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Overview of Transgenic Mouse Models of Myeloproliferative Neoplasms (MPNs)

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Abstract

Myeloproliferative neoplasms (MPNs) are a class of hematologic diseases characterized by aberrant proliferation of one or more myeloid lineages and progressive bone marrow fibrosis. In 2005, seminal work by multiple groups identified the JAK2V617F mutation in a significant fraction of MPN patients. Since that time, murine models of JAK2V617F have greatly enhanced the understanding of the role of aberrant JAK-STAT signaling in MPN pathogenesis and have provided an in vivo pre-clinical platform that can be used to develop novel therapies. From early retroviral transduction models to transgenics, and ultimately conditional knock-ins, murine models have established that JAK2V617F alone can induce an MPN-like syndrome in vivo. However, additional mutations co-occur with JAK2V617F in MPNs, often in proteins involved in epigenetic regulation that can dramatically influence disease outcomes. In vivo modeling of these mutations in the context of JAK2V617F has provided additional insights into the role of epigenetic dysregulation in augmenting MPN hematopoiesis. In this overview, early murine model development of JAK2V617F is described, with an analysis of its effects on the hematopoietic stem/progenitor cell niche and interactions with downstream signaling elements. This is followed by a description of more recent in vivo models developed for evaluating the effect of concomitant mutations in epigenetic modifiers on MPN maintenance and progression. Mouse models of other driver mutations in MPNs, including primarily calreticulin (CALR) and Tpo-receptor (MPL), which occur in a significant percentage of MPN patients with wild-type JAK2, are also briefly reviewed. © 2017 by John Wiley & Sons, Inc.

Keywords

leukemia; JAK2; myeloproliferative neoplasms; epigenetics

INTRODUCTION

Philadelphia chromosome–negative myeloproliferative neoplasms (MPNs) are a group of clonal hematopoietic disorders characterized by aberrant proliferation of one or more mature functional blood elements and progressive bone marrowfibrosis (Spivak et al., 2003). Early genetic and X-linked inactivation studies confirmed the clonal origin of MPNs, with the diseased clone originating from within the hematopoietic stem/progenitor compartment (Adamson, Fialkow, Murphy, Prchal, & Steinmann, 1976; Levine et al., 2006; Prchal, Guan, Prchal, & Barany, 1993). Seminal work in 2005 by multiple groups led to the identification of the JAK2V617F mutation in a majority of MPN patients, including 95% to 98% of those with polycythemia vera (PV), and 50% to 60% of patients with essential thrombocythemia (ET) or myelofibrosis (MF) (Baxter et al., 2005; James et al., 2005; Jones et al., 2005; Kralovics et al., 2005; Levine et al., 2005). Mutant JAK2V617F results in constitutive activation of the JAK2 tyrosine kinase, hyper-activation of downstream signaling targets (including canonical STAT proteins), and aberrant cell proliferation. These changes also result in cytokine hypersensitivity and erythropoietin-independent growth of mutant cells in vitro (James et al., 2005).

Following the discovery of the JAK2 mutation, multiple groups sought to model JAK2V617F in vivo in an attempt to determine the functional significance of mutant protein in the context of normal hematopoiesis and to develop an assay for screening potential therapies. The evolution of murine models, from early retroviral transduction models to transgenics and ultimately conditional knock-ins (Li, Kent, Chen, & Green, 2011b; Morgan & Gilliland, 2008; Mullally, Lane, Brumme, & Ebert, 2012), has enabled a detailed characterization of the JAK2V617F mutation and provided new insights into the pathogenesis of this disorder. However, many questions remain, including how a single mutation gives rise to multiple distinct clinical phenotypes, and precisely how constitutive JAK2 signaling alters the stem cell niche to propagate disease. Increasing evidence suggests that inherited genetic modifiers (Jones et al., 2009; Kilpivaara et al., 2009; Olcaydu et al., 2009; Tapper et al., 2015), differential JAK-STAT signaling regulators (Chen et al., 2010), and/or *Jak2V617F* allele burden (Passamonti & Rumi, 2009) together influence MPN phenotype and severity. The levels of *Jak2V617F* expression in patients often correlates closely with clinical subtype, with those with heterozygous JAK2V617F mutations often displaying an ET-like phenotype (characterized by high platelet count), and those with homozygous JAK2V617F mutations (i.e., through acquired copy neutral loss of heterozygosity) are associated with a PV-like phenotype (characterized more by elevated hemoglobin, splenomegaly, and low erythropoietin levels) (Barbui et al., 2004; Kralovics, Guan, & Prchal, 2002; Passamonti & Rumi, 2009; Passamonti et al., 2010; Scott, Scott, Campbell, & Green, 2006; Silver et al., 2011; Vannucchi et al., 2007). Moreover, an increasing body of evidence suggests that additional somatic mutations, either preceding or following JAK2V617F, may also influence disease outcomes in MPNs (Ortmann et al., 2015; Vainchenker, Delhommeau, Constantinescu, & Bernard, 2011). Similar in vivo modeling of these mutations in the context of JAK2V617F may further help to elucidate the phenotypic heterogeneity observed in this complex disease.

RETROVIRAL TRANSDUCTION MODELS

Retroviral transduction methods were employed to characterize the JAK2V617F mutation and to confirm the activity of mutant JAK2 protein in vivo. Retroviral transduction models, whereby plasmid constructs containing cDNA expressing a gene of interest (i.e., *Jak2V617F*) are transduced into harvested murine bone marrow cells ex vivo and then transplanted back into irradiated syngeneic mice, represent an efficient and effective in vivo approach for studying the functional effects of a mutant protein of interest. However, limitations of this method include random insertion of constructs and positional effects that ultimately affect expression levels of cDNA encoded by the vector. In this regard, expression levels of mutant protein are often much higher (~10- to 40-fold) than that of physiologic wild-type protein. In addition, dividing cells (i.e., mitotic progenitor cells) are preferentially targeted while more quiescent cells (i.e., long-term hematopoietic stem cells or LT-HSCs) are less likely to be transduced, which may inappropriately skew results and/or limit analysis.

Four groups published early retroviral transduction models of JAK2V617F (Table 14.40.1) (Bumm et al., 2006; Lacout et al., 2006; Wernig et al., 2006; Zaleskas et al., 2006) with remarkably similar findings. Transduced mice displayed a highly penetrant, PV-like disease with marked erythrocytosis, leukocytosis, and splenomegaly. Notably, platelet counts were overall normal despite increased megakaryopoiesis. Bone marrow analysis revealed trilineage hyperplasia with increased myeloid and erythroid expansion. Within 3 to 4 months, a second phase of disease was encountered, characterized by worsening pancytopenia, expanding extramedullary hematopoiesis, and progressive reticulin bone marrow fibrosis, consistent with evolving myelofibrosis and a resultant “spent phase” of disease. In addition, transplantation of mutant cells into irradiated secondary recipient mice recapitulates a PV phenotype, consistent with the cell autonomous nature of the mutant clone. Finally, all groups observed increased JAK-STAT signaling, reduced circulating erythropoietin (Epo) levels, and cytokine-independent colony growth consistent with initial studies.

Zaleskas et al. (2006) and Wernig et al. (2006) also explored strain-specific effects of JAK2V617F cells by generating models using both BALB/c as well as C57Bl/6 mice for retroviral transduction. While BALB/c mice displayed a consistent PV-like disease as described above, C57Bl/6 mice evidenced a more subtle phenotype, with only mild elevation in blood counts, less pronounced splenomegaly, and no fibrosis, even in aged animals. These data suggest that, as is seen in patients, strain-specific genetic modifiers and/or variable *Jak2V617F* gene expression levels might influence disease phenotype. Regardless, these early mouse models firmly established JAK2V617F as sufficient to induce an MPN-like condition in mice.

TRANSGENIC MODELS OF MPN

Following retroviral transduction methods, transgenic mouse models were subsequently created to better characterize the role and function of JAK2V617F in a steady state within the hematopoietic compartment (Table 14.40.1). For these models, a bacterial artificial

chromosome (BAC) construct containing murine (or human) *Jak2V617F* cDNA is primarily injected into murine oocytes during embryogenesis where it integrates randomly into genomic DNA and, under control of a tissue-specific promoter, yields stable, and genetically transmissible, gene expression. However, this procedure also results in random insertion of transgene products and therefore is vulnerable to positional effects that might alter expression levels of mutant protein.

Three groups published findings on transgenic models of JAK2V617F MPNs (Shide et al., 2008; Tiedt et al., 2008; Xing et al., 2008). All groups demonstrated that JAK2V617F is sufficient to induce an MPN-like syndrome in mice. Greater phenotypic variability among these groups was seen than with the earlier models; however, with a strong correlation of mutant JAK2 protein expression levels influencing phenotype. For example, Shide et al. (2008), expressing murine *Jak2V617F* protein under control of the *H-2Kb* promoter, observed a variable phenotype based on two different founder mouse lines, each expressing different levels of mutant protein: one with a 0.45-fold greater level transgene expression compared to wild-type *Jak2* and the other 1.35-fold greater level. A mild thrombocytosis failing to meet the criteria for ET developed in the lower expressing mutant line, with otherwise normal counts and no splenomegaly. After 9 months, however, 30% of these mice progressed to a full ET-like phenotype, with PV-like disease in another 20%. A more pronounced MPN was seen in higher expressing mice, with significant leukocytosis and thrombocytosis, hypercellular marrow with myeloid expansion and dysmegakaryopoiesis, splenomegaly, and progressive bone marrow fibrosis. Both lines demonstrated cytokine independent growth in vitro and increased STAT5 activation. These findings resembled those of Xing et al. (2008) who, using *human Jak2V617F* cDNA under control of the constitutively-expressing, hematopoietic-specific *Vav* promoter in both C57Bl/6 and XDBA/2 mice, observed similar phenotypes in both low-expressing and high-expressing mouse lines, respectively.

Tiedt et al. (2008) employed a unique conditional activation method allowing for inducible transgene protein expression within the hematopoietic compartment. Using a *human Jak2V617F* cDNA construct containing an inactive JAK2V617F kinase domain in reverse orientation and flanked by antiparallel *lox* sites, these investigators were able to induce JAK2V617F using Cre-mediated recombination under control of either the *Vav* or *Mx* promoter. The *Mx* promoter, the expression of which can be induced with varying intraperitoneal injections of the pro-inflammatory cytokine polyinosine-polycytosine (pIpC), allows for conditional induction of Cre-mediated recombination within the hematopoietic compartment. The *Vav* promoter expresses Cre constitutively at low levels during embryonic development and throughout life. This made it possible to assess effects of varying levels of transgene expression level on disease phenotype. Those mice expressing low-level *Jak2V617F* under control of the *Vav* promoter displayed significant thrombocytosis with only a slight increase in leukocytosis and normal hemoglobin, more consistent with an ET phenotype, while under control of the *Mx* promoter, mice developed a PV phenotype characterized by elevated hemoglobin/hematocrit with suppressed *Epo* and high WBC and platelet counts. These data further confirmed that transgene copy number, and consequently the *Jak2V617F* expression level, correlates closely with MPN sub-type.

KNOCK-IN MODELS

Following the development of transgenic models, several investigators turned to conditional knock-in models to most faithfully recreate mutant *Jak2* expression in its endogenous setting, under control of its native promoter. *Jak2V617F* then is induced specifically in the bone marrow compartment through Cre-mediated recombination under control of a hematopoietic-specific promoter (i.e., *Vav*, *Mx*, etc.).

There were four independent reports on individual knock-in models of *Jak2V617F* in 2010 (Table 14.40.1) (Akada et al., 2010; Li et al., 2010; Marty et al., 2010; Mullally et al., 2010). All used a 129 Sv/C57Bl/6 background mouse and all obtained a ratio of mutant-to-wild type *Jak2* expression of 1:1, in line with the nature of the conditional knock-in model.

Akada et al. (2010) utilized an *Mx*-Cre inducible model to induce *Jak2V617F* expression in the hematopoietic compartment. Following pIpC treatment, these mice displayed a highly-penetrant, severe, PV-like syndrome with high RBC, platelet, and WBC counts, splenomegaly with extramedullary hematopoiesis, and progressive fibrosis similar to previous transgene and retroviral transduction models. Similarly, the disease was transplantable, confirming the cell-autonomous nature of the mutant clone. Likewise, these investigators confirmed Epo-independent growth of cells in vitro and increased downstream phospho-STAT5 activation. Bone marrow analysis revealed expansion of early stem (LSK, Lin–Sca1+cKit+) and myeloid progenitor compartments and skewing towards erythroid differentiation. They were also able to assess homozygous JAK2V617F mutants. Homozygous mice developed an even more pronounced phenotype, with higher WBC and RBC counts, increased splenomegaly, and accelerated bone marrow fibrosis as compared to those with one dysfunctional allele. This suggested once again that allele burden might influence disease phenotype. Marty et al. (2010) reported similar findings with their knock-in model. Using mice expressing germ-line constitutive murine *Jak2V617F*, they also observed a highly-penetrant, PV-like disease with a megakaryocytic predominance, short latency, and similar splenomegaly and, by 9 months, significant fibrosis. Analysis of bone marrow revealed increased myeloid forms but no effect on cell maturation.

Mullally et al. (2010), using conditional knock-in mice in which excision is mediated by Cre recombinase driven by the *E2a* promoter, also observed a PV-like phenotype characterized by erythrocytosis, leukocytosis, splenomegaly, and extramedullary hematopoiesis. While increased megakaryopoiesis was noted in the spleen, there was minimal effect on circulating platelet counts. In addition, no fibrosis developed, even in aged (>6 months) mice. They also observed a significant reduction in overall survival (median 146 days) as compared to other models, possibly from thrombosis-related events. In bone marrow, expansion of myeloid progenitor elements, including terminal erythroid and megakaryocytic differentiation was seen; however, in contrast to Akada et al. (2010), they observed no difference in the relative size of the stem/progenitor compartment, phosphor-STAT5 activation, or gene expression changes, suggesting that JAK2 promotes cellular differentiation within the LSK compartment. In competitive transplantation experiments, only a minor selective advantage was seen with JAK2V617F mutant cells over wild-type. Notably, sorted LT-HSCs were able to engraft and develop MPNs in secondary recipient mice while other sorted populations

(including more differentiated MEPs, or GMPs) were not suggesting that JAK2V617F must be present in early stem/progenitor cells in order to recapitulate an MPN phenotype.

The model reported by Li et al. (2010) was unique in that a *human Jak2V617F* cDNA construct under control of the endogenous murine *Jak2* promoter was used with *Mx-Cre*-mediated recombination. Following pIpC treatment, these mice, in contrast to the models described earlier, developed a more ET-like phenotype, with only modest thrombosis and erythrocytosis, and no significant changes in WBC counts or splenomegaly. Approximately 10% of these mice went on to develop a PV-like disease, with increased erythrocytosis and bone marrow fibrosis. While up-regulation of *Stat5* was observed, *Epo* levels were not suppressed. In bone marrow, increased terminal erythroid and megakaryocytic differentiation was noted, along with increased numbers of lineage-restricted progenitors. Competitive transplantation assays revealed an overall *disadvantage* of mutant stem cells as compared to wild-type support cells suggesting an inherent stem cell defect. While it is unclear why this model differed from other conditional knockin models of JAK2V617F, it may be due to either the acquisition of additional genetic mutations that alter stem fitness (i.e., TET2 loss or mutations in other epigenetic modifiers) or technical aspects of incorporating human *Jak2V617F* in a murine background and the secondary effects this may have on kinase activation and/or protein binding interactions. At the same time, the Li model most closely recapitulates that which is seen in humans in that patients with a heterozygous JAK2V617F mutation are more associated with an ET phenotype. It might be anticipated therefore that homozygous JAK2V617F mice in the Li model would perhaps, like humans, display a more PV-like phenotype. While this was not addressable in their original study, these investigators would later show that mice expressing homozygous human *Jak2V617F* display a dramatic, PV-like phenotype characterized by high red cell count, low platelets (due to increased turnover), and reduced overall survival (Li et al., 2014). They also observed an even further reduction in HSC number and self-renewal capacity over time in serial transplants as compared to those heterozygous for JAK2V617F.

EVALUATION OF THE HEMATOPOIETIC STEM CELL (HSC) COMPARTMENT

Given the varying findings among the different knock-in models, the effect of JAK2V617F on the amplification of early hematopoietic cells has remained a matter of controversy (Skoda, 2010). In patients, clinical analysis has confirmed that the JAK2V617F mutation is present in both early stem and progenitor cells as well as in more mature cell lineages, including lymphocyte populations (Akada et al., 2014; Anand et al., 2011a; Baxter et al., 2005; Butcher et al., 2007; Delhommeau et al., 2007; Dupont et al., 2007; Ishii, Bruno, Hoffman, & Xu, 2006; Jamieson et al., 2006; Larsen, Christensen, Hasselbalch, & Pallisgaard, 2007). In addition, data suggest that *Jak2V617F* allele burden is higher in differentiated myeloid forms (i.e., neutrophils) as compared to early stem cells suggesting clonal expansion may occur later in myeloid differentiation (Dupont et al., 2007; Stein et al., 2011). However, in vitro studies using primary JAK2V617F human cells show variable results, with some groups reporting HSC expansion with others showing minimal to no alteration of HSC compartment size (Anand et al. 2011a; Jamieson et al., 2006). Similarly, xenograft models using human CD34+ MPN cells transplanted into NOD/SCID mice fail to outcompete normal cells, suggesting limited self-renewal (Ishii et al., 2007; James et al.,

2008). However, these studies were technically limited by poor engraftment. Thus, the effect of V617F-mutant JAK2 on the stem cell niche remains unclear.

To decipher the functional role of early HSCs in JAK2V617F-mutant MPNs, efforts were made to analyze, in greater detail, the stem cell niche of MPN murine models. Using their previously reported knock-in model, but this time with recombination under control of the *Vav* promoter, Marty et al. (2010, 2011) observed a significant, up to six-fold, increase in LSK compartment size, with expansion of early long-term and short-term hematopoietic stem cells. In addition, competitive transplants, where 30% JAK2V617F cells were mixed with 70% support cells and transplanted into irradiated recipients, demonstrated a significant outgrowth of JAK2V617F-expressing cells consistent with a robust, competitive advantage of mutant progenitors.

These findings were corroborated by Hasan et al. (2013). Using a *Vav*-Cre inducible knock-in system, a progressive increase in all progenitor cell populations was observed, including LSK/SLAM, in both bone marrow and spleen. Similarly, a strong stem cell advantage among HSCs expressing JAK2V617F was noted in competitive transplants, with increased proliferation and impaired apoptosis.

Lundberg et al. (2014) used their *MxCre-loxP*-inducible transgenic model (as per Tiedt et al., 2008) to perform detailed stem cell analysis. They too observed increased cell cycling, expansion, and outgrowth of mutant LSK populations (up to four-fold) in competitive transplants as compared to wild-type cells. In single-cell transplantation studies, individual JAK2V617F-mutant LT-HSCs successfully engrafted and recapitulated MPNs in secondary recipients, although at a lower, variable efficiency (~0% to 35%). In addition, serial transplantability of mutant LSK cell populations lent support to enhanced self-renewal. However, they also found that expression levels of mutant JAK2 protein correlated inversely with capacity for engraftment and emergence of an MPN phenotype in that LSK cells with lower expression levels of *Jak2V617F* were more likely to engraft while those with higher mutant expression were more likely to exhibit increased cell cycling and decreased self-renewal. These data suggest that levels of *Jak2* expression itself, possibly through alternative epigenetic mechanisms, affect the balance between self-renewal and differentiation. Whole-exome sequencing on sorted LSKs following transplants revealed no newly acquired mutations to suggest causality of an enhanced self-renewal phenotype.

Using their previously published knock-in model, Mullally et al. (2012) performed similar analysis of the murine BM compartment. In contrast to the studies described above, these investigators found only minimal changes in frequency of HSC-enriched LSK cells, including LT-HSCs in JAK2V617F-mutant mice. In addition, they observed no functional differences in cell cycling or JAK-STAT signaling. However, consistent with Lundberg et al. (2014), isolated LT-HSCs from mutant mice were successful in initiating the condition in a small percentage of mice in limited dilution transplants that, over time, led to myelofibrosis in secondary recipients. As reported in their initial study (Mullally et al., 2010), similar transplants using more differentiated sorted cell populations (i.e., ST-HSCs, MPPs, megakaryocytic, or erythroid progenitor cells) were unsuccessful at inducing MPN in secondary recipients. Differences in LSK population size were subtle at 16 weeks in

competitive repopulation assays, this advantage increased over time such that by 1 year, the mutant LT-HSC population had significantly grown to outcompete wild-type cells. From these findings, they concluded that disease-propagating cells in MPNs lie exclusively in the LT-HSC compartment and that, in general, HSCs convey a subtle competitive advantage in comparison to wild-type cells that manifests progressively over time.

In contrast to these findings, Kent et al. (2013), using their previously reported model (as per Li et al., 2010), demonstrated again limited self-renewal capacity of mutant HSCs and an overall decrease in numbers of mutant progenitor cells. While an increased division and expansion of early downstream progenitors was found, a minimal effect on absolute number of early LSK progenitors, including the LT-HSC compartment, was seen. This resulted in total reduced LSK cell numbers relative to other populations. This was further evidenced by decreased cell cycling, reduced apoptosis, increased senescence, and increased DNA damage in JAK2V617F HSCs as compared to the wild type. Total numbers of stem/progenitor cells, except for CMPs and MEPs, in bone marrow continued to decline as mice aged. In transplantation experiments in which wild-type support cells were mixed with mutant HSCs, mutant HSCs were slowly out-competed by wild-type cells. These results suggest that, in contrast to the studies above, JAK2V617F impairs HSC self-renewal while skewing their progeny toward differentiation and proliferation and that to propagate the condition, clonal expansion requires additional genetic lesions (i.e., TET2 loss, etc.) or other events.

Overall, the effect of JAK2V617F on HSC expansion and self-renewal in MPNs remains unclear. Most studies suggest that JAK2V617F occurring in early stem cells results in a competitive advantage in comparison to wild-type cells. The findings of Li and colleagues described above resemble patient-derived xenotransplantation models in which human V617F CD34⁺ cells from MPN patients transplanted into NOD/SCID mice do not display a robust proliferative advantage but instead manifest reduced engraftment and limited self-renewal capacity (Ishii et al., 2007). Many potential complicating factors might explain these discrepant results, including: different targeting strategies and the promoters employed; variable Cre recombination efficiencies; potential pitfalls of xenograft and transplant modeling of MPNs (including engraftment efficiency and immune- or radiation-mediated changes); use of human versus murine *Jak2V617F* cDNA constructs in murine knock-in models and their effects on differential binding interactions, kinase activity, and/or downstream signaling; acquisition of additional mutations that alter disease phenotype and/or self-renewal capacity; and epigenetic effects of JAK2 itself that might directly alter stem cell self-renewal properties beyond its canonical roles in signal transduction (Dawson et al., 2009, 2012).

SIGNALING PATHWAY ANALYSIS

The critical role of constitutive JAK2 activation in JAK2V617F mutant MPNs highlights the importance of aberrant JAK-STAT signaling in disease propagation. In addition to phosphorylation of common STAT proteins, primarily STAT5, STAT3, and STAT1, mutant JAK2 is also known to mediate a number of other downstream signaling pathways, including AKT and ERK (James et al., 2005; Levine et al., 2005). STAT proteins have important functions in both normal and malignant hematopoiesis (Dorritie, McCubrey, & Johnson,

2014). To determine the extent to which mutant JAK2 relies on STAT signaling for disease propagation in MPNs, several investigators have attempted to develop models of JAK2V617F in which individual STAT proteins are concomitantly deleted. Similarly, others explored the role of the remaining wildtype JAK2 allele in influencing disease phenotype.

STAT5

STAT5 is a direct downstream binding partner of JAK2 and is the major component of the classical JAK-STAT signaling cascade. To determine whether STAT5 activation is necessary for JAK2V617F-mediated MPN pathogenesis, Walz et al. (2012) retrovirally transduced murine *Jak2V617F* cDNA into a conditional *Stat5* knock-out background and transplanted into irradiated recipient mice (Walz et al., 2012). Mice displayed near normalization of blood counts and spleen sizes in all but one mouse. Haploinsufficiency rather than complete knock out of *Stat5* improved some parameters (i.e., leukocytosis) but not others (i.e., erythrocytosis), suggesting a possible dose-related effect. These findings were validated by Yan et al. (2012) using a similar double-mutant *Jak2V617F/Stat5^{flx/flx}* KO model. Likewise, they observed complete abrogation of a MPN phenotype, with normalization of blood parameters and spleen size comparable to wild-type controls. In bone marrow, LSK, LT-HSC, ST-HSC, and MEP cell populations, while expanded in single-mutant mice, were present in numbers similar to wild-type controls. Adding back *Stat5* in *Stat5*-deficient cells resulted in re-emergence of a PV phenotype. Likewise, transplanting *Stat5*-deficient, JAK2V617F-mutant cells into secondary recipients failed to reproduce PV-like disease, while MPN developed in mice that received cells in which *Stat5* was restored. Taken together these data confirmed that STAT5 is necessary for the initiation and maintenance of JAK2V617F-mediated PV.

STAT1

While STAT1 has important roles in megakaryocytic and erythroid differentiation (Huang et al., 2007), the consequences of STAT1 activation in the context of JAK2V617F-mutant MPNs had not been defined. Chen et al. (2010), using harvested JAK2V617F-heterozygous erythroid cells from ET and PV patients, had previously observed differential expression of STAT1 protein levels between the two sub-types with resultant effects on differential phosphorylation and downstream interferon signaling pathways, suggesting that phosphoSTAT1 levels may influence the “decision” between PV and ET phenotypes.

To determine the role of STAT1 signaling in JAK2V617F-mutant MPNs in vivo, Duek et al. (2014) crossed their *Scl-Cre Jak2V617F* conditional knock-in model with a conditional *Stat1* knock-out mouse, allowing for somatic alteration of both genes. Compared to JAK2V617F single-mutant mice, double-mutant mice displayed increased red cell mass and decreased platelets, suggesting preferential skewing towards enhanced erythroid maturation and away from megakaryopoiesis. This effect was not observed in STAT1 single-mutant knock-out mice, being evident only when erythropoiesis was already aberrantly activated (i.e., by JAK2V617F). By 10 weeks, these mice developed worsening polycythemia with evolving leukocytosis, consistent with a PV phenotype. While no compensatory increase in either STAT3 or STAT5 phosphorylation was observed, these investigators could not rule out

alterations in lineage-specific (i.e., erythroid) cell populations. These findings were consistent with the reports of Chen et al. (2010), who found that STAT1 acts normally to suppress erythroid differentiation and that with STAT1 loss JAK2V617F-mutant cells bias towards a PV phenotype.

STAT3

Like STAT1, STAT3 has been implicated in normal hematopoiesis and been found to be aberrantly expressed in a number of cancers (Ecker et al., 2009; Jenkins et al., 2007; Jenkins, Roberts, Najdovska, Grail, & Ernst, 2005; Kirito et al., 2002). Constitutive phosphorylation/activation of STAT3 is often observed in MPNs, and STAT3 knock-out results in defective hematopoietic stem/progenitor cell proliferation and differentiation (Anand et al., 2011b; Mantel et al., 2012).

Double-mutant STAT3 knock-out models were developed to evaluate its role in JAK2V617F-mutant MPNs. To this end, Grisouard et al. (2015) and Yan et al. (2015) employed similar Cre-inducible *Jak2V617F/Stat3^{fllox/flox}* KO models to generate double-mutant mice. Consistent with their previous studies (as per Tiedt et al., 2008 and Akada et al., 2010), JAK2V617F single-mutant mice displayed a PV-like phenotype with significant erythrocytosis, bone marrow trilineage hyperplasia, and increased reticulin fibrosis. This phenotype was augmented following STAT3 KO, with these animals displaying an increase in platelet counts and a trend towards an increase in fibrosis on one hand, and a reduction in red cell parameters and spleen size on the other. Although animal survival was significantly reduced in both models, it was unclear whether this was related to increased MPN-related events (i.e., thrombosis) or to a pro-inflammatory state elicited by STAT3 KO itself. In an analysis of the BM compartment, Yan et al. (2015) noted increased expansion and cycling of HSCs, myeloid progenitor, and megakaryocytic cell populations compared to JAK2V617F cells with wild-type STAT3. Single-mutant STAT3 KO had minimal effects on peripheral counts and bone marrow cell populations, although HSC and GMP populations were expanded in the spleen. A compensatory increase in STAT1 was observed in both studies, consistent with previous studies showing that STAT1 and STAT3 are reciprocally regulated (Qing & Stark, 2004) possibly as a result of decreased *Socs3* gene expression (Croker et al., 2003). These data suggest that STAT3 loss, when co-occurring with JAK2V617F, skews the MPN phenotype towards thrombocytosis and megakaryopoiesis and shortens overall survival, perhaps through a corresponding increase in STAT1 activity.

Wild-Type JAK2 Loss

Efforts were then undertaken to explore the role of the remaining wild-type *Jak2* allele in influencing disease severity in JAK2V617F-mutant MPNs. Previous studies had demonstrated that conditional loss of wild-type JAK2 within the hematopoietic compartment results in rapid loss of HSCs and progressive bone marrow failure, with worsening counts and reduced overall survival, confirming the necessary role of wild-type JAK2 in normal hematopoiesis (Akada et al., 2014; Grisouard, Hao-Shen, Dirnhofer, Wagner, & Skoda, 2014; Park et al., 2013). Similarly, early studies observed that wild-type JAK2 in JAK2V617F

mutant MPNs inhibits JAK2V617F-mediated cell growth, suggesting a negative role for wild-type JAK2 in the progression of MPNs (James et al., 2005).

Using an *Scl*-Cre conditional transgenic model (as per Tiedt et al., 2008), Kubovackova et al. (2013) explored native *Jak2* allele knock-out in the context of a JAK2V617F transgene. JAK2V617F transgenic mice with either homozygous or heterozygous JAK2 loss displayed early increases in RBC and reticulocyte counts; however, myeloid and megakaryocytic lineages were largely unaffected. The overall survival of these animals was significantly impaired, although the cause of death in most mice was unclear. This study was limited by the use of transgenic JAK2V617F, thereby preventing the analysis of wild-type JAK2 loss in the context of a paired mutant JAK2 allele.

Akada et al. (2014) used their JAK2V617F knock-in model to assess the effects of the loss of the remaining JAK2 allele. This allowed for a more physiologic assessment of allele burden on influencing disease phenotype. In this manner, these investigators also assessed mice homozygous for JAK2V617F for detailed phenotypic analysis, as was done in their original study (Akada et al., 2010). Similar to Kubovackova et al. (2013), loss of the remaining wild-type *Jak2* allele (leading to hemizygous JAK2V617F) resulted in further enhancement of a PV-like phenotype, with increases in all blood parameters, enhanced splenomegaly, and accelerated progression to myelofibrosis. This confirmed once again that wild-type JAK2 negatively regulates JAK2V617F-mediated MPNs. This phenotype was even more pronounced in homozygous JAK2V617F mice, with these animals displaying even greater increase in blood counts and more marked reticulin fibrosis. In the BM, extensive fibrosis in bone marrow resulted in decreased LSK number and frequency with a compensatory increase in spleen, consistent with extramedullary hematopoiesis. These findings again confirmed that expression levels of mutant JAK2V617F, including when in opposition to that of wild-type JAK2, dramatically influences disease phenotypes in MPNs.

MUTATIONS IN EPIGENETIC MODIFIERS IN V617F MPNS

Multiple groups identified additional mutations co-occurring with JAK2V617F in a significant percentage of MPN patients, often in genes encoding proteins involved in epigenetic regulation, including *Tet2*, *Idh*, *Ezh2*, and *Asx11*, among others (Shih, Abdel-Wahab, Patel, & Levine, 2012). Studies have since shown that the presence of these mutations can negatively affect disease outcomes in MPN patients and that the order with which mutations are acquired (i.e., TET2 first versus JAK2 first) may further influence disease progression (Lundberg et al., 2014; Ortmann et al., 2015; Schaub et al., 2010). Double-mutant models were then used to explore how mutations in epigenetic modifying proteins alter gene expression and influence outcome in JAK2V617F mutant MPNs.

JAK2/TET2 Models

Loss of TET2 mutations occur in a significant percentage of patients across the spectrum of myeloid diseases, including up to 25% of those with MPNs, and are associated with increased risk of leukemic transformation (Abdel-Wahab et al., 2009, 2010; Beer et al., 2010). TET2 is a methylcytosine dioxygenase responsible for converting 5-methylcytosine to 5-hydroxymethylcytosine (5hmC) and global epigenetic regulation of multiple gene

targets (Nakajima & Kunimoto, 2014). TET2 KO mice display increased repopulating and self-renewal capacity of HSCs in bone marrow (Li et al., 2011a). Similarly, in TET2/JAK2V617F xenotransplantation models, mice transplanted with harvested CD34+ patient cells demonstrate increased capacity to repopulate as compared to cells with V617F alone (Delhommeau et al., 2009), again suggesting an enhanced self-renewal capability of TET2-mutant hematopoietic progenitors.

To explore TET2 knock-out in the context of JAK2V617F mutant MPNs, Chen et al. (2015) crossed their JAK2V617F knock-in model with *Tet2^{flox/flox}* conditional KO mice and assessed disease phenotype and effect on the hematopoietic stem and progenitor (HSPC) compartment. In line with their initial study (Mullally et al., 2010), JAK2V617F single-mutant mice displayed a strong PV phenotype with erythrocytosis, leukocytosis, and thrombocytosis, but no significant bone marrow fibrosis. When combined with TET2 loss, the phenotype was relatively subtle, with increased splenomegaly but only minimal changes in overall blood counts. More striking effects were observed with bone marrow analysis; however, including marked expansion of LT-HSC, MPP, and myeloid progenitor compartments and an increase in megakaryocytes and megakaryocytic turnover. Competitive transplantation assays using sorted doublemutant LSKs revealed an overall growth advantage by 18 weeks, with 90% of cells in the bone marrows of recipient mice being derived from JAK2V617F/TET2 cells in comparison to wild-type cells, and recapitulation of an MPN phenotype. Attempts to transplant sorted double-mutant ST-HSCs and MPPs were unsuccessful, further suggesting that the competitive advantage seen, as in single-mutant JAK2V617F mice, was limited to that of the LT-HSC compartment. No double-mutant mice developed overt leukemia, progressed to myelofibrosis, or showed decreased overall survival as compared to JAK2V617F single-mutant mice.

Kameda et al. (2015) analyzed the effects of JAK2V617F/TET2 combined mutants using fetal liver cells expressing one or both alleles transplanted into lethally irradiated recipients. Mice transplanted with JAK2V617F cells developed a myelofibrosis-like phenotype, with leukocytosis, thrombocytosis, splenomegaly, and early bone marrow and splenic fibrosis as well as a decrease in overall survival. Similar to Chen et al. (2015), the addition of TET2 loss resulted in a more severe phenotype, with even greater leukocytosis, splenomegaly, and extra-medullary hematopoiesis, with a somewhat shorter overall survival as compared to JAK2V617F single-mutant mice. Quantitative analysis of bone marrow populations confirmed an increase in LSK frequencies in BM (but not spleen), and a strong competitive advantage over wild-type cells in competitive transplants in both TET2 KO and JAK2V617F/TET2 cells by 4 weeks post-transplant. Recipients of JAK2V617F cells showed decreased number of all-stage stem/progenitor cells and impaired serial transplantability, which is similar to what was reported by Li et al. (2010). However, it was unclear if this actually represented a cell-intrinsic effect or occurred as a result of expanding marrow fibrosis and a “spent phase” of disease. At the same time, this defect was restored when JAK2V617F was accompanied by TET2 loss.

Together, these data suggest that TET2 cooperates with JAK2V617F to promote stem cell maintenance and self-renewal while enhancing proliferation and turnover of late-stage stem/progenitor and terminally differentiated cells.

JAK2/EZH2 Models

EZH2 is a member of the polycomb repressive (PRC2) complex. It is involved in epigenetic silencing of gene expression through trimethylation of histone H3K27 marks (Volkel, Dupret, Le Bourhis, & Angrand, 2015). EZH2 also has been shown to have a number of other PRC2-independent roles, including direct regulation of gene expression and signal transduction (Kim et al., 2013; Kim & Roberts, 2016; Xu et al., 2012). Loss-of-function mutations of EZH2 have been detected in ~10% of MPN patients and, like TET2, are associated with worsened overall survival and increased risk of progression to leukemia when co-occurring with JAK2V617F in MPNs (Guglielmelli et al., 2011). In addition to myelodysplastic features, loss of EZH2 in the hematopoietic compartment results in thrombocytosis, with associated splenomegaly and enhanced extramedullary hematopoiesis (Mochizuki-Kashio et al., 2011).

Studies were conducted to assess the role of EZH2 loss in the context of JAK2V617F MPNs (Sashida et al., 2016; Shimizu et al., 2016; Yang, Akada, Nath, Hutchison, & Mohi, 2016). In general, all three employed models yielded similar results. As compared to JAK2V617F single-mutant mice alone, double-mutant mice developed an aggressive MPN-like phenotype with severe skewing towards megakaryopoiesis and thrombocytosis with associated splenomegaly and extramedullary hematopoiesis, rapid progression to myelofibrosis, and decreased overall survival. In competitive transplants, all double-mutant models displayed enhanced engraftment and repopulating capacity over JAK2V617F single-mutants, with overall expansion of the LSK stem/progenitor compartment. Likewise, MF-initiating capacity was retained in secondary transplants (Yang et al., 2016). Heterozygous EZH2 loss showed only a minimal effect on MPN phenotype in JAK2V617F mice, although a subtle decrease in promoter-specific H3K27me3 levels was noted. All three groups of investigators performed gene expression analysis on double-mutant cells. Genes involved in megakaryocytic differentiation and previously implicated in fibrosis were differentially over-expressed, including possible targets such as *Hmga2*, a DNA-binding protein part of the Lin28-Let7 pathway implicated in megakaryopoiesis (Oguro et al., 2012) and *S100a8* and *S100a9*, pro-inflammatory cytokine-like mediators implicated in cancer (Bresnick, Weber, & Zimmer, 2015). Forced expression of these genes in a JAK2V617F-mutant background led to increased megakaryopoiesis in bone marrow, thereby confirming that these genes are directly regulated by EZH2-mediated H3K27me3 (Yang et al. 2016). In the aggregate, these data suggest that EZH2 mutations, like TET2, cooperate with JAK2V617F in vivo to promote an increase in stem cell self-renewal and proliferation in MPNs.

CALR AND MPL MUTANT MPN MODELS

MPL

The prevalence of JAKV617F mutations across MPN sub-types suggested a common pathway for disease initiation, although a significant number of MPN patients were wildtype for JAK2. To address this issue, upstream and downstream components of JAK-STAT signaling were explored to identify additional driver mutations of MPNs that might account for a percentage of JAK2V617F-negative patients. Candidate proteins included upstream cytokine receptors to which JAK2 dimerizes, including Epo (EPOR), granulocyte colony-

stimulating factor (G-CSF), and Tpo (MPL) receptors. Lu et al. (2008) had earlier observed that cytokine receptor expression and JAK2 dimerization is essential for JAK2V617F-mediated transformation, suggesting a potential role for cytokine receptor mutations in MPNs. This was further evidenced by rare, inherited, activating mutations of MPL, which cause a hereditary thrombocytosis-like syndrome resembling ET (Teofili et al., 2007, 2010). Similarly, earlier murine models in which *Mpl* was constitutively overexpressed displayed an MPN-like syndrome (Cocault et al., 1996; Villeval et al., 1997).

Given this, Pikman et al. (2006) performed mutational analysis of MPL and other cytokine receptors on a large cohort of MPN patients. From this study, they identified activating mutations of MPL (MPLW515L) in ~10% of patients with JAK2V617F-negative MPNs, with enrichment in ET and MF subtypes. To explore these mutations further in vivo, these investigators generated retroviral transduction models expressing MPLW515L. These mice developed a condition that closely recapitulates the ET-like disorder in humans that is characterized by marked thrombocytosis and leukocytosis, with minimal effect on reticulocytes. They also observed myeloid and megakaryocytic expansion in both the bone marrow and spleen, with associated splenomegaly and extramedullary hematopoiesis. Progressive reticulin fibrosis developed over time. The disease was also transplantable, consistent with the cell-autonomous nature of the mutant clone. They also observed constitutive JAK-STAT signaling, confirming MPL acts as a driver mutation through the common JAK-STAT pathway in MPNs.

Subsequent studies would later utilize murine models to assess the relationship between JAK2 and the MPL receptor in the maintenance of MPNs. Bhagwat et al. (2014) employed MPLW515L murine cells retrovirally transduced with *Mx-Cre-JAK2^{flox/flox}* KO cells when they discovered normalization of blood parameters and abrogation of reticulin fibrosis following pIpC treatment, thereby confirming that wild-type JAK2 is required for maintenance of the MPLW515L clone. In addition, Sangkhae et al. (2014) observed a significant decrease in platelet and neutrophil counts, reduced splenomegaly, and limited secondary transplantability in a *Jak2V617F/Mpl^{-/-}* transgenic model. This suggests that in a reciprocal manner, mutant JAK2V617F relies on wild-type MPL for oncogenic transformation. Ng et al. (2014) demonstrated that knocking out *Mpl* specifically in megakaryocytes and platelets causes an ET-like syndrome due to an increase in TPO availability at the HSC MPL receptor, reconfirming the role of MPL/JAK-STAT signaling in MPN pathogenesis.

CALR

In addition to MPL, gene discovery studies using next generation sequencing technology have identified calreticulin (CALR) mutations in a significant portion of JAK2V617F-negative MPNs (Klampfl et al., 2013; Nangalia et al., 2013). As seen with MPLW515L, CALR mutations occur in a mutually exclusive manner to that of JAK2V617F and are enriched in ET and MF MPN sub-types. CALR is a chaperone protein that resides in the endoplasmic reticulum and regulates normal protein folding pathways (Tannous, Pisoni, Hebert, & Molinari, 2015). Mutations of CALR localize to the C-terminus and occur primarily as insertions or deletions (indels) that result in frameshift mutations. This, in turn,

leads to an altered amino acid sequence, functional loss of the entirety of exon 9 (including its KDEL ER-retention domain) and the generation of a positively charged C-terminal sequence that appears to alter its protein binding properties (Klampfl et al., 2013). While many CALR mutations exist, the two most commonly seen in humans are CALRdel52 and CALRins5 (Klampfl et al., 2013). It remains to be determined how mutations of CALR result in MPN. Early murine models using traditional retroviral and transgenic methods have been useful in elucidating CALR-mediated MPN in vivo.

Several groups have examined the effects of CALR mutations in vivo. Two of these (Elf et al., 2016; Marty et al., 2016) utilized a retroviral transduction method while Shide et al. (2016) employed a transgenic model expressing human *Calr* under the *H2Kb* promoter. All of these studies addressed specifically the CALRdel52 mutation given its prevalence in humans. The phenotypes of all three models were remarkably similar. As in MPL-mutant MPN models, mice displayed an isolated thrombocytosis with no significant effects on erythrocyte or leukocyte counts consistent with an ET phenotype. While the model by Elf et al. (2016) showed no fibrosis, Marty et al. (2016) observed progressive fibrosis by 6 months following transplantation. Bone marrow analysis revealed a general expansion of LSK and SLAM populations, and competitive transplantation assays revealed an outgrowth of CALRdel52 cells in comparison to wild-type cells, indicating a competitive advantage. Marty et al. (2016) and Shide et al. (2016) independently assessed the CALRins5 mutational phenotype. This model displays a more modest thrombocytosis in comparison to CALRdel52 and no progression to fibrosis. Their findings resemble those found with humans, in that CALRdel52 mutations tended to be associated with more progressive ET/MF disease while those with CALRins5 mutations were more enriched in chronic ET (Cabagnols et al., 2015; Tefferi et al., 2014). These groups also assessed the effect of whole *Calr* exon 9 deletion. In this case, no significant phenotype was observed, suggesting that the functional genotype of CALR mutants occurs as a result of the altered peptide sequence itself rather than from a loss of the intrinsic function of the encoded portion of exon 9. Survival was unaffected in any of the models for any mutation.

These data demonstrate that mutations in CALR, like MPLW515L and JAK2V617F itself, result in constitutive JAK-STAT signaling. It remains to be seen how mutations in an endoplasmic reticulum binding protein lead to aberrant signaling in MPNs. Cell line data suggest that mutant CALR binds with greater affinity to MPL, perhaps resulting in improved trafficking to the cell surface and/or overactive MPL signaling (Araki et al., 2016; Elf et al., 2016). Other groups have also shown that CALR absolutely requires MPL expression for CALR-mediated transformation while other growth factor receptors, including EPOR and G-CSFR are dispensable (Araki et al., 2016; Shide et al., 2016). This could explain the shared phenotypes between both MPL and CALR mutants. Another possible consideration is aberrant glycosylation of MPL by CALRdel52 leading to dimerization and overactive downstream signaling.

MURINE MODELS IN THE PRE-CLINICAL EVALUATION OF MPN THERAPIES

In addition to elucidating the pathogenesis of constitutive JAK/STAT pathway activation in MPNs, JAK2V617F and MPLW515L murine models have also been useful for screening potential MPN therapies, primarily JAK2 inhibitors. A number of studies have been conducted employing previously published or similar retroviral transduction, transgenic, or knock-in models of MPN to explore the efficacy of JAK2 inhibitor therapy in vivo (Koppikar et al., 2010; Kubovcakova et al., 2013; Mullally et al., 2010; Nakaya et al., 2014; Quintas-Cardama et al., 2010; Tyner et al., 2010; Wernig et al., 2012; Wernig et al., 2008). The results of this work generally demonstrate inhibition of JAK/STAT signaling dramatically improves blood parameters, and has positive effects on spleen sizes, reticulin fibrosis, and the overall survival of MPN-mutant mice. However, JAK2 inhibition alone does not appear to be sufficient to reduce mutant allele burden in either mice or patients. This suggests that MPN disease-propagating cells survive despite JAK2 inhibition, possibly because of inadequate inhibition, ineffective apoptosis, or signaling through alternative pathways. Koppikar et al. (2012) observed persistence of JAK2-mutant cells through heterodimerization and transactivation of JAK2 by JAK1 and TYK2 kinases allowing for persistent JAK2/STAT pathway activation despite direct JAK2 inhibition.

Given these findings, efforts have been made to develop alternative treatment modalities, with particular emphasis on those aimed at enhancing the degradation of mutant JAK2 protein or depleting MPN-propagating stem cells. For example, Marubayashi et al. (2010) used their MPL murine model to assess the efficacy of PU-H71, a heat shock 90 protein inhibitor, in promoting mutant JAK2 protein degradation. They found that chronic administration of PU-H71 normalizes blood counts, improves overall survival, and reduces mutant allele burden. Likewise, Mullally et al. (2013) explored mechanisms of IFN α , an established MPN treatment previously shown to induce remissions in patients. They observed improvement in blood parameters and extramedullary hematopoiesis as well as a reduction in size and fitness advantage of the MPN mutant stem cell pool within the HSPC compartment.

Other treatments displaying significant efficacy in murine MPN models include novel type II inhibitors, which bind and stabilize inactive JAK2, thereby preventing dimerization and subsequent downstream activation (Meyer et al., 2015), histone deacetylase inhibitors (Akada et al., 2012), Bcl-2/BBcl-xL inhibitors (Waibel et al., 2013), and aurora kinase inhibitors (Wen et al., 2015). These findings indicate that MPN models are useful pre-clinical models of this disorder that are capable of reliably predicting the therapeutic efficacy of test compounds.

CONCLUSIONS

Since the discovery of the JAK2V617F mutation in 2005, mouse models of JAK2V617F mutant MPNs have provided a great deal of information on the role of JAK2V617F mutations and JAK-STAT signaling in MPN pathogenesis. Early models established that JAK2V617F is sufficient for inducing an MPN phenotype in vivo. Yet, while

there is significant homogeneity and similarity shared among these various models, the many different methods employed for their study and the technical nuances that exist among them has only added to the difficulty of unraveling the molecular pathology associated with this condition, particularly with respect to the effects of JAK2V617F on the stem cell niche and MPN cell self-renewal capacity.

The development of double-mutant mice has allowed for the assessment of protein relationships in the setting of mutant JAK2, which has increased the understanding of the role of canonical JAK-STAT signaling in normal and malignant hematopoiesis as well as the contribution of epigenetic dysregulation in the manipulation of stem cell function. Analysis of the effect of mutations of MPL and CALR *in vivo* supports the theory of a common singular pathway in association with MPN, and that this pathway can be perturbed by a variety of epigenetic and signaling mediators that can ultimately affect the derived phenotype.

Previous studies have shown that wild-type JAK2 protein itself acts to epigenetically modify nuclear histones and alter gene expression beyond that of its canonical functions in signal transduction (Dawson et al., 2009, 2012). Advances in the understanding of how JAK2 interacts with other epigenetic modifying proteins that are mutated in MPNs, including IDH and ASXL1, will be informative. In addition, as the order with which combined mutations are acquired is known to influence disease outcomes in MPNs, *in vivo* procedures that allow for sequential mutagenesis should provide new insights into the factors responsible for MPN heterogeneity.

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Table 14.40.1

Summary of Mutant JAK2V617F MPN Models

Author	Mouse	V617F species	JAK2 promoter	Cre-promoter	Phenotype	LSK Frequency
<i>Transgenic models</i>						
Shide et al. (2008)	C57Bl/6/DBA/2	Mouse	<i>H-2Kb-</i>	n/a	Low exp: ET-like (to PV) High exp: PV-like (to MF)	n/a
Xing et al. (2008)	C57Bl/6/DBA/2	Human	<i>Vav-</i>	n/a	Low exp: mild PV-like High exp: PV-like (to MF)	n/a
Tiedt et al. (2008)	C57Bl/6	Human	Minimal human <i>Jak2-</i>	<i>Mx-</i> and <i>Vav-</i>	<i>Vav-</i> ; ET- (to MF) <i>Mx-</i> ; PV-like (to MF)	n/a
<i>Knock-in models</i>						
Akada et al. (2010)	129Sv/C57Bl/6	Mouse	<i>Jak2-</i>	<i>Mx-</i>	PV-like (to MF)	Increased
Marty et al. (2010)	129Sv/C57Bl/6	Mouse	<i>Jak2-</i>	n/a	PV-like (to MF)	Increased
Mullally et al. (2010)	129Sv/C57Bl/6	Mouse	<i>Jak2-</i>	<i>E2a-</i>	PV-like ^a	Unchanged
Li et al. (2010)	129Sv/C57Bl/6	Human	<i>Jak2-</i>	<i>Mx-</i>	ET-like	Decreased
<i>Retroviral transduction models</i>						
Wernig et al. (2006)	BALB/c and C57Bl/6	Mouse	n/a	n/a	PV-like (to MF)	n/a
Lacout et al. (2006)	C57Bl/6	Mouse	n/a	n/a	PV-like (to MF)	n/a
Zaleskas et al. (2006)	BALB/c and C57Bl/6	Mouse	n/a	n/a	PV-like (to MF)	n/a
Bumm et al. (2006)	BALB/c	Mouse	n/a	n/a	PV-like (to MF)	n/a

^aMyelofibrosis did develop in transplant recipients.