versus Type 2 calreticulin mutations in essential thrombocytemia: A collaborative study of 1027 patients. *Am. J. Hematol.* 2014, 89, E121–E124. [CrossRef]

57. Pietra, D.; Rumi, E.; Ferretti, V.V.; Di Buduo, C.A.; Milanesi, C.; Cavalloni, C.; Sant’Antonio, E.; Abbonante, V.; Moccia, F.; Casetti, I.C.; et al. Differential clinical effects of different mutation subtypes in CALR-mutant myeloproliferative neoplasms. *Leukemia* 2016, 30, 431–438. [CrossRef]

58. Decker, M.; Martinez-Morentin, L.; Wang, G.; Lee, Y.; Liu, Q.; Leslie, J.; Ding, L. Leptin-receptor-expressing bone marrow stromal cells are myofibroblasts in primary myelofibrosis. *Nat. Cell Biol.* 2017, 19, 677–688. [CrossRef]
59. Kramer, F.; Demedde, J.; Mezheyevski, A.; Tauber, R.; Micke, P.; Kappert, K. Platelet-derived growth factor receptor beta activation and regulation in murine myelofibrosis. *Haematologica* 2020, 105, 2083–2094. [CrossRef]

60. Vannucchi, A.M.; Bianchi, L.; Cellai, C.; Paoletti, F.; Rana, R.A.; Lorenzini, R.; Migliaccio, G.; Migliaccio, A.R. Development of myelofibrosis in mice genetically impaired for GATA-1 expression (GATA-1(low) mice). *Blood* 2002, 100, 1123–1132. [CrossRef]

61. Zingariello, M.; Martelli, F.; Ciaffoni, F.; Masiello, F.; Ghinassi, B.; D’Amore, E.; Massa, M.; Barosi, G.; Sancilio, L.; Li, X.; et al. Characterization of the TGF-beta1 signaling abnormalities in the Gata1low mouse model of myelofibrosis. *Blood* 2013, 121, 3345–3363. [CrossRef] [PubMed]

62. Zingariello, M.; Sancilio, L.; Martelli, F.; Ciaffoni, F.; Marra, M.; Varricchio, L.; Rana, R.A.; Zhao, C.; Crispino, J.D.; Migliaccio, A.R. The thrombopoietin/MPL axis is activated in the Gata1(low) mouse model of myelofibrosis and is associated with a defective RPS14 signature. *Blood Cancer J.* 2017, 7, e572. [CrossRef] [PubMed]

63. Chorzalska, A.; Morgan, J.; Ahsan, N.; Treaba, D.O.; Olszewski, A.J.; Petersen, M.; Kingston, N.; Cheng, Y.; Lombardo, K.; Schorl, E.; et al. Bone marrow-specific loss of ABI1 induces myeloproliferative neoplasm with features resembling human myelofibrosis. *Blood* 2018, 132, 2053–2066. [CrossRef] [PubMed]

64. Bersenev, A.; Wu, C.; Balcerek, J.; Tong, W. Lnk controls mouse hematopoietic stem cell self-renewal and quiescence through direct interactions with JAK2. *J. Clin. Investig.* 2008, 118, 2832–2844. [CrossRef]

65. Lasho, T.L.; Tefferi, A.; Finke, C.; Pardanani, A. Clonal hierarchy and allelic mutation segregation in a myelofibrosis patient with two distinct LNK mutations. *Leukemia* 2011, 25, 1056–1058. [CrossRef]

66. Pardanani, A.; Lasho, T.; Finke, C.; Oh, S.T.; Gotlib, J.; Tefferi, A. LNK mutation studies in blast-phase myeloproliferative neoplasms, and in chronic-phase disease with TET2, IDH, JAK2 or MPL mutations. *Leukemia* 2010, 24, 1713–1718. [CrossRef]

67. Bersenev, A.; Wu, C.; Balcerek, J.; Jing, J.; Kundu, M.; Blobel, G.A.; Chikwava, K.R.; Tong, W. Lnk constrains myeloproliferative diseases in mice. *J Clin Invest* 2010, 120, 2058–2069. [CrossRef]

68. Velazquez, L.; Cheng, A.M.; Fleming, H.E.; Furlonger, C.; Vesely, S.; Bernstein, A.; Paige, C.J.; Pawson, T. Cytokine signaling and hematopoietic homeostasis are disrupted in Lnk-deficient mice. *J. Exp. Med.* 2002, 195, 1599–1611. [CrossRef]

69. Shimizu, T.; Kubovcakova, L.; Nienhold, R.; Zmajkovic, J.; Meyer, S.C.; Hao-Shen, H.; Geier, F.; Dirnhofer, S.; Guglielmelli, P.; Vannucchi, A.M.; et al. Loss of Ezh2 synergizes with JAK2-V617F in initiating myeloproliferative neoplasms and promoting myelofibrosis. *J. Exp. Med.* 2016, 233, 1479–1496. [CrossRef]

70. Yang, Y.; Akada, H.; Nath, D.; Hutchison, R.E.; Mohi, G. Loss of Ezh2 cooperates with Jak2V617F in the development of myelofibrosis in a mouse model of myeloproliferative neoplasm. *Blood* 2016, 127, 3410–3423. [CrossRef]

71. Sashida, G.; Wang, C.; Tomioka, T.; Oshima, M.; Aoyama, K.; Kanai, A.; Mochizuki-Kashio, M.; Harada, H.; Shimoda, K.; Iwama, A. The loss of Ezh2 drives the pathogenesis of myelofibrosis and sensitizes tumor-initiating cells to bromodomain inhibition. *J Exp Med* 2016, 213, 1459–1477. [CrossRef] [PubMed]

72. Guo, Y.; Zhou, Y.; Yamamoto, S.; Yang, H.; Zhang, P.; Chen, S.; Nimer, S.D.; Zhao, Z.J.; Xu, M.; Bai, J.; et al. ASXL1 alteration cooperates with JAK2V617F to accelerate myelofibrosis. *Leukemia* 2019, 33, 1287–1291. [CrossRef] [PubMed]

73. Jacquelin, S.; Straube, J.; Cooper, L.; Vu, T.; Song, A.; Bywater, M.; Baxter, E.; Heidecker, M.; Wackrow, B.; Porter, A.; et al. Jak2V617F and Dnmt3a loss cooperate to induce myelofibrosis through activated enhancer-driven inflammation. *Blood* 2018. [CrossRef] [PubMed]

74. Abdel-Wahab, O.; Pardanani, A.; Patel, J.; Wadleigh, M.; Lasho, T.; Heguy, A.; Beran, M.; Gilliland, D.G.; Levine, R.L.; Tefferi, A. Concomitant analysis of EZH2 and ASXL1 mutations in myelofibrosis, chronic myelomonocytic leukemia and blast-phase myeloproliferative neoplasms. *Leukemia* 2011, 25, 1200–1202. [CrossRef] [PubMed]

75. Abdel-Wahab, O.; Patel, J.; Levine, R.L. Clinical implications of novel mutations in epigenetic modifiers in AML. *Hematol. Oncol. Clin. N. Am.* 2011, 25, 1119–1133. [CrossRef]

76. Vainchenker, W.; Delhommeau, F.; Constantinescu, S.N.; Bernard, O.A. New mutations and pathogenesis of myeloproliferative neoplasms. *Blood* 2011, 118, 1723–1735. [CrossRef]
77. Lundberg, P.; Karow, A.; Nienhold, R.; Looser, R.; Hao-Shen, H.; Nissen, I.; Girgsberger, S.; Lehmann, T.; Passweg, J.; Stern, M.; et al. Clonal evolution and clinical correlates of somatic mutations in myeloproliferative neoplasms. *Blood* 2014, 123, 2220–2228. [CrossRef]

78. Tefferi, A.; Lasho, T.L.; Finke, C.M.; Elala, Y.; Hanson, C.A.; Ketterling, R.P.; Gangat, N.; Pardanani, A. Targeted deep sequencing in primary myelofibrosis. *Blood Adv.* 2016, 1, 105–111. [CrossRef]

79. Cruz-Molina, S.; Respuela, P.; Tebart, C.; Kolovos, P.; Nikolic, M.; Fueyo, R.; Van Ijken, W.F.J.; Grosveld, F.; Frommolt, P.; Bazzi, H.; et al. PRC2 Facilitates the Regulatory Topology Required for Poised Enhancer Function during Pluripotent Stem Cell Differentiation. *Cell Stem Cell* 2017, 20, 689–705 e689. [CrossRef]

80. Margueron, R.; Reinberg, D. The Polycomb complex PRC2 and its mark in life. *Nature* 2011, 469, 343–349. [CrossRef]

81. Herrera-Merchan, A.; Arranz, L.; Ligos, J.M.; De Molina, A.; Dominguez, O.; Gonzalez, S. Ectopic expression of the histone methyltransferase Ezh2 in haematopoietic stem cells causes myeloproliferative disease. *Nat. Commun.* 2012, 3, 623. [CrossRef] [PubMed]

82. Beguelin, W.; Popovic, R.; Teater, M.; Jiang, Y.; Bunting, K.L.; Rosen, M.; Shen, H.; Yang, S.N.; Wang, L.; Ezponda, T.; et al. EZH2 is required for germline center formation and somatic EZH2 mutations promote lymphoid transformation. *Cancer Cell* 2013, 23, 677–692. [CrossRef] [PubMed]

83. Morin, R.D.; Johnson, N.A.; Severson, T.M.; Mungall, A.; An, J.; Yoda, R.; Paul, J.E.; Boyle, M.; Woolcock, B.W.; Kuchenbauer, F.; et al. Somatic mutations altering EZH2 (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. *Nat. Genet.* 2010, 42, 181–185. [CrossRef] [PubMed]

84. Guglielmelli, P.; Lasho, T.L.; Rotunno, G.; Mudireddy, M.; Mannarelli, C.; Nicolosi, M.; Pacilli, A.; Pardanani, A.; Rumi, E.; Rosti, V.; et al. MIPSS70: Mutation-Enhanced International Prognostic Score System for Transplantation-Age Patients With Primary Myelofibrosis. *J. Clin. Oncol.* 2018, 36, 310–318. [CrossRef] [PubMed]

85. Bejar, R.; Stevenson, K.; Abdel-Wahab, O.; Galili, N.; Nilsson, B.; Garcia-Manero, G.; Kantarjian, H.; Raza, A.; Levine, R.L.; Neuberg, D.; et al. Clinical effect of point mutations in myelodysplastic syndromes. *N. Engl. J. Med.* 2011, 364, 2496–2506. [CrossRef] [PubMed]

86. Gollner, S.; Oellerich, T.; Agrawal-Singh, S.; Schenk, T.; Klein, H.U.; Rohde, C.; Pabst, C.; Sauer, T.; Lerdrup, M.; Tavor, S.; et al. Loss of the histone methyltransferase Ezh2 induces resistance to multiple drugs in acute myeloid leukemia. *Nat. Med.* 2017, 23, 69–78. [CrossRef]

87. Guglielmelli, P.; Biamonte, F.; Score, J.; Hidalgo-Curtis, C.; Cervantes, F.; Manelli, T.; Ernst, T.; Winkelman, N.; Jones, A.V.; et al. EZH2 mutational status predicts poor survival in myelofibrosis. *Blood* 2011, 118, 5227–5234. [CrossRef]

88. Hirabayashi, Y.; Suzuki, N.; Tsuboi, M.; Endo, T.A.; Toyoda, T.; Shinga, J.; Koseki, H.; Vidal, M.; Gotoh, Y. Prolonging the neurogenic competence of neural precursor cells to promote astrogenic fate transition. *Neuron* 2009, 63, 600–613. [CrossRef]

89. Neff, T.; Sinha, A.U.; Khuk, M.J.; Zhu, N.; Khatib, M.H.; Stein, L.; Xie, H.; Orkin, S.H.; Armstrong, S.A. Polycomb repressive complex 2 is required for MLL-AF9 leukemia. *Proc. Natl. Acad. Sci. USA* 2012, 109, 5028–5033. [CrossRef]

90. Su, I.H.; Basavaraj, A.; Krutchinsky, A.N.; Hobert, O.; Ullrich, A.; Chait, B.T.; Tarakhovsky, A. Ezh2 controls B cell development through histone H3 methylation and IgH rearrangement. *Nat. Immunol.* 2003, 4, 124–131. [CrossRef] [PubMed]

91. Guglielmelli, P.; Zini, R.; Bogani, C.; Salati, S.; Panzaracci, A.; Bianchi, E.; Mannelli, F.; Ferrari, S.; Le Bousse-Kerdiles, M.C.; Bosi, A.; et al. Molecular profiling of CD34+ cells in idiopathic myelofibrosis identifies a set of disease-associated genes and reveals the clinical significance of Wilms’ tumor gene 1 (WT1). *Stem Cells* 2007, 25, 165–173. [CrossRef] [PubMed]

92. Harada-Shirado, K.; Ikeda, K.; Ogawa, K.; Okhawara, H.; Kimura, H.; Kai, T.; Noji, H.; Morishita, S.; Komatsu, N.; Takeishi, Y. Dysregulation of the MIRLET7/HMGAA2 axis with methylation of the CDKN2A promoter in myeloproliferative neoplasms. *Br. J. Haematol.* 2015, 168, 338–349. [CrossRef] [PubMed]

93. Boulware, J.; Perry, J.; Pellagatti, A.; Fernandez-Mercado, M.; Fernandez-Santamarina, C.; Calasan, M.J.; Larrayoz, M.J.; Garcia-Delgado, M.; Giagounidis, A.; Malcovati, L.; et al. Frequent mutation of the polycomb-associated gene ASXL1 in the myelodysplastic syndromes and in acute myeloid leukemia. *Leukemia* 2010, 24, 1062–1065. [CrossRef] [PubMed]
94. Bouloumood, J.; Perry, J.; Zaman, R.; Fernandez-Santamaria, C.; Littlewood, T.; Kusec, R.; Pellagatti, A.; Wang, L.; Clark, R.E.; Wainscoat, J.S. High-density single nucleotide polymorphism array analysis and ASXL1 gene mutation screening in chronic myeloid leukemia during disease progression. *Leukemia* 2010, 24, 1139–1145. [CrossRef]

95. Caruccia, N.; Murati, A.; Trouplin, V.; Brecqueville, M.; Adelaide, J.; Rey, J.; Vainchenker, W.; Bernard, O.A.; Chaffanet, M.; Vey, N.; et al. Mutations of ASXL1 gene in myeloproliferative neoplasms. *Leukemia* 2009, 23, 2183–2186. [CrossRef]

96. Caruccia, N.; Trouplin, V.; Gelsi-Boyer, V.; Murati, A.; Rocquain, J.; Adelaide, J.; Olschwang, S.; Xerri, L.; Vey, N.; Chaffanet, M.; et al. Mutual exclusion of ASXL1 and NPM1 mutations in a series of acute myeloid leukemias. *Leukemia* 2010, 24, 469–473. [CrossRef]

97. Chen, T.C.; Hou, H.A.; Chou, W.C.; Tang, J.L.; Kuo, Y.Y.; Chen, C.Y.; Tseng, M.H.; Huang, C.F.; Lai, Y.J.; Chiang, Y.C.; et al. Dynamics of ASXL1 mutation and other associated genetic alterations during disease progression in patients with primary myelodysplastic syndrome. *Blood Cancer J.* 2014, 4, e177. [CrossRef]

98. Gelsi-Boyer, V.; Trouplin, V.; Adelaide, J.; Bonansea, J.; Cervera, N.; Carbuccia, N.; Lagarde, A.; Prebet, T.; Nezri, M.; Sainty, D.; et al. Mutations of polycomb-associated gene ASXL1 in myelodysplastic syndromes and chronic myelomonocytic leukemia. *Br. J. Haematol.* 2009, 145, 788–800. [CrossRef]

99. Haferlach, T.; Nagata, Y.; Grossmann, V.; Okuno, Y.; Bacher, U.; Nagae, G.; Schnittger, S.; Sanada, M.; Kon, A.; Alpermann, T.; et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. *Leukemia* 2014, 28, 241–247. [CrossRef]

100. Huether, R.; Dong, L.; Chen, X.; Wu, G.; Parker, M.; Wei, L.; Ma, J.; Edmonson, M.N.; Hedlund, E.K.; Rusch, M.C.; et al. The landscape of somatic mutations in epigenetic regulators across 1,000 paediatric cancer genomes. *Nat. Commun.* 2014, 5, 3630. [CrossRef]

101. Makishima, H.; Jankowska, A.M.; McDevitt, M.A.; O’Keefe, C.; Dujardin, S.; Cazzolli, H.; Przychodzin, L.; Prince, C.; Nicoll, J.; Siddaiah, H.; et al. CBL, CBLB, TET2, ASXL1, and IDH1/2 mutations and additional chromosomal aberrations constitute molecular events in chronic myelogenous leukemia. *Blood* 2011, 117, e199–e206. [CrossRef] [PubMed]

102. Sugimoto, Y.; Muramatsu, H.; Makishima, H.; Prince, C.; Jankowska, A.M.; Yoshida, N.; Xu, Y.; Nishio, N.; Hama, A.; Yagasaki, H.; et al. Spectrum of molecular defects in juvenile myelomonocytic leukemia includes ASXL1 mutations. *Br. J. Haematol.* 2010, 150, 83–87. [CrossRef] [PubMed]

103. Thol, F.; Friess, I.; Damm, F.; Yun, H.; Weissinger, E.M.; Krauter, J.; Wagner, K.; Chaturvedi, A.; Sharma, A.; Wichmann, M.; et al. Prognostic significance of ASXL1 mutations in patients with myelodysplastic syndromes. *J. Clin. Oncol.* 2011, 29, 2499–2506. [CrossRef] [PubMed]

104. Genovese, G.; Kahler, A.K.; Handsaker, R.E.; Lindberg, J.; Rose, S.A.; Bakhour, S.F.; Chambert, K.; Mick, E.; Neale, B.M.; Fromer, M.; et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N. Engl. J. Med.* 2014, 371, 2477–2487. [CrossRef] [PubMed]

105. Jaiswal, S.; Fontanillas, P.; Flannick, J.; Manning, A.; Grauman, P.V.; Mar, B.G.; Mermel, C.H.; Nezri, M.; et al. Mutations of ASXL1 gene in myeloproliferative neoplasms. *Br. J. Haematol.* 2010, 150, 83–87. [CrossRef] [PubMed]

106. Xie, M.; Lu, C.; Wang, J.; McLellan, M.D.; Johnson, K.J.; Wendell, M.C.; Schmidt, H.K.; Yellapantula, V.; Miller, C.A.; et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N. Engl. J. Med.* 2014, 371, 2488–2498. [CrossRef]

107. Abdel-Wahab, O.; Adli, M.; LaFave, L.M.; Cai, J.; Hricik, T.; Shi, A.H.; Pandey, S.; Patel, J.P.; Chung, Y.R.; Koche, R.; et al. ASXL1 mutations promote myeloid transformation through loss of PRC2-mediated gene repression. *Cancer Cell* 2012, 22, 180–193. [CrossRef]

108. Nagase, R.; Inoue, D.; Pastore, A.; Fujino, T.; Hou, H.A.; Yamasaki, N.; Goyama, S.; Saika, M.; Kanai, A.; Sera, Y.; et al. Expression of mutant Asxl1 perturbs hematopoiesis and promotes susceptibility to leukemic transformation. *J. Exp. Med.* 2018, 215, 1729–1747. [CrossRef]

109. Shi, H.; Yamamoto, S.; Sheng, M.; Bai, J.; Zhang, P.; Chen, R.; Chen, S.; Shi, L.; Abdel-Wahab, O.; Xu, M.; et al. ASXL1 plays an important role in erythropoiesis. *Sci. Rep.* 2016, 6, 28789. [CrossRef]

110. Yang, H.; Kurtenback, S.; Guo, Y.; Lohse, I.; Durante, M.A.; Li, J.; Li, Z.; Al-Ali, H.; Li, L.; Chen, Z.; et al. Gain of function of ASXL1 truncating protein in the pathogenesis of myeloid malignancies. *Blood* 2018, 131, 328–341. [CrossRef]
111. Gelsi-Boyer, V.; Brecqueville, M.; Devillier, R.; Murati, A.; Mozziconacci, M.J.; Birnbaum, D. Mutations in ASXL1 are associated with poor prognosis across the spectrum of malignant myeloid diseases. *J. Hematol. Oncol.* 2012, 5, 12. [CrossRef] [PubMed]

112. Wang, J.; Li, Z.; He, Y.; Pan, F.; Chen, S.; Rhodes, S.; Nguyen, L.; Yuan, J.; Jiang, L.; Yang, X.; et al. Loss of Asxl1 leads to myelodysplastic syndrome-like disease in mice. *Blood* 2014, 123, 541–553. [CrossRef] [PubMed]

113. Rinaldi, L.; Datta, D.; Serrat, J.; Morey, L.; Solanas, G.; Avgustinova, A.; Blanco, E.; Fons, J.I.; Matallanas, D.; Von Kriegsheim, A.; et al. Dnmt3a and Dnmt3b Associate with Enhancers to Regulate Human Epidermal Stem Cell Homeostasis. *Cell Stem Cell* 2016, 19, 491–501. [CrossRef] [PubMed]

114. Yang, L.; Rodriguez, B.; Mayle, A.; Park, H.J.; Lin, X.; Luo, M.; Jeong, M.; Curry, C.V.; Kim, S.B.; Ruau, D.; et al. DNMT3A Loss Drives Enhancer Hypomethylation in FLT3-ITD-Associated Leukemias. *Cancer Cell* 2016, 29, 922–934. [CrossRef]

115. Ley, T.J.; Ding, L.; Walter, M.J.; McElellan, M.D.; Lamprecht, T.; Larson, D.E.; Kandoth, C.; Payton, J.E.; Baty, J.; Welch, J.; et al. DNMT3A mutations in acute myeloid leukemia. *N. Engl. J. Med.* 2010, 363, 2424–2433. [CrossRef]

116. Abdel-Wahab, O.; Pardanani, A.; Rampal, R.; Lasho, T.L.; Levine, R.L.; Tefferi, A. DNMT3A mutational analysis in primary myelofibrosis, chronic myelomonocytic leukemia and advanced phases of myeloproliferative neoplasms. *Leukemia* 2011, 25, 1219–1220. [CrossRef]

117. Stegelmann, F.; Bullinger, L.; Schlenk, R.F.; Paschka, P.; Griesshammer, M.; Blersch, C.; Kuhn, S.; Schauer, S.; Döhner, H.; Döhner, K. DNMT3A mutations in myeloproliferative neoplasms. *Leukemia* 2011, 25, 1217–1219. [CrossRef]

118. Russler-Germain, D.A.; Spencer, D.H.; Young, M.A.; Lamprecht, T.L.; Miller, C.A.; Fulton, R.; Meyer, M.R.; Erdmann-Gilmore, P.; Townsend, R.R.; Wilson, R.K.; et al. The R882H DNMT3A mutation associated with AML dominantly inhibits wild-type DNMT3A by blocking its ability to form active tetramers. *Cancer Cell* 2014, 25, 442–454. [CrossRef]

119. Yang, L.; Rau, R.; Goodell, M.A. DNMT3A in haematological malignancies. *Nat. Rev. Cancer* 2015, 15, 152–165. [CrossRef]

120. Challen, G.A.; Sun, D.; Jeong, M.; Luo, M.; Jelinek, J.; Berg, J.S.; Bock, C.; Vasanthakumar, A.; Gu, H.; Xi, Y.; et al. Dnmt3a is essential for hematopoietic stem cell differentiation. *Nat. Genet.* 2012, 44, 23–31. [CrossRef]

121. Van den Akker, E.B.; Pitts, S.J.; Deelen, J.; Moed, M.H.; Potluri, S.; Van Rooij, J.; Suchiman, H.E.; Lakenberg, N.; De Diijker, W.J.; Uitterlinden, A.G.; et al. Uncompromised 10-year survival of oldest old carrying somatic mutations in DNMT3A and TET2. *Blood* 2016, 127, 1512–1515. [CrossRef] [PubMed]

122. Young, A.L.; Challen, G.A.; Birmann, B.M.; Druley, T.E. Clonal haematopoiesis harbouring AML-associated mutations is ubiquitous in healthy adults. *Nat. Commun.* 2016, 7, 12484. [CrossRef] [PubMed]

123. Guryanova, O.A.; Shank, K.; Spitzer, B.; Luciani, L.; Koche, R.P.; Garrett-Bakelman, F.E.; Ganzel, C.; Durum, B.H.; Mohanty, A.; Hoermann, G.; et al. DNMT3A mutations promote anchracycline resistance in acute myeloid leukemia via impaired nucleosome remodeling. *Nat. Med.* 2016, 22, 1488–1495. [CrossRef] [PubMed]

124. Yue, L.; Bartenstein, M.; Zhao, W.; Ho, W.T.; Han, Y.; Murdun, C.; Mailloux, A.W.; Zhang, L.; Wang, X.; Budhathoki, A.; et al. Efficacy of ALK5 inhibition in myelofibrosis. *JCI Insight* 2017, 2, e90932. [CrossRef]

125. Chagraoui, H.; Komura, E.; Tulliez, M.; Giraudier, S.; Vainchenker, W.; Wendling, F. Prominent role of TGF-beta 1 in thrombopoietin-induced myelofibrosis in mice. *Blood* 2002, 100, 3459–3503. [CrossRef]

126. Evrard, S.; Bluteau, O.; Tulliez, M.; Rameau, P.; Gonin, P.; Zetterberg, E.; Palmblad, J.; Bonnefoy, A.; Villeval, J.L.; Vainchenker, W.; et al. Thrombospondin-1 is not the major activator of TGF-beta1 in thrombopoietin-induced myelofibrosis. *Blood* 2011, 117, 246–249. [CrossRef]

127. Chagraoui, H.; Tulliez, M.; Smayra, T.; Komura, E.; Giraudier, S.; Yun, T.; Lassau, N.; Vainchenker, W.; Wendling, F. Stimulation of osteoprotegerin production is responsible for osteosclerosis in mice overexpressing TPO. *Blood* 2003, 101, 2983–2989. [CrossRef]

128. Verstovsek, S.; Manshour, T.; Pilling, D.; Bueso-Ramos, C.E.; Newberry, K.J.; Prijic, S.; Knez, L.; Bozinovic, K.; Harris, D.M.; Spaeth, E.L.; et al. Role of neoplastic monocyte-derived fibrocytes in primary myelofibrosis. *J. Exp. Med.* 2016, 213, 1723–1740. [CrossRef]

129. Schneider, R.K.; Mullally, A.; Dugourd, A.; Peisker, F.; Hoogenboezem, R.; Van Strien, P.M.H.; Bindels, E.M.; Heckl, D.; Busche, G.; Fleck, D.; et al. Gli1(+) Mesenchymal Stromal Cells Are a Key Driver of Bone Marrow Fibrosis and an Important Cellular Therapeutic Target. *Cell Stem Cell* 2017, 20, 785–800 e788. [CrossRef]
130. Van Bon, L.; Affandi, A.J.; Broen, J.; Christmann, R.B.; Marijnissen, R.J.; Stawski, L.; Farina, G.A.; Stifano, G.; Mathes, A.L.; Cossu, M.; et al. Proteome-wide analysis and CXCL4 as a biomarker in systemic sclerosis. N. Engl. J. Med. 2014, 370, 433–443. [CrossRef]

131. Kramann, R.; Fleig, S.V.; Schneider, R.K.; Fabian, S.L.; DiRocco, D.P.; Mauroff, W.; Ong swojąensin, J.; Ikeda, Y.; Heckl, D.; Chang, S.L.; et al. Pharmacological GLI2 inhibition prevents myofibroblast cell-cycle progression and reduces kidney fibrosis. J. Clin. Investig. 2015, 125, 2935–2951. [CrossRef] [PubMed]

132. Moshai, E.F.; Wemeau-Stervinou, L.; Cigna, N.; Brayer, S.; Somme, J.M.; Crestani, B.; Maillieux, A.A. Targeting the hedgehog-glioma-associated oncogene homolog pathway inhibits bleomycin-induced lung fibrosis in mice. Am. J. Respir. Cell Mol. Biol. 2014, 51, 11–25. [CrossRef]

133. Celik, H.; Koh, W.K.; Kramer, A.C.; Ostrander, E.L.; Mallaney, C.; Fisher, D.A.C.; Xiang, J.; Wilson, W.C.; Martens, A.; Kothari, A.; et al. JARID2 Functions as a Tumor Suppressor in Myeloid Neoplasms by Repressing Self-Renewal in Hematopoietic Progenitor Cells. Cancer Cell 2018, 34, 741–756 e748. [CrossRef]

134. Fisher, D.A.C.; Miner, C.A.; Engle, E.K.; Hu, H.; Collins, T.B.; Zhou, A.; Allen, M.J.; Malkova, O.N.; Oh, S.T. Cytokine production in myelofibrosis exhibits differential responsiveness to JAK-STAT, MAP kinase, and NFκB signaling. Leukemia 2019, 33, 1978–1995. [CrossRef] [PubMed]

135. Martinez-Trillos, A.; Gay, A.; Maffioli, M.; Arellano-Rodrigo, E.; Calvo, X.; Diaz-Beya, M.; Cervantes, F. Efficacy and tolerability of hydroxyurea in the treatment of the hyperproliferative manifestations of myelofibrosis: Results in 40 patients. Ann. Hematol. 2010, 89, 1233–1237. [CrossRef] [PubMed]

136. Rampal, R.; Al-Shahrour, F.; Abdel-Wahab, O.; Patel, J.P.; Brunel, J.P.; Mermel, C.H.; Bass, A.J.; Pretz, J.; Ahn, J.; Hricik, T.; et al. Integrated genomic analysis illustrates the central role of JAK-STAT pathway activation in myeloproliferative neoplasm pathogenesis. Blood 2014, 123, e123–e133. [CrossRef] [PubMed]

137. Harrison, C.; Kiladjian, J.J.; Al-Ali, H.K.; Gisslinger, H.; Waltzman, R.; Stalbovskaya, V.; McQuitty, M.; Hunter, D.S.; Levy, R.; Knoops, L.; et al. JAK inhibition with ruxolitinib versus best available therapy for myelofibrosis. N. Engl. J. Med. 2012, 366, 787–798. [CrossRef]

138. Verstovsek, S.; Kantarjian, H.; Mesa, R.A.; Pardanani, A.D.; Cortes-Franco, J.; Thomas, D.A.; Estrov, Z.; Fridman, J.S.; Bradley, E.C.; Erickson-Viitanen, S.; et al. Safety and efficacy of INCB018424, a JAK1 and JAK2 inhibitor, in myelofibrosis. N. Engl. J. Med. 2010, 363, 1117–1127. [CrossRef]

139. Austin, R.J.; Straube, J.; Bruedigam, C.; Pali, G.; Jacquelin, S.; Brayer, S.; Somme, J.M.; Crestani, B.; Maillieux, A.A. Targeting the hedgehog-glioma-associated oncogene homolog pathway inhibits bleomycin-induced lung fibrosis in mice. Am. J. Respir. Cell Mol. Biol. 2014, 51, 11–25. [CrossRef]

140. Mascarenhas, J.; Kremyanskaya, M.; Hoffman, R.; Bose, P.; Talpaz, M.; Harrison, C.N.; Gupta, V.; Leber, B.; Sirhan, S.; Kabir, S.; et al. MANIFEST, a Phase 2 Study of CPI-0610, a Bromodomain and Extraterminal Domain Inhibitor (BETi), As Monotherapy or “Add-on” to Ruxolitinib, in Patients with Refractory or Intolerant Advanced Myelofibrosis. Am. Soc. Hematol. Orlando 2019, 134. [CrossRef]

141. Doroshow, D.B.; Eder, J.P.; LoRusso, P.M. BET inhibitors: A novel epigenetic approach. Ann. Oncol. 2017, 28, 1776–1787. [CrossRef] [PubMed]

142. Moshai, E.F.; Wemeau-Stervinou, L.; Cigna, N.; Brayer, S.; Somme, J.M.; Crestani, B.; Mailleux, A.A. Targeting the hedgehog-glioma-associated oncogene homolog pathway inhibits bleomycin-induced lung fibrosis in mice. Am. J. Respir. Cell Mol. Biol. 2014, 51, 11–25. [CrossRef]

143. Fisher, D.A.C.; Miner, C.A.; Engle, E.K.; Hu, H.; Collins, T.B.; Zhou, A.; Allen, M.J.; Malkova, O.N.; Oh, S.T. Cytokine production in myelofibrosis exhibits differential responsiveness to JAK-STAT, MAP kinase, and NFκB signaling. Leukemia 2019, 33, 1978–1995. [CrossRef] [PubMed]

144. Harrison, N.; Patriarca, A.; Mascarenhas, J.; Kremyanskaya, M.; Hoffman, R.; Schiller, G.J.; Leber, B.; Devos, T.; Kabir, S.; Senderowicz, A.; et al. Preliminary Report of MANIFEST, a Phase 2 Study of CPI-0610, a Bromodomain and Extraterminal Domain Inhibitor (BETi), in Combination with Ruxolitinib, in JAK Inhibitor (JAKi) Treatment Naïve Myelofibrosis Patients. Ann. Soc. Hematol. Orlando 2019, 134. [CrossRef]

145. Lara-Astiaso, D.; Weiner, A.; Lorenzo-Vivas, E.; Zaretsky, L.; Jaitin, D.A.; David, E.; Keren-Shaul, H.; Mildner, A.; Winter, D.; Jung, S.; et al. Immunogenetics. Chromatin state dynamics during blood formation. Science 2014, 345, 943–949. [CrossRef] [PubMed]